EVALUATION OF GROUNDNUT GENOTYPES FOR FIELD RESISTANCE TO SEED INFECTION BY *Aspergillus flavus* AND TO AFLATOXIN CONTAMINATION

Report of Work Done During May 1988 - April 1989

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EVALUATION OF GROUNDNUT GENOTYPES FOR FIELD RESISTANCE TO SEED INFECTION BY *Aspergillus flavus* AND TO AFLATOXIN CONTAMINATION

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FOREWORD

This report describes research into evaluation of responses of groundnut genotypes to field infection of seed by Aspergillus flavus and to aflatoxin contamination carried out during May 1988 - April 1989 within the Pathology Subprograms of the ISRA, Kaolack, Sénégal, and the CIRAD - IRHO, Montpellier, France. Field trials were conducted at the ISRA research stations at Bambey and Nioro and the groundnut seed samples were processed for mycoflora and aflatoxin analyses in the IRHO laboratories at the CIRAD research Center in Montpellier, France. The research was jointly carried out by Dr. V.K. Mehan, Visiting Scientist, Dr. Amadou Ba, Principal Coordinator, ISRA, Kaolack, Sénégal, and Dr. J.L. Renard, Pathologist, CIRAD - IRHO, Montpellier, France, under the ICRISAT/IRHO collaborative research program on "The management of aflatoxin contamination of groundnut".

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RESUME

Vingt-et-un génotypes d'arachide censés être résistants et sensibles à la colonisation *in vitro* des graines par *Aspergillus flavus* ont été testés pour leur résistance au champ à l'infestation des graines, en particulier avant la récolte par le champignon aflatoxigène, ainsi que pour la contamination par aflatoxine. Parmi ces génotypes se trouvaient plusieurs lignées sélectionnées, ainsi que des lignées tolérantes à la sécheresse. Les génotypes ont été évalués dans trois essais indépendants avec répétitions, sur deux sites (Nioro et Bambey) au Sénégal. Sur chaque site, les graines ont été semées à deux dates différentes (à 12-14 jours d'intervalle), assurant ainsi deux milieux de culture différents, ainsi que la meilleure possibilité d'obtenir un stress hydrique pendant le développement et la maturation des gousses, celui-ci favorisant l'infestation des gousses par *A. flavus* avant la récolte, et la contamination ultérieure par l'aflatoxine. Les deux sites et les deux dates utilisés pour l'essai ont été considéres comme étant quatre milieux différents.

La plupart des génotypes sélectionnés présentant une résistance à la colonisation *in vitro* des graines par *A. flavus* (Ah 7223, J11, U4-47-7, UF 71513, PI 337394 F, 55-437 et 73-30), présentaient également une résistance significativement plus importante à l'infection des graines au champ par *A. flavus*,

ainsi qu'une contamination par l'aflatoxine moins importante que les génotypes sensibles à la colonisation des graines (EC 76446(292) et 57-422). Certains génotypes sensibles à la colonisation *in vitro* des graines par *A. flavus* (U4-7-5, VRR 245 et Exotic 6) présentaient également une résistance à l'infestation des graines au champ, tandis que quatre parmi les cinq lignées sélectionnées résistantes qui ont été testées (ICGV 86016, ICGV 86169, ICGV 86171 et ICGV 86174) étaient très sensibles à l'infestation par *A. flavus*. Ces résultats soulignent l'absence d'un lien absolu entre la résistance à l'infestation des graines avant la récolte et la résistance à la colonisation *in vitro* des graines par *A. flavus* chez certains génotypes d'arachide.

Parmi les sept génotypes tolérants à la sécheresse qui ont été testés, les génotypes EC 21024, RMP 40, J 11 et 55-437 présentaient une résistance à l'infestation par *A. flavus*, tandis que les trois autres génotypes (57-422, ICGV 86635 et NCAc 17090) présentaient une sensibilité à l'infestation des graines par le champignon avant la récolte.

La résistance à l'infestation des graines par *A. flavus* était stable pour l'ensemble des milieux (sites et dates de semis). Quelques interactions ont été observées entre les milieux et les génotypes en ce qui concerne l'infestation par le champignon. En général, les taux d'aflatoxines étaient comparables à l'infestation des graines par *A. flavus* chez les différents génotypes/lignées sélectionnées testés dans des essais indépendants.

Les populations d'*A. flavus* étaient importantes dans les sols de toutes les parcelles étudiées. Les nombres de propagules d'*A. flavus* et d'*A. niger* fluctuaient de manière importante au cours de la saison de croissance; par contre, les conditions de sécheresse pendant le développement et la maturation des gousses facilitaient l'accumulation de l'inoculum d'*A. flavus* dans la zone de développement des gousses.

Parmi les génotypes résistants à *A flavus*, 73-30, U4-7-5, VRR 245 et J 11 présentaient des rendements en gousses relativement acceptables et de qualité commerciale.

Des études des arachides cultivées par les paysans de diverses régions agroécologiques du Sénégal ont mis en évidence des différences variétales prononcées en ce qui concerne l'infestation des graines par *A. flavus*. Des taux d'infestation peu importants (1-3%) trouvés chez le cultivar 55-437 dans toutes les régions de culture de l'arachide du nord du Sénégal ont montré sa résistance stable vis à vis de l'infestation des graines au champ par *A. flavus*. Chez d'autres cultivars sénégalais, 73-33, 28-206 et 69-101, on a mis en évidence des différences régionales prononcées vis à vis de l'infestation des graines par *A. flavus*. Les cultivars 73-33, GH 119-20 et 69-101 tendaient vers la sensibilité à l'infestation par *A. flavus*. La contamination par l'aflatoxine semble principalement avoir lieu avant la récolte dans les zones de culture de l'arachide du nord, tandis qu'elle peut avoir lieu avant ou/et après la récolte dans les zones du sud. Il est donc évident

qu'il y a lieu de réaliser des études systématiques au cours des différentes saisons, afin de déterminer les risques de contamination par l'aflatoxine aux divers stades: à la récolte, au cours du séchage au champ pendant des périodes prolongées, et pendant le stockage à la ferme dans les diverses régions agroclimatologiques du Sénégal.

SUMMARY

Twenty-one groundnut genotypes reported resistant and susceptible to *in vitro* seed colonization by *Aspergillus flavus* were tested for field resistance to seed infection, particularly preharvest infection by the aflatoxigenic fungus, and for aflatoxin contamination. These genotypes included several selected breeding lines and drought-tolerant lines. The genotypes were evaluated in three separate replicated trials at two locations (Nioro and Bambey) in Senegal. At each location sowing was done on two dates (12-14 days apart) providing two crop environments and so improving chances of obtaining drought stress during pod development and maturation, as this is favorable to preharvest pod infection by *A. flavus*, and to subsequent aflatoxin contamination. The locations and sowing dates used for the trials were regarded as four environments.

Most of the selected genotypes with resistance to *in vitro* seed colonization by *A. flavus* (Ah 7223, J 11, U4-47-7, UF 71513, PI 337394F, 55-437, and 73-30) had significantly greater resistance to field infection of seed by *A. flavus* and had lower aflatoxin contamination than had the genotypes (EC 76446(292) and 57-422) susceptible to seed colonization. Some genotypes susceptible to *in vitro* seed colonization by *A. flavus* (U4-7-5, VRR 245, and Exotic 6) also showed resistance to seed infection in the field while four of the five resistant breeding lines tested (ICGV 86016, ICGV 86169, ICGV 86171, and ICGV 86174) were highly susceptible to *A. flavus* infection. These results emphasize that there is not an absolute

relationship between resistance to preharvest seed infection and resistance to *in* vitro seed colonization by *A. flavus* in groundnut genotypes.

Of the seven drought-tolerant genotypes tested, EC 21024, RMP 40, J 11, and 55-437 showed resistance to *A. flavus* infection while the other three genotypes (57-422, ICGV 86635, and NCAc 17090) showed susceptibility to preharvest seed infection by the fungus.

Resistance to seed infection by *A. flavus* was stable across environments (locations and sowing dates). Some interactions were observed between environments and genotypes for fungal infection. In general, aflatoxin levels paralleled *A. flavus* seed infection in different genotypes/breeding lines tested in separate trials.

Soil populations of *A. flavus* were high in all experiment field plots used. Significant fluctuations in numbers of propagules of *A. flavus* and *A. niger* occurred through the growing season, however, drought conditions during pod development and maturation facilitated *A. flavus* inoculum build up in the pod zone.

Of the *A. flavus*-resistant genotypes, 73-30, U4-7-5, VRR 245, and J 11 gave reasonably acceptable pod yields and commercial quality.

Surveys of farmers' groundnuts in different agroecological regions of Senegal indicated marked varietal differences for A. flavus seed infection. Low levels of infection (1-3%) found in the cultivar 55-437 in all the northern groundnut-growing regions of Senegal indicated its stable resistance to field infection of seed by A. flavus. Marked regional differences were found for seed infection by A. flavus in other Senegalese cultivars 73-33, 28-206, and 69-101. Cultivars 73-33, GH 119-20, and 69-101 tended to show susceptibility to A. flavus infection. Aflatoxin contamination appears to be mainly preharvest in the northern groundnut-growing areas while it can be preharvest and/or postharvest in the southern regions. There is an obvious need to conduct systematic surveys in different seasons to determine aflatoxin contamination risks at different stages - at harvest, during field drying for extended periods, and on-farm storage in different agroclimatological regions of Senegal.

CONTENTS

INTRODUCTION

Aflatoxin contamination of groundnut is a serious problem in most groundnut-producing countries. It may occur pre- or post- harvest (5). Preharvest contamination is important in the semi- arid tropics (SAT), particularly under drought stress situations in rainfed groundnut-producing areas (4, 10). Late-season drought stress, a common occurrence in the SAT, is an important contributing factor to seed infection by the aflatoxin-producing fungi Aspergillus flavus and A. parasiticus, and subsequent aflatoxin contamination. Postharvest contamination can be significant under wet and humid conditions, especially resulting from improper drying and storage conditions. Levels of seed infection by the aflatoxigenic fungi, and of consequent aflatoxin contamination, can be minimized by adopting certain cultural, produce-handling and storage practices (5). These practices have been readily adopted by progressive farmers in developed countries with advanced agriculture, but have not been widely adopted by small farmers in developing countries. An alternative approach to prevention of aflatoxin contamination is to grow groundnut cultivars with resistance to seed invasion by the aflatoxigenic fungi (12, 17, 18, 19).

Since 1970 much research has been aimed at finding groundnut cultivars with resistance to seed invasion and colonization by A. flavus/A. parasiticus (1, 12, 17, 18, 19, 24, 25) and a number of genotypes and breeding lines have been reported resistant to in vitro colonization by the aflatoxin-producing fungi of rehydrated, undamaged, mature, stored seed. Resistance to A. flavus/A. parasiticus invasion and colonization of rehydrated, stored, dried

seeds has relevance when aflatoxin contamination is largely postharvest, particularly when groundnuts dried in the field or in storage are wetted, or absorb moisture from the atmosphere. A few studies (2, 4) failed to show any significant differences at harvest in A. flavus infection or aflatoxin contamination of seed of genotypes with different levels of resistance and susceptibility to in vitro seed colonization by the fungus. But some other studies (9, 13, 18, 24, 25) have shown that some genotypes with resistance to in vitro seed colonization also have resistance to field infection of seeds by A. flavus. Evaluations of resistance in groundnuts to preharvest infection by A. flavus have been limited to a few genotypes, and to very few sites. The objectives of the present study in Sénégal were (i) to evaluate for A. flavus seed infection and subsequent aflatoxin contamination in field experiments various groundnut genotypes reported resistant and susceptible to in vitro seed colonization by A. flavus, and (ii) to evaluate aflatoxin contamination of cultivars grown in different agroecological regions of Sénégal.

MATERIALS AND METHODS

Based on their resistance or susceptibility to *in vitro* seed colonization by *A. flavus*, twenty-two groundnut genotypes (Table 1) were selected for testing ; twelve resistant (Ah 7223, J11, PI337394F, UF 71513, U4-47-7, 55-437, 73-30, ICGV 86016, ICGV 86168, ICGV 86169, ICGV 86171, and ICGV 86174) and 10 susceptible (ICGV 86635, EC 21024, EC76446 (292), NCAc 17090, Exotic 6, U4-7- 5, VRR 245, RMP 40, 57-422, and GH 119-20) to *in vitro* seed colonization by *A. flavus* (1, 12, 13, 18, 19, 21, 24, 25). The genotypes NCAc 17090, ICGV 86635, 73-30, RMP 40, and 57-422 are also drought-tolerant (3, 8). Genotypes were evaluated in three separate trials for field resistance to seed infection by *A. flavus*, and for aflatoxin contamination, at two locations (Nioro and Bambey) in Sénégal. These locations are in drought-prone areas where late-season drought stress is of common occurrence, and have light, sandy soils.

All trials were carried out on fields at the ISRA research stations at Bambey and Nioro. The fields had long history of groundnut cultivation ; groundnuts being rotated with pearl millet. The trials were conducted in the 1988 rainy season, sowing dates being normal (July-August) for the locations. The trials were all rainfed. In all experiments, the fertilizer N: P: K (6: 20: 10) was applied at the rate of 150 kg ha^{-1} at land preparation. Seeds of all genotypes were treated with granox (benomyl 10 %: captafol 10 % : carbofuran 20 %) at the rate of 2g kg^{-1} a few days before sowing. Normal cultural practices were followed and care taken to lift each genotype at optimum maturity.

In addition to field trials on experiment farms, groundnut samples from farmers' fields were examined for seed infection by fungi, and for aflatoxin content.

Data on rainfall and average maximum and minimum temperatures during the season were obtained from the ISRA Meteorological Units at both locations.

Field screening of groundnut genotypes for resistance to seed infection by Aspergillus flavus and to aflatoxin contamination

TRIALS

Trial 1. Evaluation of selected groundnut genotypes for resistance to seed infection by A. flavus, and to subsequent aflatoxin contamination.

Twelve genotypes were grown in 3 X 4 rectangular lattice designs at Nioro and Bambey. These genotypes included seven resistant (Ah 7223, J11, PI 337394F, UF 71513, U4-47-7, 55-437, 73-30) and five susceptible (Exotic 6, U4-7-5, VRR 245, EC 76446(292), and 57-422) to in vitro seed colonization by A. flavus. Plots were 6 m long by 4.8 m (8 rows) wide at Nioro, and 6 m long by 4 m (8 rows) wide at Bambey. Seeds were sown singly at 15 cm spacing along the rows. At each location sowing was done on two dates (12-14 days apart) providing two crop environments and so improving chances of obtaining drought stress, particularly during late stages of pod development, as this is favourable to pod infection by A. flavus, and to subsequent aflatoxin

Table 1. Details of groundnut genotypes tested

Genotype	Botanical variety	Country of origin	Pedigree
Resistant to *in vitro* seed colonization by *A. flavus*			
Ah 7223	*vulgaris*	Nigeria	
J11	*vulgaris*	India	(Ah 4218 x Ah 4354)
PI 337394F	*fastigiata*	Argentina	
UF 71513	*fastigiata*	USA	
U4-47-7	*vulgaris*	Uganda	
55-437	*vulgaris*	Sénégal	Selection from a population probably of South American origin
73-30	*vulgaris*	Sénégal	(61-24 X 59-127)
ICGV 86016	*vulgaris*	India	[(var. 2-5 X NCAc 741) X PI 337409]
ICGV 86168	*vulgaris*	India	(J11 X PI 337394F)
ICGV 86169	*vulgaris*	India	(PI 337409 X UF 71513)
ICGV 86171	*vulgaris*	India	(J11 X PI 337394F)
ICGV 86174	*vulgaris*	India	(UF 71513 X PI 337394F)
Susceptible to *in vitro* seed colonization by *A. flavus*			
ICGV 86635	*fastigiata*	India	(NCAc 2768 x NCAc 17090)
EC 21024	*fastigiata*		
EC 76446(292)	*fastigiata*	Uganda	
NCAc 17090	*fastigiata*	Peru	
Exotic 6	*vulgaris*		
U4-7-5	*vulgaris*	USA	
VRR 245	*vulgaris*	India	
RMP 40	*hypogaea*		
57-422	*hypogaea*	Sénégal	Selection from a hybrid population (C.334-3-404)
GH 119-20	*hypogaea*	USA	[(South runner X Dixie-Giant) X Virginia runner]

contamination. Sowing dates were -Nioro (14 July and 29 July 1988), Bambey (4 August and 16 August 1988). In the second sowing of the trial at Bambey one irrigation was applied 43 days before harvest as otherwise continuous severe drought stress would have seriously reduced yields. All genotypes were harvested at maturity, and plants were arranged in windrows with pods exposed to dry for four days. Mature pods were then picked from the plants and sun-dried to a seed moisture content of 5-6 %. From each plot, 1 kg of mature, undamaged, dried pods were sampled for fungal infection and aflatoxin contamination of seeds.

In this trial, populations of A. flavus and Aspergillus niger were monitored for plots with genotypes J 11, EC 76446 (292), and 57-422 before sowing, and at 30, 70, and 85 days before harvest at both locations. Soil samples were collected from five positions at 0-5 and 5-10 cm depths both from between plants (field soil) and from below plants (plant soil) in each plot. For field soil and plant soil, individual samples were pooled for each depth of sampling. All soil samples were taken to the laboratory in polyethylene bags within 4 h of collection. After thorough mixing, from each composite sample, four subsamples (4 g each) were taken for tests. Each subsample was put into 100 ml of sterile, distilled water in 250 ml capacity flask. Appropriate dilutions were made, and 1 ml of the relevant dilution was poured onto malt salt agar medium in 9 cm diameter Petri plates, three replicate plates per sample. The plates were then incubated at 25°C in the dark. Colonies of A. flavus and A. niger growing onto the medium in each plate were counted 5-7 days after incubation and averages calculated for each subsample. Each colony was considered to

have originated from a single fungal propagule.

Trial 2. Evaluation of selected groundnut breeding lines and cultivars for resistance to seed infection by A. flavus, and to aflatoxin contamination

This trial was conducted at Bambey with eight breeding lines and cultivars. These genotypes included five resistant breeding lines (ICGV 86016, ICGV 86168, ICGV 86169, ICGV 86171, and ICGV 86174), two resistant cultivars (J11 and 55-437), and one susceptible cultivar (57-422). This trial was planted on 4 August 1988. The genotypes were grown in a randomized block design with four replications. Plots were 6 m long by 4 m (8 rows) wide with seeds sown singly at 15-cm spacing along the rows. The genotypes were harvested at maturity (90-95 days after sowing), and pods sampled for seed infection by A. flavus and aflatoxin contamination as described above.

Trial 3. Evaluation of drought-tolerant groundnut genotypes/ cultivars relative to seed infection by A. flavus and aflatoxin contamination.

This trial was conducted at Nioro and Bambey with seven genotypes. The genotypes included seven drought-tolerant lines (ICGV 86635, RMP 40, EC 21024, 55-437, 57-422, J 11, and NCAc 17090). Sowing dates were 14 July 1988 (Nioro) and 16 August 1988 (Bambey). The genotypes were grown in randomized block designs with five replications at Nioro, and three at Bambey. The test lines were each sown in 10-row plots of 6 m length. All genotypes were harvested at

maturity, and pods sampled for seed infection by A. flavus and aflatoxin contamination as described above.

Trial 4. Investigation of source of preharvest seed infection by A. flavus relative to seed position in the groundnut pod.

An experiment was conducted with three genotypes (57-422, EC 76446 (292), and GH 119-20) to investigate source of preharvest seed infection by A. flavus relative to seed position in the groundnut pods. The genotypes were grown at Nioro and Bambey in randomized block designs with five replications. The genotypes were each sown in 8-row plots of 6 m length. Seeds were sown singly at 15 cm spacing along rows that were 50 cm apart at Bambey and 60 cm apart at Nioro. The genotypes were examined at harvest for seed infection by A. flavus and other fungi. Twenty- five plants were selected at random from each plot. Mature pods were picked from these plants, hand-shelled, and 100 apical and 100 basal seeds were tested for infection by fungi using standard procedure (see below).

SURVEYS :

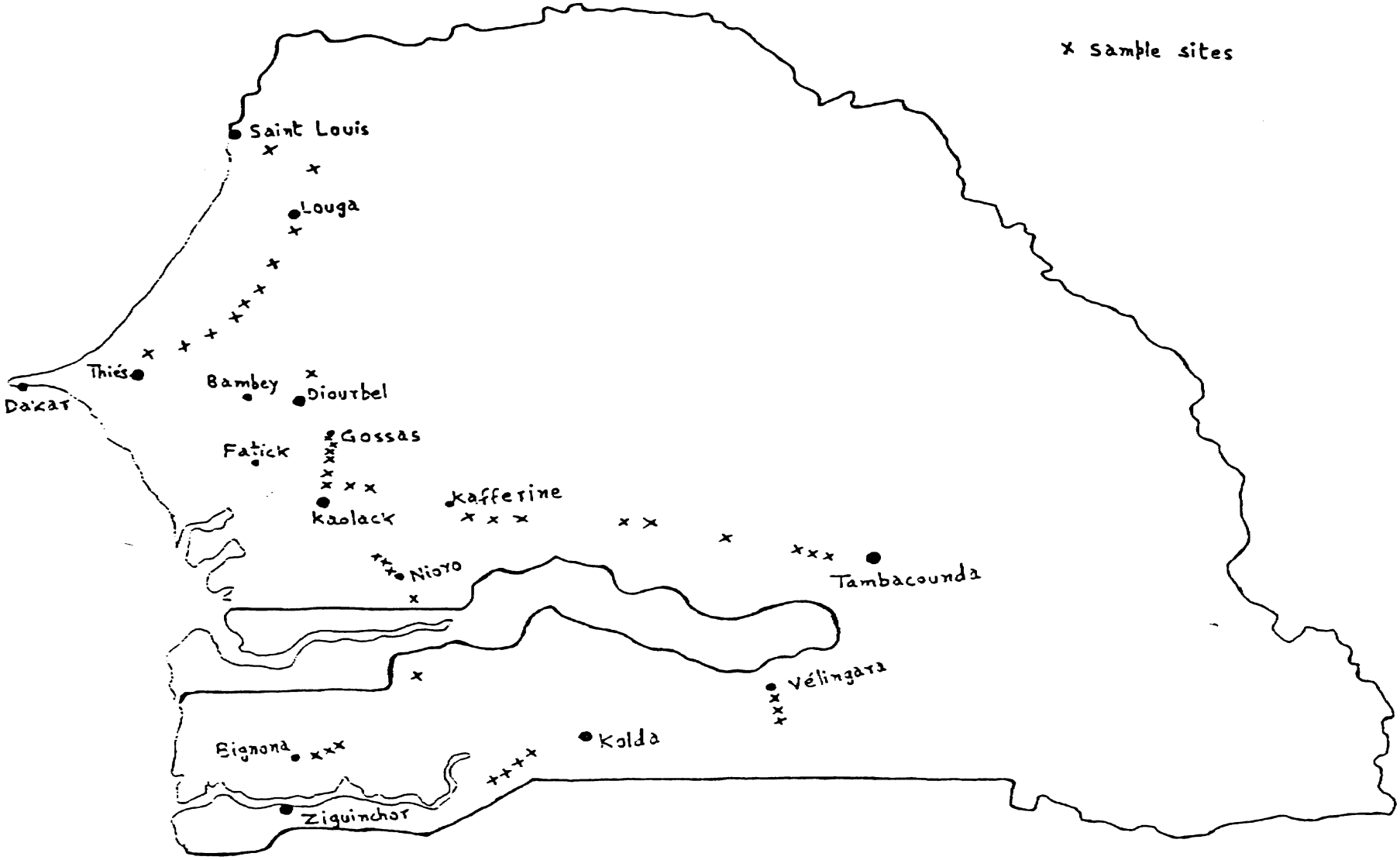
Assessment of fungal infection and aflatoxin contamination of farmers' groundnuts in Senegal.

One hundred and twenty-five samples of groundnuts were obtained from farmers' fields in different agroecological regions of Sénégal for assessing levels of seed infection by A. flavus, and of aflatoxin contamination. Pod samples were collected from the 1988 rainy season crops in farmers' fields in 46 villages of the Kaolack, Tambacounda,

Kolda, Ziguinchor, Fatick, Thiés, Saint-Louis, Louga and Diourbel regions (Figure 1). Pod samples were collected from the freshly harvested crops or from plants being dried in the fields. Approximately 1 kg pod samples (mature pods) were collected from 70-120 plants selected at random. Pod samples were brought to the ISRA, Kaolack Research Center and sun-dried to a seed moisture content of 6-7 %. The pods were hand-shelled, and seeds tested for fungal infection and for aflatoxin contamination. From each sample, 100 seeds and 50-g seed were taken for testing for fungal infection and aflatoxin content, respectively.

Seed samples from trials 1, 2, and 3, and from the surveys of farmers' crops, were sent to the IRHO laboratory at the CIRAD Research Center in Montpellier, France, for mycoflora and aflatoxin analyses.

(i) Examination of seeds for infection by A. flavus and other fungi : In all cases, 100 seeds from each replicated experimental plot/farmer's field were tested for infection by A. flavus and other fungi. The seeds were surface-sterilized by soaking for two minutes in a 0.1 % aqueous solution of mercuric choride, rinsed in two changes of sterile distilled water, and then plated onto Czapek-Dox agar medium supplemented with rose bengal in 9 cm diameter Petri plates for isolation of fungi. The plates were incubated at 25 ^{o}C in the dark and colonies of fungi growing from seeds were recorded after 5-7 days. No distinction was made between colonies of A. flavus and A. parasiticus, both being referred to as A. flavus.



gure Sa ing sites in different regions of Senega where groundnut pod samples we collected from farmers fields

For determining the proportions of A. flavus/A. parasiticus in A. flavus group isolates obtained from infected seeds, 100 isolates (selected at random from seeds of different genotypes in each trial) were examined for identity of the fungi. Isolates of A. flavus and A. parasiticus were identified based on conidiophore arrangement and colony colour characters. (ii) Aflatoxin Analyses : A 50 g sample of seed from each plot/farmer's field was used for determining aflatoxin content of seeds. Aflatoxin levels were determined using the enzyme-linked immunosorbent assay (ELISA) developed by the TRANSIA (TRANSIA. 1988. Immunoenzymatic titration of aflatoxins. pp. 14 - TRANSIA - 8 rue Saint-Jean de Dieu - 69007 Lyon, France). Aflatoxin was extracted with aqueous methanol solution (80 %, V/V). Diluted aliquots of sample extracts, and of standard aflatoxin B 1 solutions were distributed into the wells of a microtitration plate which was precoated with aflatoxin B 1. The monoclonal antibody conjugated to peroxidase was then added to each well and the plate incubated under agitation for 10 min. The plate was washed with the washing buffer, and the amount of conjugate bound to the antibody was determined after addition of the substrate, 2-2-azino-bis-ethyl-benthiazoline-6-sulfonate (ABTS). Aflatoxin levels in the sample extracts were computed from the standard curve constructed with different concentrations of standard aflatoxin B 1 solutions.

Statistical analysis

Using arc sine transformed values, analyses of variance were

performed separately for seed infection by A. flavus and by total fungi other than A. flavus, over environments. The locations and sowing dates used for the trial 1 with 12 genotypes were regarded as four environments : 1 (Nioro-sowing 1), 2 (Nioro-sowing 2), 3 (Bambey-sowing 1), and 4 (Bambey-sowing 2). Analyses of variance were also performed separately for seed infection by each of the other fungi in each environment. An analysis of variance was also performed for aflatoxin content of seed of the genotypes over environments, using $\log_e$ transformed values. An analysis was carried out for correlation between levels of seed infection by A. flavus and aflatoxin contents. Analyses of variance were also performed separately for numbers of propagules of A. flavus and A. niger in field soil and plant soil of three genotypes grown in the trial 1 conducted in environments 1 and 3.

RESULTS

Trial 1. Responses of 12 selected groundnut genotypes to seed infection by A. flavus and to aflatoxin contamination.

Environmental conditions were conducive for preharvest seed infection by A. flavus, and for subsequent aflatoxin contamination, in environments 2, 3, and 4 as moderate to severe drought stress occurred during pod development and maturation in all the groundnut genotypes tested. Drought stress was not evident in the genotypes in environment 1. There was considerable variation in rainfall between environments (locations and sowing dates) (Appendix 1). The two locations differed markedly in length of the rainy season and in rainfall pattern. Minimum and maximum air temperatures were similar at both locations.

The mean percentages of seed of the 12 groundnut genotypes infected by A. flavus are given in Table 2. Significant genotypic differences occurred for seed infection by A. flavus in all four environments. The genotypes J11, U4-47-7, UF 71513, PI 337394F, Ah 7223, 55-437, and 73-30 with resistance to in vitro seed colonization by A. flavus showed low levels of A. flavus infection (0.0-4.0 %). Of these genotypes, only 73-30 had slightly higher percentages of seed infected (2.3 - 4.0 %) in environments 2 and 3 than the other six resistant genotypes (0.6 - 3.0 %). Three genotypes Exotic 6, U4-7-5, and VRR 245, susceptible to in vitro seed colonization by A. flavus, also showed low levels of seed infection (0.0 - 3.0 %) and did not differ significantly in this respect from the seven resistant genotypes in any environment. But the other two susceptible genotypes EC 76446(292) and 57-422 had significantly

higher percentages of seed infected by A. flavus (1.0-33.3 %) than all other genotypes in all environments. Low levels of infection (0.0-5.6 %) were recorded in seeds of all genotypes in environment 1. Seed infection levels were significantly higher across genotypes in environments 3 and 4 than the other environments (Table 2). Significant interactions were found between genotypes and environments for seed infection by A. flavus. This was most discernible in the susceptible genotypes EC 76446(292) and 57-422. Genotype EC 76446(292) had the highest levels of infection in environments 1, 2, 3 whereas 57-422 had the highest level of A. flavus infection in environment 4.

Significant differences were found between genotypes for aflatoxin content of seed in environments 2, 3, and 4 (Table 3). However, only low levels of aflatoxin were detected in seed of all the 12 genotypes in environment I. The genotypes J11, U4-7-5, VRR 245, Exotic 6, UF 71513, PI 337394F, Ah 7223, 55-437 and 73- 30 had significantly lower levels of aflatoxin than the genotypes EC 76446(292) and 57-422 in environments 2,3, and 4. Significant interactions occurred between genotypes and environments for aflatoxin contamination. Genotype EC 76446 (292) had the highest level of aflatoxin in environments 2 and 4, while 57-422 had the highest level of aflatoxin in environment 3 compared to other genotypes (Table 3). Among the resistant genotypes that recorded low percentages of seed infected by A. flavus, only PI 337394F and 55-437 had higher levels of aflatoxin (15.1-24.2 ug kg^{-1}seed) in environment 4 than in other environments (0.6-3.6 ug kg^{-1}seed).

Table 2. Seed infection by Aspergillus flavus in 12 groundnut genotypes in four environments

Genotypes	Seed infected (%) Environments[a] 1	2	3	4
J11	0.0[b] (0.0)	0.6 (3.8)	2.3 (8.7)	1.6 (7.3)
U4-47-7	0.0 (0.0)	0.6 (3.8)	1.6 (7.3)	2.3 (8.7)
U4-7-5	0.0 (0.0)	0.6 (3.8)	2.6 (9.3)	2.6 (9.4)
VRR 245	0.0 (0.0)	0.6 (3.8)	2.6 (9.3)	2.3 (8.7)
Exotic 6	0.0 (0.0)	0.6 (3.8)	1.6 (7.3)	3.0 (9.9)
UF 71513	0.3 (1.9)	0.6 (3.8)	2.0 (7.9)	1.6 (7.3)
PI 337394F	0.3 (1.9)	1.6 (7.3)	2.3 (8.7)	2.0 (7.9)
Ah 7223	0.0 (0.0)	1.0 (5.7)	1.6 (7.3)	2.6 (9.4)
55-437	0.3 (1.9)	1.3 (6.5)	3.0 (9.7)	2.0 (7.9)
73-30	0.0 (0.0)	2.3 (8.7)	4.0 (11.5)	2.6 (9.4)
57-422	1.0 (5.7)	5.6 (13.8)	17.3 (24.6)	23.0 (28.6)
EC 76446(292)	5.6 (13.8)	33.3 (35.2)	30.3 (33.4)	19.0 (25.8)
SE		(± 1.136)		

[a]Environments 1 = Nioro (sowing 1), 2 = Nioro (sowing 2)
3 = Bambey (sowing 1), 4 = Bambey (sowing 2)

[b]Values in parentheses are arc sine transformations.

Table 3. Aflatoxin content (ug kg^{-1} seed) of seeds of 12 groundnut genotypes in four environments

Genotypes	Environments[a] 1	2	3	4
J11	1.8 (1.0)[b]	1.7 (0.9)	4.8 (1.7)	1.0 (0.7)
U4-47-7	1.8 (0.9)	0.5 (0.3)	1.5 (0.9)	3.9 (1.6)
U4-7-5	1.2 (0.8)	1.2 (0.8)	1.0 (0.6)	2.5 (1.3)
VRR 245	1.2 (0.6)	0.4 (0.3)	1.7 (0.9)	2.3 (1.2)
Exotic 6	1.7 (0.9)	0.7 (0.5)	1.8 (1.0)	5.8 (1.9)
UF 71513	2.0 (1.1)	2.7 (1.3)	2.8 (1.3)	4.7 (1.7)
PI 337394F	1.4 (0.8)	0.9 (0.6)	2.8 (1.3)	24.3 (3.2)
Ah 7223	0.6 (0.3)	1.2 (0.8)	6.8 (1.9)	1.2 (0.8)
55-437	1.3 (0.8)	0.6 (0.2)	3.7 (1.5)	15.1 (2.7)
73-30	2.1 (1.0)	9.4 (2.2)	16.7 (2.3)	13.0 (2.6)
57-422	7.4 (1.9)	21.5 (3.1)	90.8 (4.2)	131.2 (4.7)
EC 76446 (292)	0.5 (0.3)	61.5 (4.0)	42.6 (3.5)	240.0 (5.5)
SE		(± 0.281)		

[a]Environments 1 = Nioro (sowing 1), 2 = Nioro (sowing 2), 3 = Bambey (sowing 1), 4 = Bambey (sowing 2)

[b]Values in parentheses are $\log_e$ transformations.

Significant positive correlation was found between A. flavus seed infection and aflatoxin content in all environments except environment 1. The correlation coefficients were r = 0.033, 0.820, 0.766, and 0.811 in environments 1, 2, 3, and 4 respectively.

Significant differences were also found between genotypes for seed infection by fungi other than A. flavus in all four environments (Table 4 and Appendix 2). These fungi included A. niger, Fusarium spp., Macrophomina phaseolina, and Penicillium spp. The mean percentages of seed of the 12 genotypes infected by these fungi are shown in Appendix 2. The genotypes J11, U4-47-7, U4-7-5, VRR 245, Exotic 6, UF 71513, PI 337394F, and Ah 7223 consistently showed low percentages of seed infected (1.0-6.0 %) by these fungi in all environments (Table 4). Genotypes 55-437 and 73-30 had low to moderate levels of seed infection (3.6-11.3 %). EC 76446(292) and 57-422 had significantly higher percentages of seed infected than the other genotypes across environments. Significant interactions were found between genotypes and environments for fungal infection. Aspergillus niger, Fusarium spp., and M. phaseolina were common colonizers of seed of most genotypes in environments 3 and 4 (location Bambey) (Appendix 2). In environments 1 and 2 (location Nioro), A. niger was isolated only from seeds of a few genotypes such as EC 76446 (292), 57- 422, and 73-30. Genotype EC 76446(292) did not show any seed infection by M. phaseolina in environments 1 and 2. Penicillium spp. were found occasionally in some genotypes.

Results on soil populations of A. flavus and A. niger in the

field plots of three cultivars (J11, 57-422, and EC 76446(292), grown in the Trial 1 in environments 1 and 3 (Nioro and Bambey), are summarized in Tables 5 through 8. At sowing, there were no significant differences between the field plots for numbers of propagules of A. flavus in both environments (Nioro-sowing 1 and Bambey-sowing 1) (Tables 5 and 7). Similar observations were made for A. niger propagules in environment 3 (Bambey-sowing 1). However, significant differences were found between the field plots of three cultivars for propagules of A. niger in environment 1 (Nioro-sowing 1) (Table 6) ; the field plots of EC 76446 (292) had significantly lower numbers of propagules at both soil depths (0-5 cm and 5-10 cm) than had the plots of J 11 and 57-422. Significantly higher numbers of propagules of A. niger were recorded at 5-10 cm depth than at 0-5 cm depth in all the field plots of all three cultivars.

Significant differences occurred between sampling dates for numbers of propagules of A. flavus (Table 5). Significantly higher populations of the fungus were recovered at 85 days after sowing than at the other two sampling times (30 days and 70 days after sowing). Significant differences were found between the field plots of cultivars for A. flavus propagules per gram of soil. The plots of EC 76446(292) had significantly higher levels of A. flavus propagules than the plots of J 11 and 57-422; the latter two cultivars did not differ significantly from one another in this respect. Significant interactions were observed between cultivars and sampling dates, and also between cultivars and soil state for fungal populations. Populations of A. flavus did not vary significantly with soil depth or soil state (field soil vs plant-soil).

Table 4. Seed infection by fungi other than Aspergillus flavus in 12 groundnut genotypes in four environments

Genotypes	Seed infected (%)				
	Environments[a]				Means over environments
	1	2	3	4	
J11	1.0 (5.7)[b]	3.0 (10.0)	4.0 (11.5)	3.3 (10.5)	2.8 (9.4)
U4-47-7	1.3 (6.5)	2.3 (8.7)	2.6 (9.4)	3.6 (11.0)	2.4 (8.9)
U4-7-5	2.0 (8.1)	3.0 (9.9)	3.3 (10.3)	4.6 (12.5)	3.2 (10.2)
VRR 245	2.0 (7.9)	3.3 (10.5)	2.6 (9.4)	4.3 (12.0)	3.3 (9.9)
Exotic 6	3.0 (9.9)	4.0 (11.5)	3.3 (10.3)	6.0 (14.2)	4.1 (11.5)
UF 71513	4.3 (11.9)	3.3 (10.5)	6.0 (14.2)	5.6 (13.7)	4.8 (12.6)
PI 337394F	6.3 (14.4)	6.0 (14.2)	5.0 (12.9)	5.0 (12.9)	5.6 (13.6)
Ah 7223	3.0 (9.9)	4.0 (11.5)	3.3 (10.5)	5.0 (12.9)	3.8 (11.2)
55-437	4.6 (12.5)	5.0 (12.9)	4.6 (12.4)	8.0 (16.4)	5.5 (13.5)
73-30	3.6 (11.0)	6.6 (14.9)	8.0 (16.4)	11.3 (19.7)	7.4 (15.5)
57-422	16.6 (24.1)	19.6 (26.3)	23.0 (28.6)	25.3 (30.2)	21.1 (27.3)
EC 76446(292)	32.3 (34.6)	14.3 (22.1)	29.6 (32.9)	32.3 (34.6)	27.1 (31.1)
SE			(± 0.851)		

[a] Environments : 1 = Nioro (sowing 1), 2 = Nioro (sowing 2)
3 = Bambey (sowing 1), 4 = Bambey (sowing 2)

[b] Values in parentheses are arc sine transformations.

Table 5. Propagules of A. flavus in field soil and plant soil of three groundnut cultivars grown in trial 1 at Nioro

Sampling (Days after sowing)		Depth	Cultivar			Means over cultivars
			J 11	57-422	EC 76446 (292)	
At sowing	FS	0-5	4249	3594	4524	4122
		5-10	4839	4453	4203	4498
30	FS	0-5	1812	1695	2310	1939
		5-10	1513	1735	2845	2031
	PS	0-5	2747	1907	2000	2218
		5-10	1168	1536	1704	1469
70	FS	0-5	932	1064	1298	1098
		5-10	853	755	726	778
	PS	0-5	1114	806	1197	1039
		5-10	772	742	1282	932
85	FS	0-5	3180	3263	3535	3326
		5-10	3574	3857	4283	3905
	PS	0-5	2518	2885	4174	3192
		5-10	2292	2659	6418	3790
Means over sampling dates			1873	1909	2647	2143

At sowing		After sowing	
SE mean for comparing :			
- soil depth	± 235.25	- state of soil	± 59.93
- cultivars	± 288.08	- dates of sampling	± 73.40
- cultivars x soil depth	± 407.45	- soil depth	± 59.93
		- cultivars	± 73.40
		- cultivars x dates of sampling	± 127.09
		- cultivars x state of soil	± 103.76

FS = Field soil ; PS = Plant soil.

Soil depth : 0-5 cm ; 5-10 cm.

Table 6. Propagules of A. niger in field soil and plant soil of three groundnut cultivars grown in trial 1 at Nioro

Sampling (Days after sowing)		Depth	Cultivar			Means over cultivars
			J 11	57-422	EC 76446 (292)	
At sowing	FS	0-5	3121	2886	2346	2784
		5-10	3425	3559	2829	3271
30	FS	0-5	1607	1737	1731	1691
		5-10	2141	2325	2186	2217
	PS	0-5	2286	2197	1635	2039
		5-10	2744	2973	1847	2521
70	FS	0-5	1325	1658	1799	1594
		5-10	1889	1742	2329	1986
	PS	0-5	2131	1567	3183	2293
		5-10	2676	1557	3563	2589
85	FS	0-5	4791	4698	5473	4987
		5-10	6909	6243	6445	6532
	PS	0-5	5833	6006	3003	4947
		5-10	7835	8050	4209	6698
Means over sampling dates			3514	3396	3115	3341

At sowing		After sowing	
SE mean for comparing :			
- soil depth	± 138.01	- state of soil	± 76.59
- cultivars	± 169.01	- dates of sampling	± 93.83
- cultivars x soil depth	± 239.01	- soil depth	± 76.59
		- cultivars	± 93.83
		- cultivars x dates of sampling	± 162.48
		- cultivars x state of soil	± 132.62

FS = Field soil ; PS = Plant soil.

Soil depth : 0-5 cm ; 5-10 cm.

Table 7. Propagules of A. flavus in field soil and plant soil of three groundnut cultivars grown in trial 1 at Bambey

Sampling (Days after sowing)		Depth	Cultivar			Means over cultivars
			J 11	57-422	EC 76446	
At sowing	FS	0-5	2864	3459	3025	3116
		5-10	3603	4513	3593	3903
30	FS	0-5	2301	2032	1982	2105
		5-10	2728	2181	2890	2599
	PS	0-5	1949	1433	1773	1718
		5-10	2460	1954	3167	2527
70	FS	0-5	1637	1501	1180	1439
		5-10	1628	1333	1263	1408
	PS	0-5	1406	1134	1657	1399
		5-10	1027	1017	2584	1542
85	FS	0-5	2812	2534	3270	2872
		5-10	2993	2239	3209	2813
	PS	0-5	2039	2222	4374	2879
		5-10	2275	1975	4804	3018
Means over sampling dates			2104	1796	2679	2193

At sowing		After sowing	
SE mean for comparing :			
- soil depth	± 218.37	- state of soil	± 75.03
- cultivars	± 267.45	- dates of sampling	± 91.91
- cultivars x soil depth	± 378.23	- soil depth	± 75.03
		- cultivars	± 91.91
		- cultivars x dates of sampling	± 159.22
		- cultivars x state of soil	± 130.00

FS = Field soil ; PS = Plant soil.

Soil depth : 0-5 cm ; 5-10 cm.

Table 8. Propagules of A. niger in field soil and plant soil of three groundnut cultivars grown in trial 1 at Bambey

Sampling (Days after sowing)		Depth	Cultivar			Means over cultivars
			J 11	57-422	EC 76446 (292)	
At sowing	FS	0-5	5103	5121	5214	5146
		5-10	5888	5825	4617	5443
30	FS	0-5	3006	3058	3142	3069
		5-10	2968	3588	3313	3289
	PS	0-5	3048	3046	2845	2980
		5-10	3518	3812	3364	3565
70	FS	0-5	1911	2073	1722	1902
		5-10	2250	2515	2139	2301
	PS	0-5	2151	2263	1933	2116
		5-10	3554	3119	2323	2999
85	FS	0-5	2505	2421	2359	2428
		5-10	2146	2149	2292	2195
	PS	0-5	2907	3731	1859	2832
		5-10	3416	3204	1734	2785
Means over sampling dates			2782	2915	2419	2705

At sowing		After sowing	
SE mean for comparing :			
- soil depth	± 224.47	- state of soil	± 66.09
- cultivars	± 274.96	- dates of sampling	± 80.99
- cultivars x soil depth	± 388.86	- soil depth	± 66.09
		- cultivars	± 80.99
		- cultivars x dates of sampling	± 140.21
		- cultivars x state of soil	± 114.47

FS = Field soil ; PS = Plant soil.

Soil depth : 0-5 cm ; 5-10 cm.

In environment 3 (Bambey - sowing 1), significant differences were found between sampling dates for A. flavus populations in field plots of the three cultivars (Table 7) ; populations being higher at 85 days after sowing than at the earlier sampling times (30 days and 70 days after sowing). The field plots of EC 76446 (292) recorded significantly higher propagules of A. flavus than the field plots of other two cultivars J 11 and 57-422. Populations of the fungus varied significantly with soil depth ; A. flavus propagules being significantly higher at 5-10 cm depth than at 0-5 cm depth. There were no significant differences between states of soil for A. flavus propagules. Significant interactions were noted between cultivars and dates of sampling, between cultivars and states of soil, and between dates of sampling and depths of soil for numbers of A. flavus propagules (Table 7).

In both environments, cultivars also differed significantly for A. niger populations ; populations being higher in the field plots of 57-422 and J11 than in that of EC 76446(292) (Tables 6 and 8). The former cultivars did not differ significantly from one another in respect of populations of A. niger. Differences between sampling dates were also significant for populations of A. niger. Significant differences were observed between states of soil, and between depths of soil for A. niger populations. Propagules of A. niger were significantly higher in plant soil than in field soil. Populations of A. niger were markedly higher at 5-10 cm depth compared to 0-5 cm depth in both field soil and plant soil (Tables 6 and 8). Significant interactions were evident between cultivars and states of soil, and between dates of sampling and depth of soil for populations of A. niger.

The 12 genotypes were also evaluated for pod yield in environment 2 (Nioro-sowing 2). Genotypes differed significantly for pod yield (Table 9). The genotypes 73-30 and 57-422 had markedly higher pod yields than the other genotypes. The next in order were U4-7-5, VRR 245, and J11. All these five genotypes did not differ significantly from one another in respect of pod yield. Of the genotypes that showed resistance to seed colonization, UF 71513 and PI 337394F had low pod yields. The genotype EC 76446 (292) gave the lowest yield.

Trial 2. Responses of eight breeding lines and cultivars to seed infection by A. flavus and to aflatoxin contamination

Levels of seed infection by A. flavus, and of aflatoxin contamination, in the eight breeding lines and cultivars are given in Table 10. Significant differences were observed between genotypes (breeding lines and cultivars) for seed infection by A. flavus. Among the five breeding lines, ICGV 86168 showed the lowest and ICGV 86171 the highest levels of A. flavus infection. The cultivars J11 and 55-437 had significantly lower percentages of seed infected by A. flavus than the cultivar 57-422 and breeding lines except ICGV 86168. These two cultivars and the breeding line ICGV 86168 did not differ significantly from each other in A. flavus infection. There were also significant differences between genotypes for aflatoxin content of seeds. Cultivars J11 and 55-437 and the breeding line ICGV 86168 had significantly lower levels of aflatoxin than the other lines (Table 10). Aflatoxin contamination was highest in ICGV 86171 followed by 57-422. The breeding line ICGV 86174 that had high percentages of seed infected by A. flavus showed only moderate

Table 9. Pod yield of 12 groundnut genotypes at Nioro[1]

Genotype	Pod yield (kg ha^{-1})
73-30	721.0[2]
57-422	720.0
U4-7-5	710.7
VRR 245	705.0
J11	672.3
U4-47-7	623.3
55-437	597.0
Exotic 6	583.3
Ah 7223	537.3
UF 71513	457.7
PI 337394F	433.7
EC 76446 (292)	295.7
SE	± 56.4
CV (%)	16.6

1. Date of sowing : 29 July 1988
2. Mean of 3 replications ; plot size : 28.8 m^2

level of aflatoxin contamination.

Correlation between seed infection by A. flavus and aflatoxin content was significant ($p = 0.01$) and positive. The correlation coefficient (r) was 0.873.

Significant differences between these eight genotypes were also observed for seed infection by fungi other than A. flavus (Table 11). Macrophomina phaseolina and A. niger were the most common fungi in seed of all genotypes. Fusarium spp. were also found in seed of some of the genotypes. Cultivars J11 and 55-437 and the breeding line ICGV 86168 gave significantly lower levels of infection by these fungi than the other genotypes. Cultivar 57-422 had the highest total seed infection by these fungi.

Genotypes also differed significantly for pod yield (Table 12). Cultivar J 11 recorded the highest pod yield but it did not differ significantly from the genotypes 57-422, 55-437, ICGV 86171, ICGV 86174, and ICGV 86168. Among the five breeding lines, ICGV 86169 had the lowest yield.

Table 10. Infection by Aspergillus flavus, and aflatoxin content of seeds of eight breeding lines and cultivars at Bambey

Breeding-line/ cultivar	Seed infected (%)	Aflatoxin (ug kg seed)
J11	2.2 (8.6)[a]	2 (1.0)[b]
ICGV 86168	2.2 (8.6)	3 (1.4)
55-437	2.7 (9.4)	3 (1.4)
ICGV 86169	10.7 (18.9)	51 (3.9)
ICGV 86016	19.5 (26.0)	48 (3.6)
57-422	23.5 (29.0)	133 (4.8)
ICGV 86174	29.0 (32.5)	31 (3.3)
ICGV 86171	34.0 (35.6)	217 (5.3)
SE	(± 1.391)	(± 0.252)

[a] Values in parentheses are arc sine transformations.
[b] Values in parentheses are $\log_e$ transformations.

Table 11. Seed infection by fungi other than Aspergillus flavus in eight breeding lines and cultivars at Bambey

Breeding line/ cultivar	Seed infected (%) by : AN	Fspp	MP	Total OF
J11	1.0 (5.7)[a]	0.0 (0.0)	1.5 (6.9)	2.5 (9.0)
ICGV 86168	0.7 (4.3)	0.0 (0.0)	1.5 (6.9)	2.2 (8.6)
55-437	1.0 (4.9)	0.5 (2.9)	2.0 (8.1)	3.5 (10.6)
ICGV 86169	4.5 (12.1)	0.0 (0.0)	2.5 (9.0)	7.7 (16.1)
ICGV 86016	3.7 (11.1)	1.2 (6.3)	5.7 (13.8)	10.7 (19.1)
57-422	11.5 (19.8)	2.2 (8.6)	6.7 (15.0)	20.5 (26.9)
ICGV 86174	8.2 (16.6)	1.2 (4.5)	2.7 (9.4)	12.2 (20.4)
ICGV 86171	8.5 (16.9)	1.2 (6.3)	2.2 (8.6)	12.0 (20.2)
SE	(± 0.870)	(± 1.206)	(± 0.614)	(± 0.634)

[a] Values in parentheses are arc sine transformations.

AN = A. niger ; Fspp = Fusarium spp. ; MP = Macrophomina phaseolina ; Total OF = Total of fungi other than A. flavus

Table 12. Pod yield of eight breeding lines and cultivars at Bambey

Breeding line/ cultivar	Pod yield (kg ha^{-1})
J11	550.5
57-422	542.7
ICGV 86016	493.7
55-437	486.2
ICGV 86171	478.5
ICGV 86174	457.5
ICGV 86168	449.2
ICGV 86169	395.5
SE	± 30.8
CV (%)	12.8

1. Date of sowing : 4 August 1988
2. Mean of 4 replications

Trial 3. Responses of seven drought-tolerant genotypes to seed infection by A. flavus and to aflatoxin contamination

Levels of seed infection by A. flavus, and of aflatoxin contamination, in seven drought-tolerant genotypes are shown in Table 13. Significant genotypic differences were found for seed infection by A. flavus in both locations (Nioro and Bambey). The cultivar J 11 showed the lowest levels of A. flavus infection while 57-422 showed the highest levels of infection in both locations. EC 21024, RMP 40, and 55-437 had low percentages of seed infected by A. flavus and these genotypes did not differ significantly from J 11 in respect of A. flavus infection (Table 13). Seed infection levels in all genotypes were markedly higher in Bambey than in Nioro. The genotypes NCAc 17090 and 57-422 had significantly higher percentages of seed infected by A. flavus (16.3 - 18.6 %) than the other genotypes (2.6 - 7.3 %) in Bambey (Table 13).

Significant differences between genotypes were also observed for aflatoxin contamination of seed in Bambey (Table 13). The genotypes J 11, 55-437, and RMP 40 had significantly lower levels of aflatoxin than the other genotypes. No aflatoxin was detected in seeds of most genotypes in Nioro. Very low levels of aflatoxin were found in ICGV 86635 and NCAc 17090. Of all the seven genotypes, only 57-422 showed an appreciable level of aflatoxin in Nioro.

Genotypes also differed significantly for seed infection by fungi other than A. flavus (Table 14). The cultivars J 11 and EC 21024 showed low percentages of seed infected by fungi other than A. flavus. These fungi were A. niger, Fusarium spp, M. phaseolina, and Penicillium spp. Aspergillus niger and Fusarium spp were dominant in

seeds of most of the genotypes in Nioro while A. niger and M. phaseolina were dominant in Bambey. Penicillium spp were only occasionally isolated from seeds of some genotypes. Genotypes 57-422 and NCAc 17090 gave significantly higher percentages of seed infected by total fungi other than A. flavus than the other genotypes both in Nioro and Bambey (Table 14).

There were also significant differences between genotypes for pod yield at both locations (Table 15). Pod yields of all genotypes were markedly higher at Nioro than at Bambey. At Nioro, 57-422 gave the highest pod yield (1747.2 kg ha^{-1}) and it differed significantly ($p=0.01$) from all other genotypes. The next in order were the genotypes J 11, ICGV 86635, and RMP 40 and they did not differ significantly from one another in regard to pod yield. Genotypes 55-437 and NCAc 17090 recorded significantly lower pod yields (818.0 - 822.8 kg ha^{-1}) than the other genotypes both at Nioro and Bambey. At Bambey, EC 21024 showed the highest pod yield (343.7 kg ha^{-1}). However, it did not differ significantly from J 11 and RMP 40 in respect of yield.

Table 13. Seed infection by Aspergillus flavus and aflatoxin contamination in seven drought-tolerant groundnut genotypes grown at Nioro and Bambey.

Genotype	Nioro[1]		Bambey[2]	
	Seed infected (%)	Aflatoxin (ug kg^{-1} seed)	Seed infected (%)	Aflatoxin (ug kg^{-1} seed)
J11	0.0[3] (0.0)	0	2.3 (8.7)	3
55-437	0.0 (0.0)	0	4.3 (12.0)	4
EC 21024	0.0 (0.0)	0	2.6 (9.4)	9
RMP 40	0.0 (0.0)	0	3.0 (9.7)	6
ICGV 86635	1.8 (7.6)	1	7.3 (15.7)	9
NCAc 17090	1.8 (7.6)	1	16.3 (23.8)	12
57-422	2.4 (8.9)	10	18.7 (25.6)	22
SE	(± 0.310)		(± 1.046)	± 2.186

1. Means of five replications.
2. Means of three replications.
3. Values in parentheses are arc sine transformations.

Table 14. Seed infection by fungi other than Aspergillus flavus in seven drought-tolerant groundnut genotypes grown at Nioro and Bambey

Genotype	Nioro 1				Bambey 2			
	Seed infected (%) by :							
	AN[3]	Fspp	MP	Total OF	AN	Fspp	MP	Total OF
J11	0.0 (0.0)[4]	0.6 (3.4)	0.2 (1.1)	0.8 (4.6)	3.0 (9.9)	0.3 (1.9)	0.5 (3.8)	4.0 (11.5)
55-437	0.4 (2.3)	1.4 (6.7)	0.6 (3.4)	2.4 (8.9)	8.0 (16.4)	0.3 (1.9)	1.3 (6.5)	9.6 (18.1)
EC 21024	0.0 (0.0)	0.6 (3.4)	0.0 (0.0)	0.6 (3.4)	0.6 (3.8)	1.6 (7.3)	0.6 (3.8)	3.0 (9.9)
RMP 40	1.4 (6.0)	4.0 (11.5)	1.2 (6.2)	8.4 (16.8)	1.6 (7.3)	1.6 (7.3)	2.3 (8.7)	5.6 (13.7)
ICGV 86635	2.0 (8.1)	5.2 (13.1)	1.2 (6.2)	9.2 (17.6)	4.3 (12.0)	2.3 (8.7)	0.0 (0.0)	6.6 (14.9)
NCAc 17090	3.0 (9.9)	8.8 (17.2)	2.2 (8.5)	16.0 (23.6)	6.3 (14.5)	4.3 (12.0)	3.0 (9.9)	14.0 (21.9)
57-422	4.8 (12.6)	11.4 (19.7)	1.4 (6.7)	19.4 (26.1)	6.0 (14.1)	2.6 (9.3)	3.6 (10.9)	12.6 (20.8)
SE	(±0.793)	(±0.784)	(±0.740)	(±0.826)	(±0.832)	(±1.200)	(± 1.199)	(± 0.638)

1. Means of five replications.
2. Means of three replications.
3. AN = A. niger ; Fspp = Fusarium species ; MP = M. phaseolina ; Total OF = Total fungi other than A. flavus.
4. Values in parentheses are arc sine transformations.

Table 15. Pod yield of seven drought-tolerant genotypes at Nioro and Bambey

Genotype	Pod yield (kg ha^{-1})	
	Nioro	Bambey
57-422	1747.2	268.7
J11	954.8	308.0
ICGV 86635	926.4	280.3
RMP40	899.2	305.3
EC 21024	855.2	343.7
NCAc 17090	822.8	226.7
55-437	818.0	249.3
SE	± 23.26	± 46.70
CV (%)	5.2	28.5

1. Date of sowing at Nioro : 14 July 1988 ; at Bambey : 16 August 1988
2. Mean of 5 replications at Nioro, and of 3 replications at Bambey.

Proportions of A. flavus and A. parasiticus in A. flavus group fungi.

In each trial, A. flavus was the dominant fungus in A. flavus group isolates obtained from infected seeds of various genotypes tested. More than 90 % of A. flavus group fungi isolated from infected groundnuts in the trials 1 and 3 were A. flavus while 87 % of the isolates from the trial 2 were that of A. flavus (Table 16).

Table 16. Proportions of A. flavus and A. parasiticus isolates in A. flavus group fungi obtained from infected groundnut seeds in different trials.

Trial	No. of A. flavus group fungi isolates examined	Isolates of A. flavus	Isolates of A. parasiticus
1	100	92	8
2	100	87	13
3	100	94	6

Trial 4. Source of preharvest seed infection by A. flavus relative to seed position in groundnut pod

Fungal infection of apical and basal seed from undamaged pods of the genotypes 57-422 and GH 119-20 (grown at Nioro) and of 57-422 and EC 76446 (292), grown at Bambey, are shown in Table 17 and Table 18, respectively.

In all cases, higher infection levels were observed in basal seeds than in apical seeds (Tables 17 and 18). Seed positions differed significantly for A. flavus infection in both genotypes at Nioro. Similar observations were noted for the genotype EC 76446 (292) at Bambey. However, seed positions were not significantly different for A. flavus in case of 57-422 at Bambey. Differences between seed positions for A. flavus were most pronounced in EC 76446 (292) and GH 119-20. There were highly significant differences between seed positions for infection by other fungi in all the genotypes.

Table 17. Fungal infection of seeds in relation to their positions in the pods of two groundnut cultivars grown at Nioro.[1]

Seed position	Seed infected (%) by : AF		OF	
	57-422	GH 119-20	57-422	GH 119-20
Apical	1.2	1.2	0.6	7.4
Basal	1.8	4.2	3.6	13.8
SE	± 0.255		± 0.389	
CV (%)	27.1		13.7	

[1]Date of sowing : 14 July 1988
AF = Aspergillus flavus ; OF = Total fungi other than A. flavus

Table 18. Fungal infection of seeds in relation to their positions in the pods of two groundnut cultivars grown at Bambey.

Seed position	Seed infected (%) by : AF		OF	
	57-422	EC 76446 (292)	57-422	EC 76446 (292)
Apical	11.4	12.6	9.4	5.6
Basal	12.6	19.2	14.4	7.2
SE	± 0.760		± 0.634	
CV (%)	11.6		15.5	

[1] Date of sowing : 4 August 1988

AF = Aspergillus flavus ; OF = Total fungi other than A. flavus

Fungal Infection of farmers' seed of Commonly grown Cultivars in different regions of Sénégal

Conditions were conducive for seed infection by A. flavus in groundnut crops in all regions as considerable preharvest drought stress occurred during pod maturation. Preharvest drought stress period ranged from 35-38 days in the northern regions (Saint Louis, Louga, Thiés, Diourbel, and Fatick) and from 30-35 days in the southern regions (Kaolack, Kolda, Tambacounda, and Ziguinchor) (Fig. 1).

Natural infection by A. flavus of seed of five commonly grown groundnut cultivars in different regions of Senegal is shown in Table 19. For each cultivar, mean seed infection levels are presented separately for different regions. Marked differences between genotypes were observed for seed infection by A. flavus. Infection levels were markedly lower in seed samples of 55-437 than in the other cultivars. No significant regional variation in A. flavus infection was noted in case of 55-437, while marked regional differences were found for seed infection by A. flavus in the cultivars 73-33, 28-206, and 69-101. Most of the seed samples of 55-437 (collected from the northern groundnut growing regions of Senegal) showed only 1-2 % seed infected by A. flavus. Of the 45 samples of this cultivar tested, only one had 10 % seed infected by A. flavus. Seeds of this sample were obtained from pods showing lesions incited by Rhizoctonia solani in the Thies region. However, another similar sample from the same region showed only 2 % seed infected by A. flavus. Cultivar 73-33 tended to show higher seed infection by the fungus in the Fatick region than in the Kaolack and Tambacounda regions. Cultivar 69-101

showed markedly higher levels of A. flavus infection in the Ziguinchor region than in the Kolda and Tambacounda regions, while 28-206 had lower infection levels in Ziguinchor than in Kolda. Of the 13 samples of 28-206 tested from the Kolda region, 3 samples showed 5-26 % seed infected while others had 0-2 % seed infected. Nematode lesions on pods or termite damage (pod scarification) in various samples of cultivars 73-33, 69-101, and 28-206 did not appear to influence A. flavus infection of seed as levels of infection were similar in both samples showing pod damage by nematodes/termites and samples without any obvious damage.

There were also marked differences between genotypes for seed infection by fungi other than A. flavus in different regions of Senegal (Table 20). These fungi included A. niger, M. phaseolina, Fusarium spp, and Penicillium spp. M. phaseolina was the dominant fungus in seed samples of all five cultivars in all regions. The next most commonly found fungus was A. niger. Cultivar 55-437 showed markedly lower percentages of seed infected by these fungi than did the other cultivars. Cultivars GH 119-20 and 73-33 showed high susceptibility to M. phaseolina and A. niger.

Table 19. Seed infection by Aspergillus flavus in commonly grown cultivars in different regions of Sénégal

Cultivar	Region	Preharvest drought period (days)[1]	No of samples tested	No of samples showing infection	Seed infected (%)	
					Range	Mean
55-437	Saint Louis	35	3	3	1	1.0
	Louga	35-36	20	19	0-4	2.0
	Thiés	35-36	10	10	1-10	3.0
	Diourbel	35-36	6	6	1-4	2.1
	Fatick	34-37	6	6	1-3	2.0
73-33	Fatick	37-38	15	15	5-18	10.1
	Kaolack	30-32	24	24	1-13	4.5
	Tambacounda	33-35	6	6	2-6	4.1
28-206	Kolda	32-35	13	12	0-26	4.1
	Ziguinchor	31-32	5	4	0-2	1.0
69-101	Kolda	31-35	6	6	1-17	8.0
	Tambacounda	31-32	4	4	2-9	5.0
	Ziguinchor	30-31	3	3	13-16	14.3
GH 119-20	Kaolack	38			5-7	6.2

[1]Number of days without rainfall before harvest.

Table 20. Seed infection by fungi other than Aspergillus flavus in commonly grown cultivars in different regions of Sénégal

Cultivar	Region	Seed infected (%) by : A. niger Range	A. niger Mean	M. phaseolina Range	M. phaseolina Mean	Total fungi Range	Total fungi Mean
55-437	Saint Louis	0-1	0.6	1-2	1.3	2	2.0
	Louga	0-5	1.7	2-6	4.3	3-10	6.0
	Thiés	0-4	1.4	1-8	2.7	1-10	4.1
	Diourbel	1-3	2.0	1-6	2.1	3-8	4.5
	Fatick	1-4	2.1	1-8	4.7	4-10	7.0
73-33	Fatick	3-14	7.6	8-23	12.8	15-30	21.3
	Kaolack	1-12	5.0	3-24	7.5	4-30	12.6
	Tambacounda	4-10	6.1	4-27	14.5	12-35	22.5
28-206	Kolda	0-7	2.1	3-11	5.5	4-15	8.7
	Ziguinchor	0-1	0.4	4-5	4.6	6-7	6.2
69-101	Kolda	1-10	4.7	1-5	3.1	2-19	10.7
	Tambacounda	3-7	5.5	2-22	9.5	5-32	16.5
	Ziguinchor	3-5	4.0	2-3	2.3	8-10	9.0
GH 119-20	Kaolack	4-5	4.5	17-22	19.0	25-30	27.0

Aflatoxin Contamination of Seed of Commonly grown Cultivars in different Regions of Sénégal

Of the 125 groundnut samples collected from farmers fields in different regions of Senegal, 43 were also analyzed for aflatoxin contents of seed. These 43 samples included 16 samples of 55-437, nine of 73-33, four of GH 119-20, and seven each of 28-206 and 69-101. These samples were selected for aflatoxin analysis based on their varying A. flavus seed infection levels in different regions.

Low levels of aflatoxin were found in all samples (Table 21), with only one sample of 55-437 from the Thiés region having level of aflatoxin greater than 20 ug kg^{-1} seed. This sample showed pod lesions incited by Rhizoctonia solani. There did not appear to be any relationship between levels of A. flavus infection and aflatoxin contents of seed of different cultivars.

Table 21. Aflatoxin content of groundnut samples collected from farmers' fields in different regions of Sénégal

Cultivar	Region	Sample No.	% Seed infected by *A. flavus*	Aflatoxin (ug kg^{-1} seed)
55-437	Saint Louis			
		1	1	3
		2	1	2
		3	1	4
	Louga			
		4	4	1
		5	1	1
		6	1	6
		7	3	15
		8	4	15
	Thies			
		9	2	1
		10	2	1
		11	10	23
	Diourbel			
		12	2	1
		13	4	4
		14	2	0
		15	3	3
		16	1	0
73-33	Fatick			
		1	18	12
		2	10	8
		3	7	2
		4	6	2
	Kaolack			
		5	1	6
		6	2	9
		7	6	5
		8	11	4
	Tambacounda			
		9	6	4

Cultivar	Region	Sample No.	% Seed infected by *A. flavus*	Aflatoxin (ug kg^{-1} seed)
28-206	Kolda			
		1	0	4
		2	2	8
		3	26	3
		4	1	6
	Ziguinchor			
		5	1	8
		6	0	5
		7	2	7
69-101	Tambacounda			
		1	9	6
		2	5	5
		3	2	6
	Kolda			
		4	1	8
	Ziguinchor			
		5	13	5
		6	16	5
		7	14	5
GH 119-20	Kaolack			
		1	6	7
		2	7	0
		3	5	1
		4	7	2

DISCUSSION

Several trials were conducted to evaluate 21 selected groundnut genotypes/breeding lines (resistant or susceptible to *in vitro* seed colonization by *A. flavus*) for field resistance to seed infection by *A. flavus*, and for aflatoxin contamination. In one trial, all the seven genotypes with resistance to *in vitro* seed colonization by *A. flavus* (Ah 7223, J 11, U 4-47-7, UF 71513, PI 337394F, 55-437, and 73-30) showed significantly lower levels of natural seed infection by the fungus compared to the susceptible check genotypes 57-422 and EC 76446 (292) across the four environments. These results support the reports of significant genotypic differences in groundnuts for field resistance to seed infection by *A. flavus* in Sénégal and in India (12,19,20). Five of these seven resistant genotypes have been tested in more than one country. Of these, UF 71513 and PI 337394F have been reported as resistant to field infection of seed by *A. flavus* in Sénégal and India (12,20) while J 11 has been found resistant in North Carolina and in India (8,12).

Zambettakis et al. (20) have reported highly significant correlations between seed colonization in laboratory inoculation tests and natural field infection of seed by *A. flavus* in various genotypes tested in several field trials in Sénégal. However, the present studies did not show absolute relationships between the two aspects for all the genotypes/breeding lines tested. For example, some genotypes susceptible to *in vitro* seed colonization by *A. flavus* (VRR 245, U 4-7-5, and Exotic 6) showed resistance to seed infection in the field while four of the five resistant breeding lines tested (ICGV 86016, ICGV 86169, ICGV 86171, and ICGV 86174) were highly susceptible to *A. flavus* infection. These results are in accord with the earlier

findings of Kisyombe et al. (8) and Mehan et al. (12) and emphasize that it can not be assumed that all genotypes resistant to *in vitro* seed colonization by *A. flavus* will show resistance to natural seed infection in the field, or that all genotypes susceptible to *in vitro* seed colonization will have susceptibility to field infection of seed by the fungus.

Of the five breeding lines, ICGV 86168 showed levels of seed infection by *A. flavus* similar to that of the cultivars J 11 and 55-437 which consistently showed low levels of infection in all the trials conducted in different environments at Nioro and Bambey. Genotypic differences for *A. flavus* seed infection were most pronounced under the severe drought stress conditions that occurred at Bambey. Seed infection levels ranged from 2-33 % in different genotypes under these conditions. Such infection levels are considerably higher than those reported (0-11.7 %) by Zambettakis et al. (20) in trials conducted during 1977-1979 at Bambey and Darou, Sénégal. These differences in levels of infection may be attributed to relative susceptibility of different genotypes included in the trials, and to differences in environmental conditions. The highest levels of infection obtained in cultivars 55-437 and 57-422 in their trials were 1.7 and 6.6 %, whereas in the present trials the same cultivars recorded 3 and 23 % respectively. In the trials reported here, mean incidence of natural seed infection by *A. flavus* ranged from 0-33 % among the genotypes/breeding lines tested. Within this range we considered genotypes with 3 % or lower incidence to be resistant.

In general, aflatoxin levels paralleled A. flavus seed infection in different genotypes/breeding lines tested in different trials. However, some variation in levels of seed infection by A. flavus and aflatoxin content did occur in some cases. For example, the breeding line ICGV 86171 that had high percentages of seed infected by A. flavus showed only moderate levels of aflatoxin contamination. This may possibly be attributed to differences in genotypes in ability to support aflatoxin production (13). The low levels of A. flavus infection in the resistant genotypes and the breeding line were matched by low levels of aflatoxin contamination. The low levels of aflatoxin found in seed of these genotypes/breeding line under natural field conditions in the present study indicates that field resistance to A. flavus infection is important in conferring resistance to aflatoxin contamination.

As drought stress during pod development and maturation is known to predispose groundnuts to A. flavus seed infection it was thought that drought-tolerant genotypes might be resistant to preharvest infection by the fungus. Of the seven drought-tolerant genotypes tested, EC 21024, RMP 40, and 55-437 showed resistance to A. flavus infection similar to that of the cultivar J 11 at Bambey. The other three drought-tolerant genotypes (57-422, ICGV 86635, and NCAc 17090) showed considerable susceptibility to preharvest seed infection by A. flavus. It is interesting to note that valencia type drought-tolerant genotypes such as NCAc 17090 and ICGV 86635 gave high levels of seed infection by A. flavus. Most genotypes reported tolerant to end-of-season drought are of the valencia type, many of which appear to have weak pod shell structures which may facilitate attack by weak parasites such as A. flavus. However, the drought-tolerant valencia

genotype EC 21024 showed only a low level of seed infection. It is interesting that the drought- tolerant spanish cultivars J 11 and 55-437 show greater resistance to A. flavus infection. It is important to combine drought-tolerance with resistance to seed infection by A. flavus in groundnut cultivars for use in the semi-arid tropics, particularly in areas where end-of-seasn drought is of common occurrence. It would be interesting to determine if drought- tolerant cultivars of different botanical types and pod characters show significant differences in their reactions to A. flavus.

In all field trials, most of the A. flavus infection in all genotypes/breeding lines appeared to have originated preharvest as postharvest environmental conditions were favourable for rapid drying of groundnuts. In this context, the existence of stable resistance to field infection of seed by A. flavus in certain genotypes is important as much of the aflatoxin contamination in the SAT, under drought stress situations, occurs before harvest (4,6). A. flavus infection and subsequent aflatoxin contamination can also occur during postharvest field drying and in storage (5), and in this connection the genotypes resistant to in vitro seed colonization by A. flavus may show an advantage in minimizing the risk of aflatoxin contamination if postharvest environmental conditions favour development of the aflatoxigenic fungus. Also, under these conditions, preharvest resistance to A. flavus should prove useful since heavy preharvest infection could lead to serious build-up of aflatoxin contamination. The genotypes having resistance to both in vitro seed colonization and preharvest seed infection by the aflatoxigenic fungus should be particularly useful in minimizing

aflatoxin contamination in areas where this may occur either preharvest or postharvest or at both stages.

Significant interactions between environments and genotypes noted in the trials indicated a strong influence of environment on seed infection by A. flavus, A. niger, and M. phaseolina. Variations in levels of A. flavus infection in the genotypes in different environments may be explained by the variations in the occurrence of other fungi such as A. niger and M. phaseolina. High levels of A. flavus infection across genotypes/breeding lines in Bambey (envrionments 3 and 4) are attributed mainly to severe drought stress that occurred particularly during pod development and maturation. Drought stress during pod maturation is known to encourage preharvest fungal infection and aflatoxin contamination of seed (4,6). Genotypes with field resistance to A. flavus, in general, appeared to show greater resistance to seed infection by A. niger, M. phaseolina, and F. spp. than the A. flavus-susceptible genotypes. Resistance to seed infection by these pathogenic fungi is important for maintaining seed quality for planting.

Of the A. flavus resistant genotypes, 73-30, U4-7-5, VRR 245, and J 11 gave reasonably acceptable pod yields and commercial quality, and should be tested under farmers' conditions to determine whether the resistance can confer a definite advantage in terms of low fungal infection and aflatoxin contamination in comparison with currently grown Senegalese groundnut cultivars. Of these, cultivar 73-30 has considerable seed dormancy and this trait should be useful in conditions of drought stress being released by rains just at maturity.

Soil populations of A. flaus were markedly higher in all experiment field plots than those reported by other workers in Sénégal (18). Significant fluctuations in numbers of propagules of A. flavus and A. niger during the periods of the trial 1 can be explained by the soil moisture levels. Late-season drought conditions facilitated build-up of inoculum of A. flavus in the geocarposhpere. Significant differences between genotypes for numbers of viable propagules of A. flavus and A. niger reflect differential effects of cultivars upon the populations of these fungi. The presence of adequate inoculum and late-season drought conditions especially in light, sandy soils in Sénégal provide very congenial conditions for A. flavus infection of groundnuts.

Some researchers have suggested that A. flavus may invade groundnuts through the flowers, travel down the pegs and become established in the developing seed (19, 20). If such is the case, then it may be that basal (i.e. proximal) seeds in multi-seeded pods were more likely to be infected by A. flavus than are apical (i.e. distal) seeds. The higher levels of infection found in basal seeds in some of the cultivars examined in the present studies appear to support this hypothesis, but the infection may have originated through peg or shell. There is no indication that undamaged pods can not be infected directly through the shell particularly under conditions of fluctuating moisture stress. It is known that certain valencia type groundnut cultivars have weak pod areas, especially prominent beak and such pod characters would presumably expose the apical seeds to invasion by A. flavus. More research needs to be done to determine if seed position in the groundnut pod has any significant relation to fungal infection and aflatoxin contamination in various groundnut

cultivars of different botanical types.

Surveys of farmers' groundnuts indicated marked varietal differences for A. flavus seed infection. Low levels of A. flavus infection (1-3 %) found in the cultivar 55-437 in all the northern groundnut-growing regions of Sénégal indicated its stable resistance to field infection of seed by A. flavus. Cultivars 73-33, 60-101, and GH 119-20 tended to show susceptibility to A. flavus seed infection. However, mean infection levels found in these cultivars (1.0-14.3 %) are considerably lower than those reported (31-62 %) by Pettit (17). Different seed surface-disinfectants used in the various studies are likely causes of variation in levels of infection detected. Differential responses of the cultivars 73-33, 69-101, and 28-206 to A. flavus infection in different regions may possibly be attributed to the variations in the occurrence of other fungi such as M. phaseolina and A. niger. Nematode lesions on pods or limited termite damage (pod scarification) found in various samples of cultivars 73-33, 69-101, and 28-206 did not appear to influence seed infection by A. flavus. Several studies have failed to establish a definite link between nematode infestations and A. flavus infection or aflatoxin contamination in groundnuts (14, 15).

Only low levels of aflatoxin were found in all the 43 samples of farmers' groundnuts tested. Low levels of aflatoxin found in samples of the cultivar 55-437 in the northern regions can be attributed to their low levels of A. flavus seed infection. However, it is interesting to note that some samples of the cultivars 73-33, 69-101, and 28-206 with high percentages of seed infected (13-26 %) by A. flavus also had only low levels of aflatoxin. This suggests that postharvest conditions favoured rapid drying of produce thus limiting

further development of A. flavus in seeds. High temperatures in this period may have inhibited aflatoxin production by the fungus already present in the seeds. Earlier studies in Sénégal (6) have reported high levels of aflatoxin (130-600 ug kg seed) in groundnuts sampled from different regions, levels being higher in the northern regions than the southern regions. Such large differences in aflatoxin levels might be possible due to variations in susceptibility to aflatoxin contamination of cultivars sampled, environmental conditions, and sampling procedures. It is important to emphasize that in the present studies only seeds from well dried, intact pods were tested for fungal infection and aflatoxin contamination since pod damage of any kind is likely to override resistances to A. flavus in groundnuts.

CONCLUSIONS AND IMPLICATIONS

The trials have shown that several groundnut genotypes have stable resistance to field infection of seed by A. flavus. Most seed infection in the test genotypes is considered to have originated prior to harvest, bearing in mind the severe preharvest drought and favourable postharvest drying conditions in the season. In this situation, it is interesting to note the presence of resistance to preharvest seed infection by A. flavus in some selected genotypes with resistance to in vitro seed colonization by the fungus. However, there is not an absolute relationship between resistance to preharvest infection and resistance to in vitro seed colonization by A. flavus in groundnuts. This conclusion is derived from the presence of resistance or susceptibility to field infection of seed by A. flavus in both the groups of genotypes with or without resistance to in vitro

seed colonization. The lack of complete agreement between results of resistance measured by the laboratory inoculation test and results of field test indicates the risk involved in relying entirely upon the laboratory inoculation method for resistance screening. Resistance to pod/seed invasion in the field could be due to resistance in the shell and seed, but it might also be at least in part due to factors operating in the geocarposphere. It is imperative to give more emphasis to resistance of the groundnut fruit to A. flavus infection rather than to focus solely on seed resistance to invasion by A. flavus under in vitro conditions.

It would be useful to compare the A. flavus-resistant genotypes with commercial cultivars in farmers' fields to assess their comparative advantage in terms of prevention or substantial reduction in aflatoxin contamination. The aflatoxin contamination status of all components of the saleable yield should be determined as most assessements have concentrated on undamaged, mature seeds. It would be important to evaluate such materials in areas where aflatoxin contamination occurs preharvest, postharvest or at both stages.

Differential reactions of drought-tolerant genotypes to A. flavus infection suggest that the resistance of the groundnut pod is associated with certain structural and biochemical characters of both pod and seed. Drought-tolerant spanish cultivars appear to have greater resistance to A. flavus seed infection than drought-tolerant valencia genotypes. More research is needed to answer the important question : Can the drought-tolerance of a cultivar reduce stress on pod and seeds and thus reduce the chances of invasion by A. flavus in the soil ? Nevertheless, it would be desirable to combine resistance to preharvest seed infection by A. flavus with drought-tolerance in

groundnut cultivars for rainfed areas where late-season drought stress is of common occurrence.

Preliminary results have shown significant differences between apical and basal seeds for A. flavus infection. More research needs to be done to determine if seed position in the groundnut pod has any influence on infection by A. flavus, using multi-seeded pods of various cultivars in different botanical types. The possibility of invasion of groundnut fruit in the soil being initiated through infection of flowers and pegs needs to be properly investigated under both normal and drought-stress situations. This would be important in terms of improving sampling procedures for monitoring A. flavus seed infection and /or aflatoxin contamination.

Surveys of farmers' groundnuts have provided additional evidence of the presence of resistance to seed infection by A. flavus in the Senegalese cultivar 55-437. Other Senegalese cultivars 73-33, GH 119-20, and 69-101 are susceptible to A. flavus. Aflatoxin contamination appears to be mainly preharvest in the northern groundnut growing areas while it can be both preharvest and/or postharvest in the southern regions. Only limited work has been done to assess aflatoxin contamination in commonly grown cultivars in different regions of Sénégal. There is an obvious need to conduct systematic surveys in different seasons to determine the extent to which groundnuts are contaminated with aflatoxin at different stages - at harvest, during field drying, and on-farm storage in different agroclimatological regions. It should then be possible to identify high-, low-, and no-aflatoxin contamination risk areas. Such information would help in establishing a plan for effective control of aflatoxin contamination.

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Appendix 1. Rainfall received by groundnut crops during the 1988 rainy season at Nioro and Bambey

Place	Sowing date	Total rainfall received (mm)	Rain received by the crops at different stages of crop maturity			
			Days after sowing			
			1-30	31-60	61-90	91-100
Nioro	14 July	869.4	369.1	342.7	157.6	0
Nioro	29 July	796.8	496.3	257.9	42.6	0
Bambey	4 August	592.2	436.5	154.3	1.4	0
Bambey	16 August	561.0	460.6	99.9	0.5	0

Appendix 2. Seed infection by fungi other than Aspergillus flavus in 12 groundnut genotypes in four environments

Genotypes	Environments 1			2			3			4		
	Seed infected (%) by											
	AN	Fsp	MP	AN	Fsp	MP	AN	Fsp	MP	AN	Fsp	MP
J 11	0.0[a] (0.0)	0.7 (3.8)	0.7 (3.8)	0.0 (0.0)	1.7 (7.3)	1.3 (6.5)	0.7 (3.8)	1.0 (5.7)	2.0 (8.1)	1.3 (6.5)	0.0 (0.0)	2.0 (7.9)
U4-47-7	0.0 (0.0)	1.0 (5.7)	0.3 (1.9)	0.0 (0.0)	1.7 (7.3)	0.7 (3.8)	0.7 (3.8)	0.3 (1.9)	1.7 (7.3)	1.3 (6.5)	0.0 (0.0)	2.3 (8.7)
U4-7-5	0.0 (0.0)	1.7 (7.3)	0.3 (1.9)	0.0 (0.0)	1.7 (7.3)	1.3 (6.5)	1.7 (7.3)	0.3 (1.9)	1.3 (6.5)	2.3 (8.7)	0.0 (0.0)	2.3 (8.7)
VRR 245	0.0 (0.0)	1.3 (6.5)	0.3 (1.9)	0.0 (0.0)	1.7 (7.3)	1.7 (7.3)	0.3 (1.9)	0.3 (1.9)	2.0 (8.1)	1.3 (6.5)	0.7 (3.8)	2.3 (8.7)
Exotic 6	0.3 (1.9)	1.7 (7.3)	1.0 (5.7)	0.0 (0.0)	2.3 (8.7)	1.7 (7.3)	1.3 (6.5)	0.7 (3.8)	1.3 (6.5)	2.6 (9.4)	0.0 (0.0)	3.3 (10.5)
UF 71513	0.3 (1.9)	2.7 (9.4)	1.7 (7.3)	0.0 (0.0)	1.3 (6.5)	1.7 (7.3)	3.7 (11.0)	0.7 (3.8)	1.7 (7.3)	3.0 (9.9)	0.7 (3.8)	2.0 (8.1)
PI337394F	0.0 (0.0)	2.7 (9.4)	3.0 (9.9)	0.3 (1.9)	2.7 (9.4)	2.7 (9.4)	3.0 (9.9)	0.3 (1.9)	1.7 (7.3)	2.3 (8.7)	0.0 (0.0)	2.7 (9.3)
Ah 7223	0.0 (0.0)	1.3 (6.5)	1.0 (5.7)	0.0 (0.0)	2.0 (8.1)	1.3 (6.5)	1.0 (5.7)	1.0 (5.7)	1.3 (6.5)	2.3 (8.7)	0.0 (0.0)	2.7 (9.4)
55-437	0.0 (0.0)	2.3 (8.7)	2.3 (8.7)	0.0 (0.0)	2.3 (8.7)	2.3 (8.7)	1.0 (4.6)	0.7 (3.8)	3.0 (9.9)	3.3 (10.5)	0.3 (1.9)	4.3 (12.0)
73-30	0.0 (0.0)	2.3 (8.7)	1.3 (6.5)	1.7 (7.3)	1.7 (7.3)	2.7 (9.3)	3.3 (10.3)	1.7 (7.3)	3.0 (9.9)	4.3 (12.0)	2.7 (9.1)	4.0 (11.5)
57-422	4.7 (12.5)	4.0 (11.5)	6.3 (13.4)	7.7 (16.0)	4.7 (12.5)	6.3 (14.5)	12.0 (20.2)	2.3 (8.7)	7.3 (15.7)	19.7 (26.3)	0.7 (3.8)	4.3 (12.0)
EC 76446 (292)	17.0 (24.3)	12.7 (20.8)	0.0 (0.0)	9.7 (18.1)	4.0 (11.1)	0.0 (0.0)	19.0 (25.8)	4.7 (12.4)	2.7 (9.4)	23.3 (28.9)	5.3 (13.3)	3.7 (11.0)
SE	(±0.91)	(±0.90)	(±1.27)	(±0.66)	(±0.86)	(±0.98)	(±1.49)	(±1.54)	(±0.77)	(±0.75)	(±1.27)	(±0.72)

[a]Values in parentheses are arc sine transformations.