Resistance of Groundnut Varieties to *Aspergillus flavus* in Senegal

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Abstract

In four rainy seasons (1977-1980) some 40 groundnut genotypes were screened for field resistance to seed invasion by the aflatoxigenic fungus, Aspergillus flavus *in trials at Bambey and Darou research stations in Senegal. Significant varietal differences were observed at harvest in respect of levels of naturally occurring seed infestation by* A. flavus. *Field resistances were positively correlated with previously measured resistance to in vitro seed colonization by* A. flavus *in laboratory inoculation tests.*

The commercially grown variety 55-437 had high levels of resistance to A. flavus *in both field and laboratory screening, while two other varieties (73-30 and 73-33) also grown in Senegal had moderate levels of resistance.*

In associated investigations it was found that genotypes with seed resistance to A. flavus *had a lower proportion of* A. flavus *in their rhizosphere mycoflorae than had genotypes susceptible to seed invasion by this fungus. Varieties, through their effects on rhizosphere mycoflorae may influence the composition of the soil mycoflora of groundnut fields.*

Résumé

Recherche sur la résistance des variétés d'arachide à *Aspergillus flavus* : *Une quarantaine de génotypes ont été évalués au cours de quatre campagnes pluviales (1977-1980) pour la sélection de matériel résistant à l'invasion des graines par le champignon aflatoxinogène* Aspergillus flavus *dans les champs. Ces essais ont été entrepris sur les stations de recherche de Bambey et de Darou au Sénégal. Des différences variétales significatives ont été observées dans le taux de contamination naturelle des variétés à la récolte. Il y a une corrélation positive entre la résistance dans les champs et la résistance mesurée antérieurement à la colonisation in vitro par* A. flavus *au cours des tests d'inoculation, réalisés au laboratoire.*

La variété commerciale 55-437 s'est montrée très résistante à la fois dans les champs et au laboratoire. Deux autres variétés (73-30 et 73-33) également cultivées au Sénégal présentent une résistance moyenne.

*D'autre part, le pourcentage d'*A. flavus *dans la mycoflore de la rhizosphère est inférieur pour les génotypes résistant à l'invasion des graines par* A. flavus *par rapport aux génotypes sensibles.*

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Compte tenu de l'interaction entre les variétés et la mycoflore de leur rhizosphère, celles-ci peuvent également influencer la composition de la mycoflore du sol des champs d'arachide.

Resúmene

La resistencia de variedades de cacahuate a *Aspergillus flavus* en Senegal : *Durante cuatro temporadas de lluvias (1977-1980), aproximadamente 40 genotipos de cacahuate fueron evaluados y seleccionados por su resistencia, bajo condiciones de campo, a la invasión de sus semillas por el hongo aflatoxigénico* Aspergillus flavus, *en pruebas realizadas en las estaciones experimentales de Bambey y Darou, en Senegal. Se observaron diferencias significativas entre las variedades estudiadas en el momento de la cosecha, en los niveles de infección con* A. flavus que ocurren en la semilla bajo condiciones de campo. Las resistencias de campo observadas resultaron estar positivamente correlacionadas con las resistencias a la colonización de las semillas por A. flavus, *observadas previamente bajo condiciones* in vitro *en pruebas de inoculación realizadas en el laboratorio.*

La variedad comercial 55-437 tuvo altos niveles de resistencia a A. flavus, *tanto en las pruebas de campo como de laboratorio, mientras que otras dos variedades (73-30 y 73-33), que también se siembran en Senegal, solamente tuvieron niveles medianos de resistencia.*

En investigaciones complementarias, se ha encontrado que los genotipos que poseen resistencia en sus semillas a A. flavus *tenían una menor cantidad de* A. flavus *en la microflora de sus rhizosferas que los genotipos susceptibles a la invasión de sus semillas por el hongo citado. Las diferentes variedades, a través de los efectos sobre la micoflora de sus rhizosferas, pueden influir en la composición de la micoflora de los campos cacahuateros.*

Introduction

Groundnuts are very important to the economy of Senegal, both for local consumption and export to obtain foreign exchange. The significance of the aflatoxin problem in groundnut: relation to public health and to the future of the export trade has been recognized in Sene High priority was given to research on the problem and this was undertaken in a collaborat program involving the Institut senegalais de recherches agricoles (ISRA), the Institut recherches pour les huiles et oléagineux (IRHO), and the Cryptogamy Laboratory of the Mu national d'histoire naturelle (MNHN) in Paris. This paper describes some of the research carr out during the years 1977–79 on varietal resistance in groundnut to seed infection by aflatoxin-producing *Aspergillus flavus* Link ex Fries together with preliminary studies 1981/82 of the rhizosphere mycofloras of two genotypes, one resistant and one susceptibl seed infection by *A. flavus*.

Materials and Methods

Varietal Trials

During the rainy seasons of 1977, 1978, and 1979, two field trials with 36 groundnut genoty were carried out at two locations (Bambey and Darou) in Senegal. At each location, one t

was sown at the normal time and the other was sown one month later. Genotypes ranged in duration from 90 to 130 days, and were harvested when judged to have attained optimum maturity.

Bambey is in the north central region of Senegal where the rainy season is short (104±34 days) whereas Darou is in southern Senegal and has a rather longer rainy season (115±18 days). Soils at Bambey are light and sandy, and at Darou are typical Alfisols.

Trials were laid out as randomized blocks with two replications of treatments (genotypes). Five seeds were sown in each hole at 40-cm spacing along 50-cm wide ridges. Plots were composed of six ridges, each 6m-long.

After lifting, plants were dried in windrows in the field until seed moisture contents were below 10%. Samples of undamaged, mature pods were collected from each plot for testing for natural seed infection by *Aspergillus flavus*, and for screening for resistance in rehydrated seed to colonization by *A. flavus*.

To determine natural infection of seeds by *A. flavus*, 300 undamaged mature pods were handshelled and the seeds surface sterilized by immersion for 3 min in a 0.2% aqueous solution of sodium hypochlorite. Following three rinses in sterile distilled water the seeds were transferred aseptically onto moistened filter paper in sterilized petri dishes. The dishes were incubated at room temperature (25 ± 1°C) for 7 days and fungi growing from the seeds were identified and recorded.

The method of Mixon and Rogers (1973) was followed to determine resistance to seed colonization by *A. flavus* in laboratory inoculation tests. Seeds sterilized as described above were hydrated to 20% water content and surface inoculated with a suspension of spores of *A. flavus*. The inoculated seeds were then incubated in petri dishes at 25 ± 1°C for 7 days and the numbers of seed colonized by *A. flavus* were then recorded.

Rhizosphere Studies

Two groundnut genotypes, 55-437 which had been found resistant to seed infection by *A. flavus*, and 75-16 (PI 343419) which had been found susceptible to *A. flavus*, were grown in the 1981 and 1982 rainy seasons at Darou. On the day of sowing, the soil mycoflora of the field was examined, and the rhizosphere mycoflora was examined 15 days later and then at 15-day intervals until harvest. Three samples were taken at each examination from each of three replicate plots. The soil dilution plate technique was employed using acidified potato dextrose agar medium. Colonies of various genera of fungi were subcultured onto specific media for identification to species (Rouxel 1978, Alabouvette 1983). Mycofloras were described both quantitatively and qualitatively.

Results

Field and Laboratory Screening

Full information on all varieties screened for natural seed infection by *A. flavus* and for reaction to seed colonization has been presented in a series of publications (Waliyar 1978, Zambettakis et al. 1977, 1981). In this paper we review data from work in 1977, 1978 and 1979 on 36 genotypes that were common to the trials.

Natural seed infection with *A. flavus* was greater in the late sowings than in the earlier sowings in all but the 1977 trials in Bambey where infection was much higher in the seeds from the earlier sowing (Table 1). This result from Bambey was probably due to the drought that occurred in the late stages of pod maturation in the early-sown crop. Mean levels of *A. flavus* infection were highest in 1978 which was a drought year in Senegal. This again is a strong indication as to the influence of drought stress on seed infection. Of the 36 genotypes scored, 5 showed significantly lower than average levels of *A. flavus* seed infection, and the mean infection levels for these genotypes over years are compared with those of the susceptible control cultivar PI 343419. Three of the resistant genotypes, 55-437, 73-30, and 73-33, are released commercial cultivars in Senegal, 55-437 being the most widely grown.

The mean levels of *A. flavus* infection for combined genotypes were generally higher in Bambey than in Darou, and this was also the case for the resistant genotypes (Tables 1 and 2).

The genotypes with low levels of natural seed infection by *A. flavus* had lower than average levels of seed colonization by this fungus in laboratory inoculation tests (Waliyar 1978). Dates of sowing influenced seed colonization by *A. flavus* in inoculation trials for all genotypes, levels being higher from seed produced by the late-sown crops. This is thought to be occasioned by higher seed testa damage occurring in the late-sown material, which results from greater drought stress in that material and possibly quicker drying of plants later in the year.

In addition to varietal differences, other factors also play a role in the contamination ɔed by *A. flavus*. Among these factors soil type (Mehan et al. 1986) influences the groundnut

Table 1. Field contamination by *Aspergillus flavus* of groundnut seeds from two sowings, Bambey and Darou, Senegal, 1977-79.

	Contaminated seed (%)			
	Bambey		Darou	
Year	1st sowing	2nd sowing	1st sowing	2nd sowing
1977	23.5	9.1	9.9	12.2
1978	39.4	42.5	19.1	30.3
1979	9.2	20.3	3.2	18.1

Table 2. Field contamination of various groundnut genotypes by *Aspergillus flavus*, Bambey and Darou, Senegal, 1977-79.

	Contaminated seed (%)			
	Bambey		Darou	
Genotypes	1st sowing	2nd sowing	1st sowing	2nd sowing
PI 337409	1.23	1.70	0.14	1.40
PI 337394 F	1.43	2.57	0.37	2.30
55-437	0.84	0.74	0.30	0.90
73-30	0.54	0.87	0.43	2.30
73-33	0.57	3.10	0.22	0.56
PI 343419[1]	4.13	5.09	3.81	9.49

1. Susceptible control genotype.

infection. Other factors such as damage caused by different insects, termites, and millipedes facilitate seed infection by *A. flavus*.

The underground development of groundnut pods makes them particularly vulnerable to attacks by different fungi likely to facilitate *A. flavus* contamination. Soil mycoflora increases in the presence of groundnut and this may influence the next crop's reaction to diseases.

Rhizosphere Studies

Comprehensive reports on studies on groundnut rhizospheres have been published by Waliyar (1986a, 1986b). From 45 days after sowing (DAS) until harvest, numbers of fungal propagules per gram of dry soil were considerably higher in the rhizosphere of the *A. flavus*-susceptible cultivar 75-16 than in the rhizosphere of the *A. flavus*-resistant cultivar 55-437. Numbers of species of fungi present in the rhizosphere were also greater in the cultivar 75-16 from 45 DAS. These changes in numbers and composition of the components of the mycofloras may be related to the onset of pod development.

Numbers of propagules of *A. flavus* g^{-1} of dry rhizosphere soil were fairly similar for the two cultivars at the first three times of sampling but in the samples taken from 45 DAS until harvest the numbers were higher for the susceptible cultivar 75-16 (Table 3).

Table 3. Numbers of propagules (× 10^3) of *Aspergillus flavus* g^{-1} dry rhizosphere soil, Senegal, 1981.

	Time of sampling (DAS)[1]						
Variety	0	15	30	45	60	75	90
55-437	8	3	7	4	12	24	58
75-16	4	2	6	14	82	42	96

1. DAS = Days after sowing.

Conclusions

The variety 55-437, which is a commercial variety of excellent agronomic character, well suited to conditions in Senegal, is as resistant to natural seed infection by the aflatoxigenic *A. flavus* as the two resistant genotypes PI 337409 and PI 337394F. The varieties 73-30 and 73-33, also commercial varieties in Senegal, have tolerance to seed invasion by *A. flavus* and are also drought tolerant. The three varieties now represent around 80% of the groundnut crop area in Senegal, and their use on this scale should have a significant effect in reducing the overall aflatoxin contamination levels in Senegal's groundnut crop.

The positive correlation found between resistance to natural infection in the field and resistance in laboratory inoculation tests indicates that either method could be used in evaluating groundnut cultivars and breeding lines for seed resistance to infection by *A. flavus*.

There are indications that varieties may have differential influences upon the buildup and constitution of soil mycoflora including *A. flavus*. Effects may be manifest on the infection of pods and seeds of the growing crop or on those of subsequent crops.