Effects of adding inoculum of *Aspergillus flavus* to pod-zone soil on seed infection and aflatoxin contamination of peanut genotypes (1)

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Summary. — Several peanut genotypes resistant or susceptible to *in vitro* seed colonization by *Aspergillus flavus* were grown in replicated field trials at ICRISAT Center, Patancheru, India, during 1981 to 1984. Addition of inoculum of an aflatoxigenic strain of *A. flavus* to the pod-zone soil increased preharvest *A. flavus* seed infection and aflatoxin contamination. Levels of aflatoxin B1 contamination were positively correlated with percentage of seed infected by *A. flavus*, irrespective of whether or not inoculum was added to the soil. Genotypic differences for seed infection by *A. flavus* and for aflatoxin contamination were about the same in both control and inoculum treated plants. Levels of seed infection by other soil fungi were not significantly influenced by addition of *A. flavus* inoculum to the pod-zone soil. Most of the genotypes included as resistant to *in vitro* seed colonization by *A. flavus* of rehydrated, mature, undamaged, stored seed, also showed resistance to invasion by the fungus in the field.

INTRODUCTION

When screening peanut genotypes for resistance of seeds to preharvest invasion by the aflatoxigenic fungus *Aspergillus flavus* Link ex Fr., we found that under the conditions of soil (Alfisols) and climate normally prevalent in the peanut growing season, levels of seed infection and of aflatoxin contamination were usually very low [Mehan *et al.*, 1986]. Preliminary studies indicated that addition of inoculum of *A. flavus* to the pod-zone soil could increase seed invasion by the fungus [Mehan and McDonald, 1984]. This paper presents data from replicated field trials at ICRISAT Center to examine the effects of applying inoculum of an aflatoxigenic strain of *A. flavus* to pod-zone soil of selected peanut genotypes at different growth stages of the crop on seed infection by *A. flavus* and other fungi, and on aflatoxin contamination.

MATERIALS AND METHODS

All trials were carried out on Alfisols at ICRISAT Center, Patancheru (17°3′N Lat., 78°16′E Long., alt. 541 m), near Hyderabad (India). Rainy season crops were sown in late June and harvested in October ; postrainy season irrigated crops were sown in late November or early December and harvested in April. In trials in the postrainy seasons, irrigation was withheld 20 days before harvest. In the rainy seasons, the trials were all rainfed. In all trials, 60 kg/ha of P_2O_5 were applied at land preparation. Seeds were sown singly at 15 cm spacing along ridges 75 cm apart. The genotypes used in the trials were resistant, susceptible, and highly susceptible to *in vitro* seed colonization by *A. flavus* (IVSCAF) (3).

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(3) IVSCAF : *In vitro* seed colonization by *A. flavus* of rehydrated, mature, stored, undamaged seed.

Experiment 1.

In the 1981 rainy and 1981/82 postrainy seasons 15 peanut genotypes were sown in field trials using a split-plot design with 3 replications. The main-plots were assigned to compare inoculation treatment versus control (no inoculation) and the sub-plots to test genotype reaction. Plots were 9 m long by 1.5 m (2 ridges) wide. Ten plants were selected at random from each plot in the experiment and inoculum of the aflatoxigenic strain (AF 8-3-2A) of *A. flavus* was applied to the pod-zone soil of each of these plants at 30 and again at 20 days before harvest.

Mass inoculum of the aflatoxigenic strain of *A. flavus* (AF 8-3-2A) was produced on Czapek-Dox broth supplemented with 0.7 p. 100 yeast extract in Corning flasks (250 ml capacity). The liquid medium (100 ml in each flask) was inoculated with a spore suspension of 8-day-old culture of the strain, and then incubated at 25 ± 1 °C in dark for 8-9 days. After incubation, the fungal cultures in the flasks were filtered through Whatman No. 1 filter paper and the mycelial mats and spores were thoroughly washed with deionized water. A spore suspension was prepared in sterile deionized water containing 0.1 p. 100 Tween 20 and adjusted to a concentration of approximately 15×10^6 conidia/ml. The washed mycelial mats were broken into small bits.

Soil was carefully removed from around pegs and pods by brushing it aside by hand, and 500 ml of spore suspension and 20 g of mycelial bits were added to the pod-zone soil of each plant and the soil was replaced. Care was taken to avoid disturbance and injury to the pegs and pods at each inoculation time.

Genotypes were harvested at maturity and plants arranged in inverted windrows in the field to dry. After windrow drying (for 3 days in the rainy season and 2 days in the postrainy season) the pods were hand-picked and dried in the shade until the seed moisture content was below 8 p. 100. Dried pods were then stored in cloth bags at room temperature until tested for seed infection by *A. flavus*, the pods from inoculated plants being kept separate from the others.

Experiment 2.

In the 1982/83 and 1983/84 postrainy and the 1984 rainy seasons 8 genotypes were sown in field trials using a split-plot design with 3 replications. The main-plots were assigned to genotypes and the sub-plots to inoculation treatments. Plots were 9 m long by 3.75 m (5 ridges) wide. For the inoculation treatments, 20 plants were selected at random from each plot and inoculum of the *A. flavus* strain applied to the pod-zone soil as described above. The inoculation treatments were : *(a)* no inoculation (T1), *(b)* inoculation at pegging stage and also during pod development (T2), *(c)* inoculation at pegging stage, during pod development and also at 30 days before harvest (T3), and *(d)* inoculation during pod development and at 30 days before harvest (T4).

The genotypes were harvested, sampled, and pods stored as described in experiment 1.

For all trials in both the experiments, 100 undamaged, mature seeds from each plot were examined for infection by *A. flavus* and other fungi using the method described by Mehan and McDonald [1984].

For all trials in experiment 2, seeds were also tested for aflatoxin content. Two 50 g samples of seed from each plot were tested for aflatoxin content using the method of Pons *et al.* [1966].

Statistical analysis.

Using arc sine transformed values, analyses of variance were performed separately for seed infection by *A. flavus* for each of the trials. Analyses of variance were also done for aflatoxin B1 content of seed of 8 genotypes for the 1982/83 and 1983/84 postrainy, and 1984 rainy season samples, using log transformed values.

RESULTS

Experiment 1.

The mean percentages of seed of the 15 peanut genotypes infected by *A. flavus* are given in Table I. The addition of *A. flavus* inoculum to soil around developing pods significantly increased *A. flavus* infection of seeds in both seasons. The increase in percentage of seed infected in different genotypes ranged from 1.7 to 9.0 in the 1981 rainy season, and from 2.3 to 9.7 in the 1981/82 postrainy season (Table I). Marked differences were found between genotypes for seed infection by *A. flavus* in both seasons.

TABLE 1. — **Infection with *A. flavus* of seeds of peanut genotypes following inoculation of developing pods at 30 days and again at 20 days prior to harvest**

(Infestation par A. flavus *des graines de génotypes d'arachide, suivant l'inoculation des gousses en voie de formation à 30 jours puis à 20 jours avant la récolte)*

	Rainy season *(Saison des pluies)* 1981			Postrainy season *(Saison sèche)* 1981/82		
	% seed infected *(% graines infestées)*		Mean increase in infection *(Augmentation moyenne de l'infestation)*	% seed infected *(% graines infestées)*		Mean increase in infection *(Augmentation moyenne de l'infestation)*
Genotypes	NI(a)	I(a)	(I-NI)	NI	I	(I-NI)
PI 337394F(c)	0.4 (3.8)(b)	2.3 (8.7)	1.7	0.4 (3.8)	4.3 (11.9)	3.7
PI 337409(c)	0.4 (3.8)	2.6 (9.4)	2.0	1.3 (6.5)	4.3 (12.0)	3.0
UF 71513(c)	0.1 (1.9)	1.9 (7.9)	1.7	0.4 (3.8)	3.0 (9.9)	2.3
J 11(c)	0.1 (1.9)	1.6 (7.3)	1.3	0.4 (3.8)	3.6 (11.0)	3.0
Ah 7223(c)	0.1 (1.9)	2.6 (9.3)	2.3	0.2 (2.7)	3.0 (9.9)	2.3
Var. 27(c)	1.0 (5.7)	4.3 (12.0)	3.3	1.3 (6.5)	5.6 (13.7)	4.3
Exotic 2(d)	0.4 (3.8)	2.6 (9.4)	2.0	1.6 (7.3)	5.3 (13.3)	3.7
U-4-47-7(d)	0.4 (3.8)	3.6 (11.0)	3.0	1.3 (6.5)	4.3 (12.0)	3.0
U-4-4-1(d)	0.4 (3.8)	6.3 (14.5)	5.7	0.6 (4.6)	6.6 (14.9)	5.7
Ah 7299(d)	1.0 (5.7)	5.3 (13.3)	4.3	1.3 (6.5)	6.3 (14.5)	5.0
NC Ac 841(d)	0.4 (3.8)	6.6 (14.9)	6.0	1.3 (6.5)	6.3 (14.5)	5.0
TMV 2(e)	1.3 (6.5)	4.6 (12.4)	3.3	2.3 (8.7)	7.3 (15.7)	5.0
EC 76446(e) (292)	1.6 (7.3)	7.3 (15.7)	5.7	2.6 (9.3)	10.3 (18.7)	7.7
Gangapuri(e)	2.0 (8.1)	11.0 (19.4)	9.0	3.6 (10.9)	13.2 (21.3)	9.7
U-1-2-1(f)	0.4 (3.8)	3.6 (11.0)	3.0	1.6 (7.3)	5.6 (13.7)	4.0
SE mean for comparing *(Ecart-type pour comparaison de)* :						
— treatments *(traitements)*	(± 0.46)			(± 0.24)		
— genotypes *(génotypes)*	(± 0.83)			(± 0.78)		
— treatment × genotypes *(traitement × génotype)*	(± 1.17)			(± 1.10)		

(a) NI = No *(sans)* inoculation ; I = Inoculation.

(b) Values in parentheses are arc sine transformations *(Les valeurs entre parenthèses sont des transformations d'arc sinus).*

(c) IVSCAF-resistant = less than 15 p. 100 seeds colonized by *(Résistant à la CIVGAF = < 15 p. 100 des graines colonisées par) A. flavus.*

(d) IVSCAF-moderately resistant = 16 to 30 p. 100 seeds colonized by *(Moyennement résistant à la CIVGAF = entre 16 et 30 p. 100 des graines colonisées par) A. flavus.*

(e) IVSCAF-susceptible = 30 to 50 p. 100 seeds colonized by *(Sensible à la CIVGAF = entre 30 et 50 p. 100 des graines colonisées par) A. flavus.*

(f) IVSCAF-highly susceptible = over 50 p. 100 seeds colonized by *(Hautement sensible à la CIVGAF = > 50 p. 100 des graines colonisées par) A. flavus.*

The IVSCAF-resistant genotypes (J 11, Ah 7223, UF 71513, PI 337394F, and PI 337409) had relatively lower levels of seed infection than had the IVSCAF-susceptible genotypes [TMV 2, Gangapuri and EC 76446 (292)]. However, the IVSCAF-resistant genotype Var. 27 gave slightly higher percentages of seed infected by *A. flavus* than the other IVSCAF-resistant genotypes and the IVSCAF-moderately resistant genotypes U4-47-7 and Exotic 2.

Levels of seed infection by *A. flavus* for all genotypes were slightly higher in the postrainy season than in the rainy season.

Experiment 2.

The mean percentages of seed of the 8 genotypes infected by *A. flavus* are shown in Table II, and mean aflatoxin B1 contents of their seeds are given in Table III. Analysis of variance for seed infection by *A. flavus* for the data pooled from 3 seasons is shown in Table IV. All inoculation treatments significantly ($p < 0.001$) increased *A. flavus* infection of seeds for all genotypes in all seasons (Table II). The extent of increase in *A. flavus* infection by inoculation treatments was consistent across seasons, ranging from an increase of 2.7 to 31.0 percentage of seed infected. The inoculation treatment T3 gave the highest levels of seed infection, but it did not differ significantly from the other two inoculation treatments T2, and T4, in respect of seed infection in most individual genotypes across seasons. The IVSCAF-resistant genotypes (J 11, Ah 7223, UF 71513, and PI 337394F) had significantly lower levels of seed infection by *A. flavus* than had the IVSCAF-susceptible genotypes [TMV 2, Gangapuri, EC 76446(292), and OG 43-4-1] in all treatments in all seasons.

Levels of natural seed infection by *A. flavus* (no inoculation) were higher in the 1984 rainy season than in the two postrainy seasons.

In general, aflatoxin contamination paralleled *A. flavus* infection of seeds although these two tests were carried out on separate sub-samples. The inoculation treatments influenced seed contamination with aflatoxin B1 in accord with their effects on seed infection by *A. flavus* (Tables II and III). All the correlations between seed infection by *A. flavus* and contamination with aflatoxin B1 were significantly positive. The correlation coefficients were $r = 0.707$, 0.799, 0.807, and 0.899 for T1, T2, T3, and T4 treatments, respectively, in the 1982/83 postrainy season while they were $r = 0.833$, 0.733, 0.777, and 0.649 for these treatments in the 1983/84 postrainy season. In the 1984 rainy season, the correlation coefficients were $r = 0.766$, 0.778, 0.868, and 0.789 for T1, T2, T3, and T4 treatments, respectively. However, some variation in levels of seed infection and aflatoxin B1 content occurred in the IVSCAF-susceptible genotypes.

TABLE II. **Seed infection with *A. flavus* of 8 peanut genotypes following application of inoculum of the aflatoxigenic *A. flavus* to the pod-zone soil at different growth stages of the crop, in three seasons**

(Infestation par A. flavus *des graines de 8 génotypes d'arachide suivant l'addition d'un inoculum de la souche aflatoxigène de* A. flavus *dans la zone du sol occupée par les gousses à des stades différents de croissance de la culture au cours de 3 saisons)*

	P. 100 of seeds infected with *(P. 100 de graines infestées par)* A. flavus														
	Postrainy season *(Saison sèche)* 1982/83 Inoculation treatments *(Traitements d'inoculum)*					Postrainy season *(Saison sèche)* 1983/84 Inoculation treatments *(Traitements d'inoculum)*					Rainy season *(Saison des pluies)* 1984				
Genotypes	T1	T2	T3	T4	Mean *(Moyenne)*	T1	T2	T3	T4	Mean *(Moyenne)*	T1	T2	T3	T4	Mean *(Moyenne)*
J 11(b)	0.1 (1.9)(a)	4.7 (12.5)	5.3 (13.3)	3.7 (11.0)	2.8 (9.7)	0.1 (1.9)	4.3 (11.9)	5.0 (12.9)	3.6 (11.0)	2.7 (9.4)	1.6 (7.3)	4.7 (12.5)	7.3 (15.7)	4.7 (12.5)	4.3 (12.0)
Ah 7223(b)	0.4 (3.8)	4.3 (12.0)	4.3 (11.9)	4.0 (11.5)	2.9 (9.8)	0.2 (2.7)	3.6 (11.0)	4.7 (12.5)	4.0 (11.5)	2.7 (9.4)	1.6 (7.3)	5.7 (13.8)	7.7 (16.1)	6.6 (14.9)	5.1 (13.0)
UF 71513(b)	0.4 (3.8)	4.0 (11.5)	5.3 (13.3)	4.3 (11.9)	3.1 (10.1)	0.1 (1.9)	4.0 (11.5)	5.0 (12.9)	3.3 (10.5)	2.6 (9.2)	1.6 (7.3)	4.6 (12.4)	6.7 (15.0)	4.3 (12.0)	4.1 (11.7)
PI 337394F(b)	1.0 (5.7)	4.0 (11.5)	6.3 (14.5)	4.7 (12.5)	3.6 (11.0)	0.4 (3.8)	4.7 (12.5)	5.7 (13.8)	4.7 (12.5)	3.4 (10.6)	2.3 (8.7)	5.7 (13.8)	8.4 (16.8)	5.7 (13.8)	5.5 (13.3)
OG 43-4-1(d)	2.3 (8.7)	9.7 (18.1)	16.3 (23.8)	10.9 (19.3)	9.0 (17.5)	1.6 (7.3)	8.6 (17.1)	11.6 (19.9)	9.3 (17.7)	7.1 (15.5)	3.7 (11.0)	23.5 (29.0)	24.6 (29.3)	18.9 (25.8)	16.3 (23.8)
TMV 2(c)	2.3 (8.7)	10.7 (19.1)	15.0 (22.8)	11.6 (19.9)	9.1 (17.6)	2.3 (8.7)	8.6 (17.1)	13.3 (21.4)	8.0 (16.4)	7.5 (15.9)	4.6 (12.4)	15.3 (23.0)	24.6 (29.7)	14.0 (22.0)	13.8 (21.8)
Gangapuri(c)	2.7 (9.4)	16.6 (24.0)	18.3 (25.3)	16.3 (23.8)	12.4 (20.6)	2.6 (9.3)	12.3 (20.5)	17.3 (24.6)	12.9 (21.0)	10.4 (18.8)	4.6 (12.4)	21.5 (27.6)	28.9 (32.5)	22.5 (28.3)	18.1 (25.2)
EC 76446(c) (292)	3.0 (9.9)	14.7 (22.5)	19.0 (25.8)	15.7 (23.3)	12.2 (20.4)	2.7 (9.4)	12.3 (20.5)	18.7 (25.6)	15.0 (22.8)	11.3 (19.6)	8.3 (16.7)	24.3 (29.5)	39.3 (38.8)	18.3 (25.3)	21.5 (27.6)
Mean *(Moyenne)*	1.3 (6.5)	8.0 (16.4)	10.3 (18.7)	8.2 (16.6)		1.0 (5.6)	7.0 (15.3)	9.5 (17.9)	7.1 (15.4)		3.3 (10.4)	11.9 (20.2)	16.8 (24.2)	10.9 (19.3)	
SE mean for comparing *(Ecart-type pour comparaison de) :*															
— treatments *(traitements)*								(± 0.37)						(± 0.43)	
— genotypes *(génotypes)*		(± 0.37)						(± 0.67)						(± 0.53)	
— treatment × genotype *(traitement × génotype)*		(± 0.55) (± 1.05)						(± 1.04)						(± 1.20)	

(a) Values in parentheses are arc sine transformations *(Les valeurs entre parenthèses sont des transformations d'arc sinus).*
(b) IVSCAF-resistant *(Résistant à la CIVGAF).*
(c) IVSCAF-susceptible *(Sensible à la CIVGAF).*
(d) IVSCAF-highly susceptible *(Hautement sensible à la CIVGAF).*

Levels of aflatoxin B1 in the no-inoculation treatment were low in seeds of all genotypes except EC 76446 (292) and Gangapuri in all three seasons.

Fusarium spp. and *Aspergillus niger* were the only fungi other than *A. flavus* to commonly occur in seed of all genotypes in all 3 seasons. *Penicillium* spp. were occasionally found in some genotypes, particularly in the 1983/84 season. The inoculation treatments did not significantly influence seed infection by *Fusarium* spp. or by *A. niger* across seasons. However, in the 1984 rainy season, T3 gave relatively higher levels of seed infection by *Fusarium* spp. than the other treatment, including control (no inoculation). The percentages of seed infected by *Fusarium* spp. were 0.7-4.0, 0-3.0, and 1.3-10.0 for the 1982/83, 1983/84, and 1984 seasons, respectively. The percentages of seed infected by *A. niger* were 0.7-3.0, 0.3-3.0, and 1.0-3.7 in the 1982/83, 1983/84, and 1984 seasons. Significant differences occurred between genotypes for seed infection by *Fusarium* spp. across seasons. Genotypes J 11, Ah 7223, and UF 71513 had the lowest percentages of seed infected by *Fusarium* spp., while EC 76446 (292) and Gangapuri had the highest percentages of seed infected.

DISCUSSION

Over several seasons and years of testing seed from many peanut genotypes at ICRISAT Center for natural infection by *A. flavus* and other fungi, levels of infection were often low. Large numbers of seeds had to be examined to confirm levels of infection that were often less than 1 p. 100 in some genotypes. This confirmation could be done by testing a minimum of 300 seeds from each sample, and it was possible to show significant genotypic differences for natural seed infection by *A. flavus* in screening trials [Mehan *et al.*, 1986]. The benefits from dealing with higher levels of infection in identifying resistance to the fungus are obvious. Hence we attempted to establish resistance or susceptibility to *A. flavus* infection in selected peanut genotypes under enhanced inoculum pressure in the field.

Addition of *A. flavus* inoculum increased the levels of seed infection by the fungus, e.g. the percentage of seed infected in genotype J 11 increased from 0.1 p. 100 to about 5 p. 100 in the 1982/83 and 1983/84 postrainy seasons, and from 1.6 to 7.3 p. 100 in the 1984 rainy season (Table II). This result may permit use of smaller seed samples when testing for resistance to seed infection, an important matter when seed number is limited. The relative differences between *A. flavus* resistant and susceptible genotypes were not significantly altered by the inoculation treatments. Data from these trials support previous findings on the existence of varietal resistance to seed invasion by *A. flavus* and other fungi [Kisyombe *et al.*, 1985 ; Mehan *et al.*, 1986 ; Zambettakis *et al.*, 1981]. Although most of the IVSCAF-resistant genotypes tested showed field resistance to seed invasion by *A. flavus*, it should not be assumed that all IVSCAF-resistant genotypes have resistance to seed infection by the fungus in the field [Kisyombe *et al.*, 1985 ; Mehan *et al.*, 1986]. It is

TABLE III. — **Aflatoxin B1 content of seeds of 8 peanut genotypes following application of inoculum of the aflatoxigenic *A. flavus* to the pod-zone soil at different growth stages of the crop, in three seasons**

(Teneur en aflatoxine B1 des graines de 8 génotypes d'arachide suivant l'addition d'un inoculum de la souche aflatoxigène de A. flavus *dans la zone de sol occupée par les gousses à des stades différents de croissance de la culture pendant 3 saisons)*

	Aflatoxin B1 (µg/kg seed - *de graines)*														
	Postrainy season *(Saison sèche)* 1982/83					Postrainy season *(Saison sèche)* 1983/84 Inoculation treatments *(Traitements d'inoculum)*					Rainy season *(Saison des pluies)* 1984				
Genotypes	T1	T2	T3	T4	Mean *(Moyenne)*	T1	T2	T3	T4	Mean *(Moyenne)*	T1	T2	T3	T4	Mean *(Moyenne)*
J 11(b)	1 (0.8)(a)	244 (5.5)	364 (5.9)	299 (5.7)	89 (4.5)	0 (0)	133 (4.9)	180 (5.2)	133 (4.9)	44 (3.8)	5 (1.8)	199 (5.3)	244 (5.5)	133 (4.9)	80 (4.4)
Ah 7223(b)	1 (0.9)	244 (5.5)	445 (6.1)	244 (5.5)	89 (4.5)	0 (0)	180 (5.2)	199 (5.3)	163 (5.1)	48 (3.9)	15 (2.8)	133 (4.9)	220 (5.4)	120 (4.8)	89 (4.5)
UF 71513(b)	1 (0.7)	220 (5.4)	298 (5.7)	269 (5.6)	73 (4.3)	0 (0)	133 (4.9)	147 (5.0)	133 (4.9)	39 (3.7)	6 (1.9)	147 (5.0)	244 (5.5)	133 (4.9)	73 (4.3)
PI 337394F(b)	1 (0.8)	199 (5.3)	329 (5.8)	199 (5.3)	73 (4.3)	0 (0)	180 (5.2)	180 (5.2)	163 (5.1)	48 (3.9)	14 (2.7)	120 (4.8)	269 (5.6)	147 (5.0)	89 (4.5)
OG 43-4-1(d)	10 (2.4)	445 (6.1)	492 (6.2)	402 (6.0)	180 (5.2)	32 (3.5)	298 (5.7)	402 (6.0)	298 (5.7)	180 (5.2)	12 (2.6)	298 (5.7)	364 (5.9)	269 (5.6)	133 (4.9)
TMV 2(c)	10 (2.4)	445 (6.1)	897 (6.8)	543 (6.3)	220 (5.4)	36 (3.6)	298 (5.7)	364 (5.9)	329 (5.8)	180 (5.2)	15 (2.8)	445 (6.1)	601 (6.4)	492 (6.2)	220 (5.4)
Gangapuri(c)	19 (3.0)	734 (6.6)	1096 (7.0)	811 (6.7)	329 (5.8)	66 (4.2)	734 (6.6)	897 (6.8)	664 (6.5)	445 (6.1)	32 (3.5)	664 (6.5)	1211 (7.1)	897 (6.8)	402 (6.0)
EC 76446(c) (292)	11 (2.5)	811 (6.7)	1096 (7.0)	664 (6.5)	298 (5.7)	44 (3.8)	544 (6.3)	664 (6.5)	402 (6.0)	298 (5.7)	73 (4.3)	897 (6.8)	1338 (7.2)	991 (6.9)	544 (6.3)
Mean *(Moyenne)*	4 (1.7)	364 (5.9)	543 (6.3)	364 (5.9)		6 (1.9)	269 (5.6)	329 (5.8)	244 (5.5)		15 (2.8)	269 (5.6)	445 (6.1)	269 (5.6)	
SE mean for comparing *(Ecart-type pour comparaison de)* :															
— treatments *(traitements)*	(± 0.11)							(± 0.40)					(± 0.09)		
— genotypes *(génotypes)*	(± 0.19)							(± 0.10)					(± 0.14)		
— treatment × genotype *(traitement × génotype)*	(± 0.33)							(± 0.12)					(± 0.27)		

(a) Values in parentheses are log transformations *(Les valeurs entre parenthèses sont des transformations logarthmiques).*
(b) IVSCAF-resistant *(Résistant à la CIVGAF).*
(c) IVSCAF-susceptible *(Sensible à la CIVGAF).*
(d) IVSCAF-highly susceptible *(Hautement sensible à la CIVGAF).*

particularly interesting that levels of aflatoxin contamination parallelled levels of seed infection by *A. flavus*. As this result held good for materials from the no-inoculation treatment (T1) as well as the inoculation treatments, the implication is that the strains of *A. flavus* in ICRISAT Center soils are mostly aflatoxigenic. However, some variation in levels of seed infection by *A. flavus* and aflatoxin B1 content did occur in some of the IVSCAF-susceptible genotypes. This result can possibly be attributed to differences in their ability to support aflatoxin production [Mehan and McDonald, 1983 ; 1984].

The benefits of having higher levels of seed infection when comparing genotypes may be offset by the laborious method of inoculum application. However, the fact that adding inoculum to the pod-zone soil increased infection indicates that enhancement of infection using simpler inoculation techniques might have relevance when resistance screening has to be done in soils not conducive to *A. flavus* infection of peanut pods and seeds. Further, there were no significant differences between the three inoculation treatments for seed infection in most of the genotypes tested. Applying inoculum three times in the T3 treatment only slightly increased seed infection levels compared with applying inoculum twice in the other inoculation treatments.

CONCLUSIONS

Seed infection by *A. flavus* and subsequent aflatoxin contamination in peanut genotypes could be significantly enhanced by adding inoculum of an aflatoxigenic strain of the fungus to soil around developing pods. This technique is useful for screening peanut genotypes for resistance to seed infection by *A. flavus*/aflatoxin contamination in environments not conducive for seed infection by the toxigenic fungus. Significant varietal differences were confirmed for infection of peanut seed by *A. flavus* under enhanced inoculum pressure in the field. Some IVSCAF-resistant genotypes also showed resistance to *A. flavus* infection in the field.

TABLE IV. — **Analysis of variance for seed infection by *A. flavus* for data pooled from 3 seasons (Experiment 2)**
(Analyse de variance pour l'infestation des graines par A. flavus
à partir de données confondues provenant de 3 saisons - expérience 2)

Source of *(de la)* variation	DF	MS	F PR
Seasons. Blocks Stratum *(Saisons, blocs)*			
Seasons *(Saisons)*	2	662.602	<0.001
Residual *(Résidu)*	6	2.671	
Total	8	167.654	
Seasons. Blocks. Cultivar Stratum *(Saisons, blocs, cultivar)*			
R *vs.* S	1	6617.433	<0.001
Seasons *(Saisons)* (R *vs.* S)	2	122.652	<0.001
R	3	13.387	0.031
S	3	142.718	<0.001
Seasons *(Saisons)* R	6	1.659	0.872
Seasons *(Saisons)* S	6	7.526	0.116
Residual *(Résidu)*	42	4.109	
Total	63	119.980	
Seasons. Blocks. Cultivar. Treatment Stratum *(Saisons, blocs, cultivar, traitement)*			
C *vs.* I	1	6216.618	<0.001
Seasons *(Saisons)* (C *vs.* I)	2	0.354	0.907
(R *vs.* S). (C *vs.* I)	1	373.521	<0.001
I	2	235.344	<0.001
Seasons *(Saisons)* (R *vs.* S). (C *vs.* I)	2	50.606	<0.001
R. (C *vs.* I)	3	2.463	0.567
S. (C *vs.* I)	3	16.367	0.005
Seasons *(Saisons)* I	4	13.231	0.007
(R *vs.* S). I	2	38.057	<0.001
Seasons *(Saisons)* R. (C *vs.* I)	6	1.932	0.784
Seasons *(Saisons)* S. (C *vs.* I)	6	4.169	0.339
Seasons *(Saisons)* (R *vs.* S). I	4	6.538	0.132
R. I	6	1.667	0.838
S. I	6	6.413	0.111
Seasons *(Saisons)* R. I	12	0.689	0.999
Seasons *(Saisons)* S. I	12	9.017	0.006
Residual *(Résidu)*	144	3.636	
Total	216	37.498	
Grand Total *(Total général)*	287		
Grand Mean *(Moyenne globale)*	15.57		
Total number of observations *(Nbre total d'observations)*	288		

R = IVSCAF-resistant genotypes *(Génotypes résistants à la CIVGAF)*.
S = IVSCAF-susceptible genotypes *(Génotypes sensibles à la CIVGAF)*.
C = Control (No Inoculation) (T1) *(Témoin - sans inoculation - T1)*.
I = Inoculation treatments *(Traitements d'inoculum)* (T2, T3 & T4).

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RÉSUMÉ

Effets provoqués par l'addition d'un inoculum d'*Aspergillus flavus* dans la zone du sol occupée par les gousses sur l'infestation des graines et sur la contamination par aflatoxines de génotypes d'arachide.

V. K. MEHAN, D. McDONALD et N. RAMAKRISHNA, *Oléagineux*, 1988, **43**, N° 1, p. 21-28.

Plusieurs génotypes d'arachide résistants ou sensibles à la colonisation *in vitro* des graines par *Aspergillus flavus* ont été cultivés dans des essais au champ, à plusieurs répétitions, au centre de l'ICRISAT, Patancheru, Inde entre 1981 et 1984. L'inoculum d'une souche aflatoxigène de *A. flavus* rajouté à la zone du sol occupée par les gousses a donné lieu à une augmentation avant la récolte de l'infestation des graines par *A. flavus* et de la contamination par aflatoxines. Il existait une corrélation positive entre les niveaux de contamination par aflatoxines **B1** et le pourcentage de graines infestées par *A. flavus*, ceci avec ou sans addition de l'inoculum dans le sol. Les différences génotypiques relatives à l'infestation des graines par *A. flavus* et à la contamination par aflatoxines étaient presque identiques pour les plantes témoins et pour les plantes ayant été exposées à l'inoculum. Les taux d'infestation par d'autres champignons dans le sol n'ont pas été influencés de façon significative par l'addition de l'inoculum de *A. flavus* dans la zone du sol occupée par les gousses. La plupart des génotypes classés comme résistants à la colonisation *in vitro* des graines par *A. flavus* dans les cas de graines réhydratées, matures, non abîmées et stockées, font également preuve de résistance à l'invasion par le champignon au champ.

RESUMEN

Efectos provocados por la adición de un inóculum de *Aspergillus flavus* en el área del suelo ocupada por los frutos, sobre la infestación de los granos y sobre la contaminación de genotipos de maní por aflatoxinas.

V. K. MEHAN, D. McDONALD y N. RAMAKRISHNA, *Oléagineux*, 1988, **43**, N° 1, p. 21-28.

Varios genotipos de maní resistentes o sensibles a la colonización *in vitro* de las semillas por *Aspergillus flavus* se cultivaron en pruebas de campo, con varias replicaciones, en el Centro del ICRISAT, en Patancheru, India, entre 1981 y 1984. El inóculum de una fuente aflatoxígena de *A. flavus* añadido a la zona del suelo ocupada por las vainas resultó en un aumento de la infestación de semillas por *A. flavus* antes de la cosecha, y en una contaminación por aflatoxinas. Habia una correlación positiva entre los niveles de contaminación por aflatoxinas **B1** y el porcentaje de semillas infestadas por *A. flavus*, con adición del inóculum en el suelo o sin ella. Las diferencias genotípicas relativas a la infestación de semillas por *A. flavus* y a la contaminación por aflatoxinas eran casi idénticas para las plantas testigo y para las plantas expuestas a la acción del inoculum. Los coeficientes de infestación por otros hongos en el suelo no experimentaron ninguna influencia significativa por la adición del inoculum de *A. flavus* en la zona del suelo ocupada por los frutos. La mayoría de los genotipos clasificados como resistentes a la colonización *in vitro* de las semillas por *A. flavus* en los casos de semillas rehidratadas, maduras, no alteradas y almacenadas, también demuestran su resistencia a la invasión por el hongo en el campo.

Effets provoqués par l'addition d'un inoculum d'*Aspergillus flavus* dans la zone du sol occupée par les gousses sur l'infestation des graines et sur la contamination par aflatoxines de génotypes d'arachide (1)

V. K. MEHAN, D. McDONALD et N. RAMAKRISHNA (2)

INTRODUCTION

Au cours d'une sélection de génotypes d'arachide pour la résistance des graines à la colonisation prérécolte par le champignon aflatoxigène *Aspergillus flavus* Link ex. Fr., on a constaté que les taux d'infestation des graines de contamination par aflatoxines étaient généralement très bas dans les conditions pédologiques (Alfisols) et climatiques prévalant habituellement pendant la saison de culture de l'arachide [Mehan *et al.*, 1986]. Des études préliminaires ont montré que l'addition d'un inoculum de *A. flavus* dans la zone du sol occupée par les gousses pourrait augmenter le taux d'infestation des graines par le champignon [Mehan et McDonald, 1984]. Cet article présente des données obtenues à partir d'essais au champ mis en place avec plusieurs répétitions au Centre de l'ICRISAT afin d'examiner l'effet de l'addition de l'inoculum d'une souche aflatoxigène de *A. flavus* à la zone du sol occupée par les gousses de génotypes d'arachide sélectionnés, et ce aux différents stades de croissance, sur l'infestation des graines par *A. flavus* et par d'autres champignons, ainsi que sur la contamination par aflatoxines.

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