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Effects of Genotype and Date of Harvest on Infection of Peanut Seed by *Aspergillus flavus* and Subsequent Contamination with Aflatoxin[1]

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ABSTRACT

Several peanut genotypes reported as resistant, susceptible or highly susceptible to *in vitro* colonization of rehydrated, mature, stored, undamaged seed by *Aspergillus flavus* (IVSCAF) were tested for natural seed infection by *A. flavus* and other fungi in two or more replicated field trials at ICRISAT Center, Patancheru, India, in 1979 - 1984. Undamaged pods were sampled before maturity, at optimum maturity (normal harvest) and when over - mature (late harvest) and seed examined for infection by *A. flavus* and other fungi. In the 1983 and 1984 rainy and 1983/84 postrainy seasons, only four genotypes (one resistant and three susceptible) were tested, and seed were also tested for aflatoxin content. In all seasons the genotypes reported as IVSCAF - resistant had significantly lower levels of seed infection with *A. flavus* and other fungi than did genotypes reported as IVSCAF - susceptible. Genotypic differences in levels of seed infection by *A. flavus* were consistent over seasons. The resistant cultivar J11 had a significantly lower aflatoxin content than the other three IVSCAF - susceptible genotypes tested in the 1983 - 1984 seasons. Drought stress in the 1984 season apparently increased susceptibility to seed infection by *A. flavus* and other fungi, and to aflatoxin contamination, in all cultivars. Seed infection by *A. flavus* and other fungi, and aflatoxin contamination increased with increasing maturity of pods, indicating the importance of lifting the peanut crop at optimum maturity.

Key Words: Groundnut, preharvest seed infection, *Aspergillus flavus*, mycotoxin

In the last 25 years there has been much research into infection of peanut (*Arachis hypogaea* L.) pods by soil fungi, particularly by the aflatoxigenic fungi (*Aspergillus flavus* group), and into aflatoxin contamination of seeds (1, 7, 9, 17). It is now well established that peanuts can be invaded by aflatoxigenic fungi while still in the ground, during postharvest curing and drying, and in storage. Levels of seed invasion by *A. flavus*, and of aflatoxin contamination, can be much reduced by prevention of drought stress during crop growth, by avoidance of damage to pods, by timely harvesting, by rapid drying of produce, and by clean, dry storage (5). However, there are some limitations to carrying out these practices, particularly in less developed countries where they have not been widely adopted by farmers. Attention has therefore been focussed on breeding peanut cultivars with resistance to seed infection by *A. flavus* (11, 12, 13). In laboratory inoculation tests a number of peanut genotypes have been identified as resistant to *A. flavus* invasion of rehydrated, undamaged, mature, stored seed (11, 12, 13). It is obviously important to establish if these genotypes also have seed resistance to invasion by *A. flavus* before harvest. Davidson *et al.* (4), in Georgia, USA, failed to show any differences at harvest in *A. flavus* invasion or aflatoxin contamination of seed from two cultivars with different levels of resistance to *in vitro* seed colonization by *A. flavus*. However, workers in North Carolina, USA, have obtained indications that some genotypes with resistance to *in vitro* seed colonization also had resistance to preharvest invasion by *A. flavus* (18).

This paper reports on the effects of genotype and harvest date on seed infection by *A. flavus*, and other fungi, and on aflatoxin contamination of seeds.

Materials and Methods

All trials were carried out on ICRISAT Center farm at Patancheru (17° 3 N lat., 78° 16 E long.; alt. 541 m), near Hyderabad, India. The crops were grown on red soil fields (alfisols) in the rainy season (June - October), except for one trial that was grown with irrigation in the postrainy season (November - April). Details of seasons, sowing and harvest dates, and number of genotypes tested in each season are given in Table 1. The genotypes used in the trials included entries resistant, susceptible and highly susceptible to *in vitro* seed colonization by *A. flavus* (IVSCAF)[3] (10). These genotypes are characterized in Table 2. Data on weekly rainfall, and weekly average maximum and minimum temperatures during the rainy and postrainy seasons were obtained from the ICRISAT Meterological Unit.

1. Investigation of resistance of peanut seed to natural preharvest infection with *A. flavus* and other fungi (1979 - 1982 rainy seasons)

In four consecutive rainy seasons (1979 - 1982) sets of ten genotypes (Tables 1 and 2) were grown in field trials using randomized block designs with three replications. Plots were 9 m long by 2.25 m (3 ridges) wide, and seed were sown singly at 15 cm spacing along the

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[3]IVSCAF = *in vitro* colonization of rehydrated, mature, stored, undamaged seed by *A. flavus*.

Table 1. Details of field trials: 1979 - 1984.

Year	Season	Genotypes		Date of sowing	Harvest date		
		No. tested	Code[a] No.		H1[b]	H2	H3
1979	Rainy	10	1,2,6,10, 11,12,13, 15,16,18	16/6	6/9	5/10	15/10
1980	Rainy	10	1,2,6,10, 11,12,13, 15,16,18	18/6	10/9	9/10	19/10
1981	Rainy	10	1,2,3,5, 6,9,13, 14,16,18	20/6	14/9	14/10	24/10
1982	Rainy	10	1,3,4,5, 6,7,8, 13,14,16	18/6	9/9	8/10	18/10
1983	Rainy	4	6,13,16, 17[c]	24/6	20/9	20/10	30/10
1984	Rainy	4	6,13,16, 17[c]	15/6	20/9	21/10	31/10
1983/84	Postrainy	4	6,13,16, 17[c]	26/12	3/4	2/5	12/5

a These numbers are related to the "genotypes" listed in Table 2 and to the "coded" bars in the figures.

b H1 = early harvest (approximately 30 days before normal harvest)
H2 = normal harvest
H3 = late harvest (10 days after normal harvest)

c Genotype M 13 (Code No. 17) was harvested 20 days later than other genotyes.

Table 2. Details of groundnut genotypes tested for natural infection of seeds by soil fungi.

Genotype ICG No.	Identity	Botanical variety	Country of origin	Code[a] No.
[b]Resistant to in vitro seed colonization by A. flavus				
4749	PI 337394F	fastigiata	Argentina	1
4750	PI 337409	fastigiata	Argentina	2
7633	UF 71513	fastigiata	USA	3
4601	Var. 27	fastigiata	Australia	4
3700	Ah 7223	vulgaris	Nigeria	5
1326	J 11	vulgaris	India	6
2224	Faizpur	vulgaris	India	7
4562	C 55-437	vulgaris	Senegal	8
3263	U 4-47-7	vulgaris	Uganda	9
[c]Susceptible to in vitro seed colonization by A. flavus				
6446	NC 3033	hypogaea	USA	10
4747	PI 259747	fastigiata	Peru	11
1697	NC Ac 17090	fastigiata	Peru	12
2716	EC 76446(292)	fastigiata	Uganda	13
751	Gangapuri	fastigiata	India	14
4590	Florigiant	vulgaris	USA	15
221	TMV 2	vulgaris	India	16
156	M 13	hypogaea	India	17
[d]Highly susceptible to in vitro seed colonization by A. flavus				
7867	OG 43-4-1	vulgaris	India	18

a Numbers used to designate genotypes in figures.
b Resistant = up to 15% seeds colonized by A. flavus
c Susceptible = 30 to 50% seeds colonized by A. flavus
d Highly susceptible = over 50% seeds colonized by A. flavus

ridges. The fertilizer P_2O_5 at the rate of 60 kg/ha was applied at land preparation. The genotypes were examined for seed infection by fungi while immature (H1 = 30 days before normal harvest), when mature (H2 = at normal harvest time), and when over-mature (H3 = 10 days after normal harvest). Twenty-five plants were selected at random from each plot in the experiment at the relevant harvest date. Full sized, undamaged pods were collected from these plants, hand-shelled, and seed examined for infection by fungi.

2. Investigation of resistance of peanut seed to natural preharvest infection with *A. flavus* and other fungi, and aflatoxin contamination (1983 and 1984 rainy seasons and 1983/84 postrainy season).

Four genotypes (J11, TMV 2, EC 76446(292), and M 13) were grown in three seasons (Tables 1 and 2) in field trials using a split-plot design with three replications. The main-plots were assigned to genotypes and sub-plots to harvest time. Plots were 9 m long by 3.75 m (5 ridges) wide and seed were sown singly at 10 cm spacing along the ridges for all the genotypes except M 13 which was sown at 15 cm spacing. Fertilizer application was 20 kg/ha of P_2O_5 at land preparation. The trials were grown under rainfed conditions in the 1983 and 1984 rainy seasons, and with irrigation in the 1983/84 postrainy season. The genotypes were harvested at different maturity stages, and pods sampled as described for the first set of trials.

Examination of seeds for infection by fungi. For all trials, 100 undamaged seeds from each plot at each harvest time (H1, H2, and H3) were tested for fungal infection within 24 h of the plants being harvested. The seed were surface - sterilized by soaking for 3 minutes in a 0.1% aqueous solution of mercuric chloride, rinsed in two changes of sterile distilled water and then plated onto Czapek-Dox Rose Bengal Streptomycin Agar medium in 10 cm diameter Petri plates for isolation of fungi. The plates were incubated at 25 C in the dark and colonies of fungi growing from seeds were recorded after 5 - 7 days.

Determination of aflatoxin content of seed. For the second set of trials, two 50 g samples of seed from each plot at each harvesting date were tested for aflatoxin content using the method of Pons *et al.* (15).

Statistical analysis. Using square - root transformed values, analyses of variance were performed separately for seed infection by *A. flavus*, by several other fungal species, and by all fungi other than *A. flavus*, for each of the trials. A combined analysis of variance was also carried out for pooled data from seven seasons on seed infection by *A. flavus* of three genotypes, J11, TMV 2, and EC 76446 (292). Analyses of variance were also done for aflatoxin B_1 content of seed of four genotypes for the 1983 and 1984 rainy, and 1983/84 postrainy seasons, using $\log_e$ transformed values.

Results

Rainfall varied between seasons. In the 1984 rainy season the crop suffered from both mid-season and end - of - season drought. Temperatures were much higher during the late pod - filling stage in the 1983/84 postrainy season than in the rainy seasons.

1. 1979 - 1982 trials: Natural infection of seed of test genotypes by *A. flavus* is shown in Fig. 1. Within each set of ten entries, seed infection data are graphed separately for IVSCAF - susceptible and IVSCAF - resistant genotypes. In general, levels of seed infection by *A. flavus* were higher in all genotypes tested in the 1982 rainy season than in the other rainy seasons. Seed infection by *A. flavus* increased with increasing maturity, being markedly higher in all the genotypes at H3 than at H2 or H1 (Fig. 1). Percentages of seed infected by *A. flavus* at H1 and H2 did not differ significantly between IVSCAF - resistant genotypes in any season. However, IVSCAF - susceptible genotypes had markedly higher levels of seed infection by the fungus at H2 than at H1 in the 1979 and 1982 rainy seasons. In all seasons, IVSCAF - resistant genotypes showed significantly lower percentages of seed infected by *A. flavus* than did IVSCAF - susceptible genotypes. The three IVSCAF -

resistant genotypes PI 337394F, PI 337409, and J11 tested in the 1979 and 1980 rainy seasons did not differ significantly from each other. Six and seven genotypes with resistance to IVSCAF were tested against three and four IVSCAF - susceptible genotypes in the 1981 and 1982 rainy seasons, respectively. In the 1982 rainy season, genotypes J11 and UF 71513 had significantly lower levels of natural seed infection by *A. flavus* than all the other genotypes, but in the 1981 season they did not differ significantly in this respect from other IVSCAF - resistant genotypes.

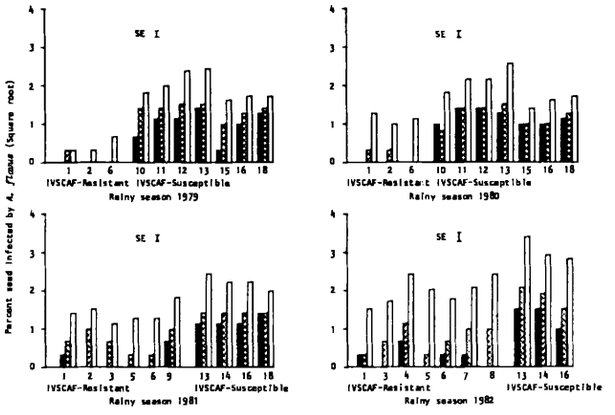

Fig. 1. Seed infection by *A. flavus* in peanut genotypes resistant or susceptible to *in vitro* seed colonization by the fungus, at three harvest dates in four crop seasons. H1 = early harvest (30 days before normal harvest) (■), H_2 = normal harvest (▨), H_3 = late harvest (10 days after normal harvest) (□).

Among the IVSCAF - susceptible genotypes, EC 76446 (292) showed the highest levels of seed infection by *A. flavus* in all seasons. However, OG 43-4-1, a genotype highly susceptible to IVSCAF, had relatively lower levels of seed infection at harvest than had most of the IVSCAF - susceptible genotypes.

Significant differences between genotypes were also observed in all seasons for seed infection by fungi other than *A. flavus*. These included *Fusarium* spp., *Aspergillus niger, Macrophomina phaseolina,* and *Penicillium spp.* Seed infection by these fungi increased with crop duration, being significantly higher in all genotypes at H3 than at H2, and at H2 than at H1. Percentages of seed infected by fungi were higher in all genotypes in the 1982 rainy season than in the other seasons. Cultivar J11 showed the lowest levels of seed infection by other fungi, while EC 76446 (292) recorded the highest seed infection by fungi in all seasons. *Fusarium* spp. were the most common fungi in seed of all genotypes regardless of harvest date or season. The next most dominant fungus observed in all seasons was *M. phaseolina.* Significant differences occurred between genotypes for seed infection both by *Fusarium* spp. and *M. phaseolina.* Genotypes with resistance to IVSCAF had significantly lower levels of seed infection by these fungi than did IVSCAF - susceptible genotypes. Seed infection by *A. niger* and *Penicillium* spp. was low in all seasons.

2. 1983 - 1984 trials. Natural infection of seed of the four genotypes in one postrainy and two rainy seasons is shown in Fig. 2 for *A. flavus.* Significant differences occurred between genotypes for seed infection by *A. flavus* (Fig. 2). Cultivar J11 had significantly lower percentages of seed infected by *A. flavus* than the other three genotypes in the three seasons while EC 76446 (292) had the highest percentages of seed infected by *A. flavus* in all seasons. For all genotypes, levels of seed infection by *A. flavus* were highest in the 1984 rainy season and lowest in the 1983/84 postrainy season. Seed infection by *A. flavus* increased with increase in duration, being considerably higher at H3 than at H1 or H2 for all genotypes in all seasons. In all genotypes except J11, seed infection by *A. flavus* was markedly higher at H2 than at H1.

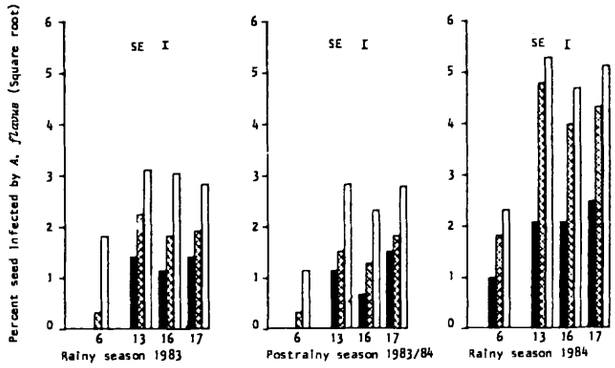

Fig. 2. Seed infection by *A. flavus* at three harvest dates for four peanut genotypes in three crop seasons. H1 = early harvest (30 days before normal harvest) (■), H2 = normal harvest (▨), H3 = late harvest (10 days after normal harvest) (□).

There were also significant differences between genotypes for seed infection by fungi other than *A. flavus.* J11 had significantly lower percentages of seed infected by other fungi (*Fusarium* spp., *M. phaseolina, A. niger* and *Penicillium* spp.) than the other three genotypes in all seasons. The ranges of seed infection were 0 - 9%, 1 - 35%, 3 - 24% and 3 - 49% for J11, TMV 2, M 13 and EC 76446 (292), respectively. *Fusarium* spp. were predominant in all seasons, infection increasing with crop duration. *M. phaseolina* was common only in the 1983 rainy season and *Penicillium* spp. in the 1984 rainy season. Seed infection by *A. niger* was frequent in the 1984 rainy and the 1983/84 postrainy seasons, but the four genotypes did not differ significantly in this respect in either season.

Aflatoxin contamination. Levels of aflatoxin contamination in the four genotypes for all seasons are shown in Fig. 3. Aflatoxin contamination was highest for all genotypes in the 1984 rainy season. The four genotypes did not differ significantly from each other in aflatoxin contamination in the 1983 rainy season. J11 had the lowest and EC 76446 (292) the highest levels of aflatoxin B_1 contamination in all seasons. In the 1984 rainy and the 1983/84 postrainy seasons, J11 gave significantly lower levels of aflatoxin contamination than the other three genotypes. Aflatoxin contamination increased with increase in crop duration for all genotypes. In the 1983 rainy and 1983/84 postrainy seasons, no aflatoxin contamination of seeds at H1 in any genotype was observed. Aflatoxin contamination was markedly higher at H3 than at H2 in all seasons. Significant interactions between

genotypes and harvest dates were observed for levels of aflatoxin contamination in the 1984 rainy and 1983/84 postrainy seasons; aflatoxin contamination being significantly higher at H_3 than at H_2 for EC 76446(292) and M13. Highly significant correlations were found between percentages of seed infected by *A. flavus* and levels of aflatoxin contamination in all seasons (r = 0.76, 0.56 and 0.87 in the 1983 and 1984 rainy and 1983/84 postrainy seasons, respectively.

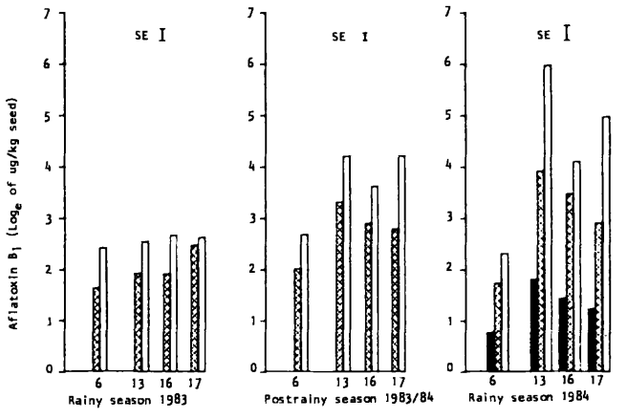

Fig. 3. Aflatoxin contamination at three harvest dates for four peanut genotypes in three crop seasons. H1 = early harvest (30 days before normal harvest) (■), H2 = normal harvest (▩), H3 = late harvest (10 days after normal harvest) (□).

Discussion

Most of the interest in the 'seed resistance' possessed by certain peanut genotypes has been in relation to their use to prevent or reduce *A. flavus* invasion and colonization and aflatoxin contamination in rehydrated, mature, stored seed (2, 11, 12, 13, 20). Only recently have some of the genotypes with resistance to *in vitro* seed colonization by *A. flavus* and *A. parasiticus* been tested for resistance to preharvest pod and seed infection by these fungi and consequent aflatoxin contamination in the field (3, 4, 20). Field trials in Georgia, USA, with some IVSCAF - resistant genotypes did not show any advantage over the commonly grown cultivar Florunner (reported to have moderate resistance to IVSCAF) in reducing levels of seed infection by *A. flavus* and /or aflatoxin contamination in freshly harvested peanuts (3,4). However, in the present investigations we found that, for the genotypes used, those with resistance to IVSCAF had significantly lower levels of natural seed infection by the fungus than had the IVSCAF - susceptible genotypes. These results were generally consistent over several seasons and strongly support the reports of significant genotypic differences in peanuts with regard to preharvest resistance to seed infection by *A. flavus* in Senegal (19, 20). Zambettakis *et al.* (20) have also reported that IVSCAF - resistant genotypes showed resistance to field infection by *A. flavus* in Senegal. The laboratory test determines the ability of *A. flavus* to penetrate the testa and colonize the seed whereas in the field, seed invasion by *A. flavus* is influenced by factors such as shell resistance and drought stress (4). This is supported by the fact that genotypes OG 43 - 4 - 1, that has very high susceptibility to IVSCAF, had lower levels of natural seed infection by *A. flavus* than most IVSCAF - susceptible genotypes tested (Fig. 1).

The finding that seed infection by *A. flavus* and aflatoxin contamination increased with increasing crop duration of peanuts confirms earlier reports of the gradual increase of fungal infection and aflatoxin contamination of seeds as they age (7,9). Significantly higher levels of seed infection by *A. flavus* and aflatoxin B1 contamination were found at late harvest in the 1984 rainy season than in the other seasons. This may be attributed to the occurrence of significant drought stress late in the 1984 rainy season. Drought stress during pod maturation, and over - maturity of peanut pods, are known to encourage fungal infection and aflatoxin contamination of seed (9, 14, 16). However, genotypic differences were evident and consistent for natural seed infection by *A. flavus* in late harvests across all seasons. Cultivar J11 had consistently low seed infection by *A. flavus* while genotype EC 76446 (292) showed high seed infection across the seven seasons (Fig. 4). TMV 2 was intermediate in this respect except in the 1984 season where this showed high seed infection.

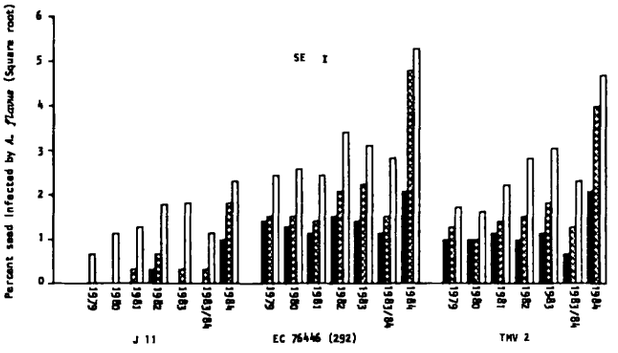

Fig. 4. Seed infection by *A. flavus* at three harvest dates for three peanut genotypes in seven crop seasons. H1 = early harvest (30 days before normal harvest) (■), H2 = normal harvest (▩), H3 = late harvest (10 days after normal harvest) (□).

Cultivar differences for *A. flavus* seed infection were conspicuous in the 1984 rainy season which had a significant drought stress during pod maturation. These results indicate an obvious advantage in growing cultivars that have very low levels of seed infection by *A. flavus* even under delayed harvest conditions. These results also reveal the dangers of leaving the crop in the soil beyond normal duration and emphasize the significance of harvesting as soon as the crop is mature.

Genotypes resistant to IVSCAF, in general, also had markedly lower percentages of seed infected by fungi other than *A. flavus* across all seasons than did the IVSCAF - susceptible genotypes. *Fusarium* spp. were predominant at all harvest times across all seasons. *Fusarium* spp. have been found dominant in the seed mycoflora in the USA (6), in Nigeria (7) and in India (17). *M. phaseolina* was also dominant in immature and mature seed, and was particularly common in over - mature seed of all genotypes in all rainy seasons, except the 1984 rainy season. In Nigeria, *M. phaseolina* has also been

found dominant in seed from over-mature pods (7), but was less important in seed from mature pods. Environmental conditions influenced seed infection by the different fungal species. For instance, *Penicillium* spp. occurred commonly in seed from the 1984 rainy season crop, but were less frequent or absent in seed from the 1983 rainy and 1983/84 postrainy seasons.

The above results clearly indicate resistance to field infection of seed by *A. flavus* and other fungi in genotypes that possess resistance to IVSCAF. In the present investigations all the tests were done on seed from crops grown on alfisols at ICRISAT Center. It will be necessary to test the stability of resistance in these genotypes in different locations and in different soil types. We have started this process in India, giving priority to drought - prone areas with light sandy soils that provide a favorable environment for development of *A. flavus* and for seed infection by the fungus.

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