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Comparison of Pod and Seed Screening Methods on *Aspergillus* spp. Infection of Peanut Genotypes[1]

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ABSTRACT

Six peanut genotypes grown at three locations (sources) were tested in laboratory studies for the influence of pod and seed inoculation methods on seed colonization by *Aspergillus parasiticus* Speare and/or incidental prior contamination (uninoculated method) in the field or storage by *A. flavus* Link ex Fr. or A. *parasiticus*. The genotypes has been identified as having varying levels of resistance to seed colonization by *Aspergillus* spp. in laboratory screening. There was no difference between the pod and seed inoculation on the mean percentage of seed colonization, but pod inoculation resulted in a noticeable reduction in seed colonization of the more susceptible genotypes when compared to inoculated seed (genotype x method interaction). Uninoculated seed incubated similarly to the inoculated samples exhibited considerably less colonization. For all three methods, seed colonization was consistently less for the resistant genotypes than for the 'Florunner' variety or the highly susceptible check P. I. 343419. A source x genotype interaction resulted from the difference in the magnitude of percent colonization but the resistant genotypes were colonized less frequently than susceptible genotypes. The seed screening method currently in use (seed inoculation) was equally or more effective than the pod inoculation or uninoculated seed method in identifying genotypes resistant to *A. parasiticus*.

Key Words: *A. Flavus*, Peanut Seed Contamination, Mycotoxin, Fungal Resistance.

Several workers have reported peanut genotypes with pod or seed resistance to *Aspergillus* spp. in fruit contaminated with this fungus (3, 9, 10, 13, 16, 18). Since *Aspergillus* spp. are ubiquitous throughout peanut-growing areas of the world, the exposure of peanut fruit to unfavorable drying conditions for several days greatly increases contamination and development of aflatoxin (2, 12). Many environmental conditions favorable for the fungal growth and invasion of the fruit have been documented (4, 5, 11, 17).

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Attempts have been made to explain differences in seed colonization of peanuts evaluated in laboratory studies. The structure and arrangement of testa cells (14), the relative permeability of intact testa (6), the difference in waxy exterior (6, 14), the tannin components (7, 8, 15), and total and specific amino acids (1), have been implicated in the resistance to seed colonization.

Most screening of peanut genotypes for seed colonization has been carried out in the laboratory using a seed inoculation technique (6, 9, 15). The study reported here was undertaken to compare seed and pod screening techniques, and to determine if pods of some peanut genotypes impede penetration and subsequent seed colonization by *Aspergillus* spp.

Material and Methods

Six peanut genotypes (Table 1) were grown near Tifton, Georgia in 1977 and 1978, and in Puerto Rico in the winter of

Table 1. Peanut genotypes reaction to seed colonization by *Aspergillus* sp.

Entry	Genotype Identity	Seed Colonization Category†
1	PI 337409‡	Resistant
2	A 72120§	Resistant
3	A 72118§	Resistant
4	A 7309§	Resistant
5	Florunner	Mod. Resistant
6	PI 343419¶	Susceptible

† Based on prior laboratory screening.

‡ Accession from Argentina.

§ Advanced lines from crosses in F_8 generation of selection for resistance.

¶ Accession from Israel.

1977-78. The first four entries had been evaluated and selected previously as having considerable resistance to seed colonization by *A. flavus* Link ex Fr. and *A. parasiticus* Speare, using a seed screening procedure similar to the seed inoculation method described below. Peanuts were grown using recommended land preparation and cultural procedures in plots spaced 91.4 cm apart in two adjacent rows 6.1 m long at Tifton and single-row plots 7.6 m long and 91.4 cm apart in Puerto Rico. At Tifton, pods were hand picked from plants that had been inverted and field dried for 4 to 6 days. At Puerto Rico, pods were hand picked from freshly dug plants, and dried by forced-draft ambient or intermittant forced air heated to 32C and non-forced ambient air for 5 days. Pods from each genotype were bulked, transported to Tifton and stored at room temperature for 30 to 60 days.

Seed Screening. For the two seed screening metods [Seed Inoculation (Inoc.) and Uninoculation (Uninoc.)], a portion of the pods was hand shelled and sound seed with intact testa were selected. Two separate six-replicate tests of 20-g samples (5.0 to 6.0% seed moisture) were placed in 250-ml beakers for the Inoc. and Uninoc. screening methods, respectively. Seed were soaked for two 6-min. intervals in 100 ml of sterile-demineralized water with 0.005% surfactant added to the first soaking. The seed were drained after each soaking, and after the second soaking were inoculated with a 1-ml suspension (ca. 4.0 x 10^6 spores/ml) of *A. parasiticus* (NRRL 2999 strain*). Spores were from 2- to 4-week-old colonies of the fungus grown on Czapek agar.

Seed were placed in petri dishes (100 x 15 mm) and sterile water was added to adjust to 20% seed moisture (seed weight basis). After incubating for 7 days at 27C in a humidity chamber (98 ± 2% relative humidity), the percentage of seed colonizaed by *A. parasiticus* and/or incidental *Aspergillus* spp. contamination in the field or in storage was recorded. Seed were considered colonized when conidiophores of the fungus erupted through the testa.

Pod Inoculation. Six 50-g samples of sound-mature pods of each genotype placed in large petri dishes (140 x 20 mm) were treated with propylene oxide (1 ml/1000 cc) in glass desiccators at 71.8 cm Hg negative pressure for 24 hrs. Desiccators were then opened and pods were aerated for 18 hrs before inoculating with a 1-ml spore suspension as described. Pod moisture was adjusted to 25% (pod wt basis) by adding sterile water into each dish; seed were incubated at 27C in the humidity chamber for 3 days, and then placed into the forced draft dryer at 40C for 3 days. Pods were hand shelled and 20 g of sound seed with intact testae were placed in 250 ml beakers following surface sterilization for three min in 100 ml of 0.5% sodium hypochlorite solution. Water was decanted following this sterilization and two successive soakings for 3 minutes in 100 ml of sterile demineralized water. Seed were placed in petri dishes. Moisture was adjusted to 20% (seed weight basis), seed were incubated for 4 days without inoculation, and seed colonization was recorded.

* Obtained from the Northern Region Research Laboratory, USDA-SEA-AR, Peoria, Illinois.

Results and Discussion

Results of seed colonization by *A. parasiticus* following separate inoculation of the pods and seed, and/or by incidental contamination (uninoculated seed) with *Aspergillus* spp. in the field or in storage for the peanut genotypes are presented in Table 2. The data were analyzed as a factorial using Duncan's multiple range test of probability levels (0.05 or 0.01). There was significant genotype x method, genotype x source, and genotype x method x source interactions, but not between source and method. There was no difference in

Table 2. Seed of six peanut genotypes colonized by *Aspergillus* spp.

		Method of Screening†			Sources of Peanuts‡			
Entry	Genotype	Seed Inoc.	Pod Inoc.	Seed Uninoc.	1977-Tifton	Winter§ 1977-78	1978-Tifton	Genotype x̄
		-%-			%			
1	P.I. 337409	5.4 a¶	10.8 a	0.8 a	6.5 a	3.5 a	6.9 a	5.7 a
2	A 72120	11.4 c	14.1 b	1.8 a	11.8 b	8.6 b	6.9 a	9.1 b
3	A 72118	11.2 bc	14.4 b	2.1 a	9.8 ab	10.3 b	7.6 a	9.2 b
4	A 7309	8.1 ab	25.2 c	0.9 a	7.1 a	14.3 c	12.7 b	11.4 c
5	Florunner	36.6 d	30.7 d	5.3 b	25.6 c	20.2 d	26.7 c	24.2 d
6	P.I. 343419	69.6 e	46.4 e	5.7 b	39.8 d	35.0 e	46.9 d	40.6 e
		23.7 B	23.6 B	2.7 A	16.8 B	15.3 A	18.0 B	

† Averaged for 3 sources.

‡ Averaged for 3 screening methods.

§ From winter nursery in Puerto Rico.

¶ Column means with lower case letters and the horizontal means in grouping with upper case letters not followed by common letter differ at 0.05 level of probability (DNMR) test.

Interactions: Source x method, non-significant at 0.05 level; source x genotype, genotype x method, and source x genotype x method significant at 0.01 level of probability.

the average percentage of seed colonization between the pod and seed inoculation methods, but colonization was less for the uninoculated seed. For all three methods of screening, seed colonization of resistant genotypes (entries 1 through 4) was consistently less than for the moderately resistant (Florunner) and susceptible genotype (P. I. 343419). There was a highly significant genotype x screening method interaction partially because the pod inoculation method resulted in less seed colonization of the more susceptible genotypes when compared to the seed inoculation method. The uninoculated seed method (incubated similar to the seed inoculation method) is thought to be an exaggerated index of the field and post harvest contamination by *Aspergillus* spp. It is abvious that the more resistant genotypes had less natural field or incidental contamination potential.

Although there was a highly significant source x genotype interaction, the more resistant genotypes maintained their general resistance potential compared to the more susceptible Florunner and P. I. 343419.

From this study it is concluded that the seed inoculation method currently in use, is as effective as the pod inoculation method in identifying genotypes with resistance to seed infection. Also, the former method was also effective in identifying genotypes that has less seed contamination resulting from field contamination.

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