Effects of Meloidogyne arenaria, Aspergillus flavus, and Curing Time on Infection of Peanut Pods by Aspergillus flavus

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ABSTRACT

Studies were conducted to determine effects of *Meloidogyne arenaria, Aspergillus flavus,* and curing time on infection of peanut pods by the fungus. Plants were grown in field microplots of fumigated soil inoculated with either *A. flavus, M. arenaria,* or *A. flavus* + *M. arenaria.* Pods from *M. arenaria*-inoculated plants were heavily galled, but the incidence of *A. flavus* and total fungi was not affected. Length of curing time also did not affect the incidence of *A. flavus* or total fungi. Only in pods from *A. flavus*-inoculated plants was the incidence of *A. flavus* increased. Aflatoxin contamination was not affected by any of the treatments. We concluded that *M. arenaria* damage to peanut pods did not affect *A. flavus* infection. Phytopathology 61:1038-1039.

Additional key words: Arachis hypogaea.

Studies have been conducted at the Georgia Coastal Plain Experiment Station since 1965 on the effects of nematode damage on fungal invasion of freshly dug peanut (*Arachis hypogaea*) pods (1, 2, 3, 4). Particular emphasis has been placed on *Aspergillus flavus* and aflatoxin contamination. These studies suggested that the lesion nematode, *Pratylenchus brachyurus,* does not increase the incidence of either *A. flavus* or aflatoxins in freshly dug pods, although the total number of fungi isolated was increased (2). Of the root knot nematodes studied, *Meloidogyne hapla* increased the incidence of *A. flavus* in kernels but not in shells (3), whereas *M. arenaria* sometimes increased *A. flavus* in both kernels and shells (4). Neither root knot nematode affected aflatoxin contamination of pods.

Since data from earlier studies suggested that *M. arenaria* sometimes increased *A. flavus* in freshly dug pods (uncured pods), we felt that it would be desirable to re-evaluate this in view of the economic importance of aflatoxins in peanuts. We also wanted to determine if various lengths of curing time would increase *A. flavus,* and possibly aflatoxin contamination and total fungi, in *M. arenaria*-damaged pods.

Field microplots were established in concrete drain tiles, 0.7 m (diam) × 1.0 m (length), with two-thirds of their length buried in the ground. Each tile was filled with Tifton sandy loam and fumigated with methyl bromide. Spanish peanut, *A. hypogaea* L. 'Argentine', was planted on 1 May 1969, and 12 plants were established in each microplot.

Twelve microplots were inoculated with larvae of *M. arenaria* (Neal) Chitwood at the time of planting. Larvae were obtained from infected tomato (Rutgers) roots by mist chamber extraction, surface-disinfested in 0.001% 8-hydroxyquinolin sulfate for 30 min, and rinsed with tap water. The larvae were suspended in water and poured into 4-cm deep holes, distributed randomly over the soil surface area, and covered with methyl bromide-treated soil.

Twelve microplots, including six which had been previously inoculated with *M. arenaria* larvae, were inoculated 60 days after planting with *A. flavus* Link using isolate NRRL-2999, a known aflatoxin producer. The inoculum was obtained by growing *A. flavus* in 500-ml Erlenmeyer flasks containing 100 ml of 2% malt extract liquid. Fungal growth from 25 flasks was pooled and fragmented in a blender containing distilled water and diluted to 30 liters with distilled water. Each microplot was inoculated by flooding the entire soil surface with 2.5 liters of the resulting fungal suspension, then covering the surface with 1 cm of steam-sterilized soil. The resulting treatments, *A. flavus* alone, *M. arenaria* alone, *A. flavus* + *M. arenaria,* and control, were replicated 6 times.

Plants were dug at maturity and pods were rated for root knot galling (pod-gall index) on a 1-5 scale (Table 1). One-third of the pods from each replicate were harvested immediately. The remaining pods were left attached to the plants and placed on a wire mesh greenhouse bench to dry. Five and 12 days later, additional harvests were made, collecting one-half the remaining pods each time.

Fungal assays were made immediately after each harvest. Twenty-five two-seeded pods were soaked 5 min in 0.5% (w/v) NaOCl, rinsed in sterile distilled water, and opened aseptically. Then 50 half-shells and 50 kernels were plated separately, five/dish, on warm rose bengal-streptomycin agar. After 5 days' incubation at 28 C, the fungal and bacterial colonies growing from kernels and shells were counted.

The remaining pods were dried and stored at (—) 4 C for ca. 80 days, and the kernels were analyzed for aflatoxins by the aqueous-acetone method (5).

Pods of plants inoculated with nematodes were heavily galled, whereas those of plants not inoculated with nematodes were free of galls (Table 1). Nematodes did not affect the incidence of *A. flavus* (Table 1) or total fungi (Table 2) in either shells or kernels. In general, however, the incidence of *A. flavus* and total fungi was increased in shells and kernels of plants inoculated with *A. flavus* (Tables 1, 2). The length of curing time did not appear to increase the incidence of *A. flavus* or total fungi (Tables 1, 2); in fact, the in-

TABLE 1. Effect of inoculation with *Aspergillus flavus* and/or *Meloidogyne arenaria* on the number of *A. flavus* colonies isolated from peanut shells and kernels after 0, 5, and 12 days of curing

		Days cured					
Treatment	Pod-gall index[a]	0	5	12	0	5	12
		A. flavus (shells)			*A. flavus* (kernels)		
Control	1.0 a[b]	0.3 a	0.5 a	0.7 a	0.3 a	0.0 a	0.3 a
A. flavus	1.0 a	25.7 b	8.3 b	17.3 c	3.7 c	0.0 a	4.5 b
M. arenaria	4.1 b	4.8 a	0.2 a	0.7 a	0.7 ab	0.0 a	0.7 a
A. flavus + *M. arenaria*	4.1 b	22.5 b	12.2 b	4.3 b	2.3 bc	2.5 b	0.8 a

[a] Pod-gall index: 1 = no galls; 2 = light galling; 3 = moderate galling; 4 = heavy galling; 5 = very heavy galling.
[b] Mean values within a column followed by the same letter are not significantly different at the .05 level (Duncan's multiple range test, average of six replicates).

TABLE 2. Effect of inoculation with *Aspergillus flavus* and/or *Meloidogyne arenaria* on the number of total fungi isolated from peanut shells and kernels after 0, 5, and 12 days of curing

	Days cured					
Treatment	0	5	12	0	5	12
	Total fungi (shells)			Total fungi (kernels)		
Control	67.0 a[a]	62.2 a	78.8 b	33.6 a	25.5 b	15.0 a
A. flavus	88.1 b	74.0 ab	81.5 b	23.3 a	27.7 b	13.8 a
M. arenaria	63.0 a	69.2 a	69.3 ab	15.2 a	11.8 a	16.3 a
A. flavus + *M. arenaria*	81.8 ab	87.2 b	60.8 a	19.2 a	27.8 b	12.7 a

[a] Mean values within a column followed by the same letter are not significantly different at the .05 level (Duncan's multiple range test, average of six replicates).

cidence decreased with increased curing time in many of the treatments.

The following fungi, with few exceptions, were present in all treatments: *A. flavus; A. niger* van Tiegh.; *Colletotrichum* sp.; *Curvularia* sp.; *Fusarium* spp.; *Penicillium* spp.; and *Trichoderma* sp.

Aflatoxin contamination was not affected by any of the treatments, since only one sample (*A. flavus* + *M. arenaria,* 12th day harvest) contained aflatoxins.

We concluded that *M. arenaria* damage to peanut pods does not affect the incidence of *A. flavus,* total fungi, or aflatoxin contamination under our experimental conditions. In view of our data and previous data on *P. brachyurus* and *M. hapla,* nematode damage to peanut pods probably contributes little to the general problem of aflatoxin contamination in peanuts in the Georgia peanut belt.

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