Peanut Pod Invasion by *Aspergillus flavus* in the Presence of *Meloidogyne hapla*[1]

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Abstract: 'Argentine', 'Early Runner' and 'Florigiant' peanut cultivars were grown in methyl bromide treated soil in field microplots inoculated with: (i) *Aspergillus flavus* or (ii) *A. flavus* + *Meloidogyne hapla*. Nematode infection produced heavy root galling and light pod galling equally on all cultivars. *A. flavus*, *A. niger*, *Cephalosporium* spp., *Colletotrichum* sp., *Curvularia* spp., *Fusarium* spp., *Penicillium* spp. and *Trichoderma viride* were isolated from shells and kernels. A significantly greater incidence and density of *A. flavus* was obtained from kernels of plants inoculated with both organisms than from kernels of plants receiving only the fungus. Differences were not significant, however, for incidence and density of *A. flavus* in shells or for the total of all fungal propagules in shells and kernels. Shells of 'Early Runner' contained significantly greater incidence and density of *A. flavus* than the other two cultivars; also, kernels of this cultivar contained more fungal propagules than kernels of 'Argentine.' A significantly larger number of total fungi was isolated from kernels of 'Argentine' than from 'Florigiant.' Aflatoxins were found only in two shell samples and not in kernels.

An earlier report (2) indicated infection of peanut (*Arachis hypogaea* L.) pods by the fungus *Aspergillus flavus* (Lk.) Fr. increased when root-knot nematodes [*Meloidogyne arenaria* (Neal) Chitwood] were present. It failed to establish that *A. flavus* in pods was always increased by the nematode. Aflatoxins content of kernels was not related to presence of the nematode. Another study (1) gave virtually no indication that the lesion nematode [*Pratylenchus brachyurus* (Godfrey, Filipjev, & Schuurmans-Stekhoven)] in peanut pods increased the incidence of *A. flavus*, although total numbers of all fungi were increased. The infrequency of aflatoxins among replications also tended to confirm lack of a *P. brachyurus*/*A. flavus* relationship. The present experiment was designed to determine whether *M. hapla* Chitwood increases the incidence and density of *A. flavus* and aflatoxins in peanut pods.

Received for publication 14 April 1969.

[1]Cooperative investigations of Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture and the University of Georgia College of Agriculture Experiment Stations, Coastal Plain Station, Tifton. Journal Series Paper No. 475.

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MATERIALS AND METHODS

Twenty-four field microplots were established in concrete drain tiles 0.7 m (diam) × 1 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. Peanut cultivars 'Early Runner' and 'Florigiant' were planted May 7, 1968, 14 days before 'Argentine', so all cultivars would mature simultaneously. Three plants of each cultivar were established randomly in each microplot. Each of 12 microplots received 16,000 and 15,000 *M. hapla* larvae on June 5 and June 10, respectively. Nematode larvae were obtained from infected tomato ('Rutgers') roots by mist chamber extraction and were surface disinfested in 0.001% 8-hydroxyquinolin sulfate for 30 min and then rinsed with tap water. Larvae suspended in water were poured into trenches 4 cm deep and 4 cm from the base of plants and into holes 4 cm deep distributed randomly over remaining soil area and all covered with methyl bromide-sterilized soil.

A. flavus was cultured 12 days in 500-ml Erlenmeyer flasks on 100 ml of 2% malt extract liquid. All microplots were inoculated July 1 and again July 19 using the total

TABLE 1. Comparison of mean root-knot indices of peanut roots and shells, and fungus incidence in shells and kernels of three peanut cultivars grown in microplots.

Soil infestation treatment or cultivar	Root-knot indices[a]		Number of fungal colonies			
			Aspergillus flavus		Total fungi	
	root	shell	shell[b]	kernel[c]	shell[b]	kernel[c]
TREATMENT:						
A. flavus alone	1.0 a[d]	1.0 a	36.2 a[e]	1.0 a	647.2 a	13.4 a
A. flavus + *Meloidogyne hapla*	4.0 b	1.7 b	42.6 a	1.9 b	658.9 a	15.0 a
PEANUT CULTIVARS:						
'Argentine'	2.5 a	1.5 a	5.2 a	0.2 a	430.4 a	18.3 b
'Early Runner'	2.5 a	1.1 a	131.2 b	2.6 b	1061.0 a	14.2 ab
'Florigiant'	2.5 a	1.3 a	26.0 a	2.2 ab	541.0 a	9.9 a

[a] Root-knot index: 1 = no galls, 2 = light galling, 3 = moderate galling, 4 = heavy galling, 5 = very heavy galling.
[b] Mean numbers per shell.
[c] Mean numbers from 20 kernels.
[d] Data subjected to split-plot analysis. Means with same letter not significantly different at the 5% level.
[e] Fungal data transformed to $\sqrt{n+1}$ for statistical analysis.

fungal growth from 25 cultures each time. The fungus was fragmented in a blender in distilled water and diluted so that 2.5 l and 6 l of fungal suspension were applied to each microplot the first and second date, respectively. In addition to filling 26 holes 4 cm deep with the suspension on July 1, the entire soil surface was flooded and then covered with 1 cm of steam-sterilized soil. On July 19 the soil surface was flooded with the inoculum and was not covered with soil. The treatments, *A. flavus* and *A. flavus* + *M. hapla*, were replicated 12 times.

Roots and pods were harvested on September 26 and rated for nematode galling on a 1–5 scale (Table 1).

Ten shells from each replication were washed in tap water and fragmented in 100 ml of sterile water with a blender. Two 5-ml portions of the resulting suspension were mixed with warm rose bengal-streptomycin agar and dispensed into petri dishes. After incubation for 7 days at 28 C, fungi growing from shell fragments were enumerated.

Ten pods from each replication were soaked 5 min in 0.5% (v/v) NaOCl, opened aseptically, and 20 kernels were placed (5 per petri dish) on warm rose bengal-streptomycin agar. Discrete fungal colonies growing from kernels were enumerated after 7-days incubation at 28 C.

Washed fresh pods were stored at –23 C for 90 days and assayed for aflatoxins by the aqueous-acetone method (3). For aflatoxin analyses, the number of replications per treatment was reduced to four by combining replications 1-3, 4-6, 7-8, and 10-12. Shells and kernels were assayed separately.

RESULTS AND DISCUSSION

Roots of plants inoculated with nematodes were severely galled with no apparent differences among cultivars (Table 1). Light pod damage also occurred but was much less severe than that previously reported for *M. arenaria* (2). This difference was expected since *M. arenaria* in both field and greenhouse studies has caused more severe pod damage on these cultivars than *M. hapla*. Roots and pods of plants not inoculated with nematodes were free of galls. *A. flavus* was isolated from shells and kernels of nematode-inoculated and non-inoculated plants. Other fungi, principally *A. niger* van Tiegh., *Cephalosporium* spp., *Colletotrichum* sp.,

Curvularia spp., *Fusarium* spp., *Penicillium* spp., and *Trichoderma viride* (Per.) Fr., were present in all treatments. Presence of numerous contaminating fungi is unavoidable in field plots. Mean numbers of colonies of *A. flavus* and total fungi from shells and kernels of all cultivars were higher for plants inoculated with nematodes but the only significant difference was for *A. flavus* in kernels. Incidence and density of the fungus were significantly greater for kernels from plants that received both organisms than from those receiving only the fungus. Thus, the data indicate that nematodes may have played a role, even though a minor one, in increasing the incidence and density of *A. flavus* in pods.

Shells of 'Early Runner' contained significantly larger incidence and density of *A. flavus* than the other two cultivars; also, kernels of this cultivar contained more of this fungus than did kernels of 'Argentine'. Approximately twice as many total fungi were isolated from shells of 'Early Runner' as from shells of the other cultivars, but differences were not significant. Interaction analysis (not shown) indicated *M. hapla* did not significantly increase fungal infection of 'Early Runner'. A significantly greater total number of all fungi was isolated from kernels of 'Argentine' than from 'Florigiant.' Pods of many samples were discolored. Dark colored pods were more numerous among 'Early Runner' samples than the other two cultivars. The high density of fungi in 'Early Runner' shells indicated that pod discoloration was probably related to fungi. One might conclude that 'Early Runner' pods were more susceptible to the fungi present. However, results of an earlier experiment did not reveal a higher level of susceptibility for 'Early Runner' when inoculated with *P. brachyurus* + *A. flavus* and containing a similar, but not identical, contaminating fungal flora (1).

Aflatoxins were not detected in any kernel samples and in only two shell samples—one from 'Argentine' inoculated with *A. flavus* and one from 'Florigiant' inoculated with both organisms. The low incidence and density of *A. flavus* in kernels and of aflatoxins in shells, plus the absence of aflatoxins in kernels do not support the hypothesis of an *M. hapla-A. flavus* interaction tending to increase aflatoxins in peanut pods.

Literature Cited

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