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An outbreak of yellow mold of peanut seedlings in Texas

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Abstract

Yellow mold of peanut (*Arachis hypogaea*) seedlings caused by *Aspergillus flavus* was first observed during May 1984 in a commercial peanut farm in south Texas. The mold caused preemergence rotting of peanut seed and seedlings. On emerged seedlings the infection was largely restricted to cotyledons. The diseased plants were chlorotic, stunted, and leaflets were reduced in size with pointed tips and vein-clearing. Aflatoxins were found in cotyledons of infected seedlings but not in roots, hypocotyls, or leaves. *A. flavus* was the predominant fungus in the seed lot planted by the grower. Six isolates of *A. flavus* isolated from the seed and diseased seedlings were pathogenic to peanut in greenhouse tests.

Introduction

During May 1984, a seedling disease of peanut (*Arachis hypogaea* L.) was observed in a commercial peanut farm in Atascosa County, Texas. Approximately 300 acres of the peanut crop was affected, resulting in a stand reduction of ca. 70%. According to the grower, the peanut was a Virginia market type (cultivar not specified). Apparently the seedling disease problem was associated with only one seed lot produced in North Carolina and marketed by a seed company in south Texas. The same fields were subsequently replanted with another lot of seed (cv. Florigiant).

In this paper, we report the results of mycoflora analysis of seed used by the grower, isolation of the causal organism, and the results of pathogenicity tests.

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Materials and methods

Seed mycoflora

Samples of the peanut seed lot used by the grower were obtained for mycoflora examination and returned to the laboratory in paper sacks. In the first test, 300 seed were randomly selected, washed by agitating in sterile distilled water for 5 min, and placed on Czapek-Dox agar (Difco Laboratories, Detroit, MI) supplemented with 50 ppm rose-bengal and 10 ppm streptomycin. The plates were incubated at 25 °C in the dark and examined after 10 days. The fungal species growing out of the seed were identified, and the percentage of dominant and subdominant fungi was determined. In the second test, 600 seed were washed in running tap water, for 10 min, surface disinfected by immersion in 0.52% sodium hypochlorite solution containing traces of Tween 80 (polyoxyethylene sorbitan monooleate) for 5 min, and examined for mycoflora as described above. In the third test, 200 seed were surface disinfected and seed coats, cotyledons and embryos were excised from each seed under aseptic conditions. Pieces of

seed coat from each of 200 seed, one cotyledon from each of 200 seed, and all embryos were examined for fungal infection as described in the first test.

Isolation of fungi from diseased plants

Infected seedlings were dug from different fields and brought to the laboratory in polyethylene bags. Small pieces of diseased tissue (cotyledons) were excised, surface disinfected with 0.52% sodium hypochlorite solution containing traces of Tween 80 for 5 min, and placed on Difco potato-dextrose agar (PDA). Plates were incubated in the dark at 25 °C. Colonies of a fungus identified as *Aspergillus flavus* Link ex Fries consistently grew from almost all the diseased tissues within a week.

Pathogenicity

Two *A. flavus* isolates (AF-S1 and AF-S2) isolated from seed supplied by the grower and four (AF-SR, AF I, AF II, and AF III) from infected seedlings were used in pathogenicity tests. In the first test, isolates were grown on PDA slants for a week at 25 °C in the dark, and conidial suspensions (1×10^4 spores/ml) were prepared in sterile distilled water containing traces of Tween 80. Seed of the Tamnut 74 cultivar were surface disinfected with sodium hypochlorite, inoculated with *A. flavus* by immersing in the conidial suspension for 5 min, air dried over-night, and planted in plastic pots (17 cm dia) containing sandy loam soil fumigated with methyl bromide. Five seed were sown in each pot and ten pots were planted for each isolate. Pots were maintained in the glasshouse at 25 to 35 °C. Disease incidence was assessed 15 days after sowing by counting the number of seedlings having disease symptoms.

In the second test, the isolates were cultured in 250 ml Erlenmeyer flasks containing a mixture of moist sterile river-sand and polished rice (90:10, W/W). Fifteen days after incubation at 25 °C in the dark, the inoculum was prepared by homogenizing the contents in the flask and mixing with sandy loam soil (9:95, W/W) in plastic pots. Five seed of Tamnut 74 cultivar were sown in each of ten pots, and the disease incidence was assessed as described in the first test. Uninoculated controls were maintained in both tests.

Aflatoxin analysis of diseased plants

Peanut seedlings were collected from four locations in the grower's fields. Cotyledons, hypocotyls, leaves, and roots were separated and analyzed for aflatoxins using high pressure liquid chromatography (Waters Associates, Inc., Milford, MA.) by the method of Pons [5].

Results and discussion

Field symptoms

Brown necrotic lesions were observed on the cotyledons, radicles, and hypocotyls of ungerminated seed and seedlings that failed to emerge. Both the infected seed and seedlings were shriveled, dried, dark brown and heavily covered with yellow or yellow-green spores. Necrotic lesions were also present on the cotyledons of emerged seedlings. The lesions were sunken with reddish brown and commonly covered with yellowish-green spore masses (Fig. 1a). Normally, this necrosis terminated at or near the cotyledonary axis; in some cases lesions extended to hypocotyls. The diseased plants were chlorotic and stunted. Leaflets were reduced in size with pointed tips and vein-clearing. The root system was also poorly developed (Fig. 1b).

Seed mycoflora

Aspergillus flavus was the predominant fungus followed by *A. niger* and species of *Fusarium*. In general, more fungi were isolated from surface sterilized seed than from unsterilized seed. The process of surface sterilization could have removed or diluted the fungicide present on the seed thus enabling more fungi to grow out of the seed. Most of the fungi were isolated from seed coats rather than from cotyledons and embryos; *A. flavus* was isolated from

Fig. 1. Symptoms of yellow mold of peanut caused by *Aspergillus flavus* in south Texas. (a) Necrotic lesions on cotyledons covered with yellowish-green spore masses. (b) Diseased seedlings showing stunting and poorly developed root system.

cotyledons and embryos in higher frequencies than other fungi, suggesting that *A. flavus* infection was deeply embeded in the seed (Table 1). The literature on pre- and post-harvest conditions affecting *A. flavus* invasion of peanut pods has recently been reviewed by Diener *et al.* [2]. *Aspergillus flavus* can invade peanut pods at any stage of crop development, but the post-harvest conditions are often especially favorable for invasion. *Aspergillus flavus* invasion of pods is generally high when the crop is subjected to drought stress during pod development. Invasion of pods by other soil fungi and fauna may predispose the pods to *A. flavus* infection. Poor harvest and post-harvest conditions such as mechanical damage during lifting, slow drying, and improper storage facilitating rewetting of pods are ideal for *A. flavus* invasion [2]. Although *A. flavus* is a common component of peanut seed mycoflora in the United States, the frequency of its occurrence in sound mature seed is generally low [2]. The levels of *A. flavus* infection in the present peanut seed sample were extremely high (58%), especially for seed intended for planting purposes.

Table 1. The percentage of peanut seeds[a] infected by various fungi.

Fungal species	Seed or seed parts infected with fungi				
	Unsterilized seed[b]	Surface sterilized[c]	Seed parts[d]		
			Seed coat	Cotyledons	Embryo
	(%)	(%)	(%)	(%)	(%)
Aspergillus flavus Link ex Fries	36.0	58.0	54.5	12.0	7.5
A. niger van Tiegh.	6.0	17.5	28.0	3.0	2.0
A. fumigatus Fres.	2.5	5.8	0	0	0
Aspergillus spp. (*A. amstelodami* (Mangin) Thom & Church *A. chevalieri* (Mangin) Thom & Church *A. ochraceus* Wilh. *A. parasiticus* Speare *A. terreus* Thom)	0.6	1.6	4.8	3.0	0.3
Fusarium spp. (*F. oxysporum* Schl. *F. semitectum* Berk. & Rav. *F. solani* (Mart.) App. & Wr.)	4.5	10.5	5.5	3.0	1.0
Rhizoctonia solani kuhn	2.0	4.5	2.5	1.5	0
Rhizopus spp. (*R. arrhizus* Fischer *R. stolonifer* (Ehrenberg ex Fries) (Vuillemin))	3.0	4.3	5.0	1.5	0
Other fungi (Species of *Alternaria, Epicoccum, Gliocladium, Penicillium* and *Trichoderma*)	0.7	1.4	3.5	1.3	0.8
Number of seed/seed parts tested	300	600	200	200	200

[a] Peanut seed used by the grower in south Texas for planting.

[b] Seed were washed in sterile distilled water and placed in petri dishes containing Czapek-Dox agar supplemented with rose bengal and streptomycin (CDRBSA).

[c] Seed were surface sterilized with 0.52% sodium hypochlorite and placed on CDRBSA medium.

[d] Seed were surface sterilized, seed coats, cotyledons and embryos were excised aseptically, and placed on CDRBSA medium.

Pathogenicity of A. flavus

All the isolates of *A. flavus* tested were equally pathogenic to peanut seedlings (Table 2). The disease incidence was higher when the fungus was inoculated into the soil than when the fungus was applied to the seed. There was no marked difference in the degrees of virulence among isolates. Unemerged seed and seedlings had lesions on the cotyledons, plumules, and radicles. In advanced stages of disease development, both the infected seed and seedlings were reduced to a dark brown mass covered with greenish-yellow fructifications of the pathogen. Most of the seedlings which emerged from the soil had necrotic lesions on the cotyledons. Necrosis always terminated at or near the cotyledonary axis. Lesions were not observed on hypocotyls, roots, stems, and leaves. The lesions on the cotyledons were covered with conidia of the pathogen. Plants were stunted and chlorotic. Leaflets were small, had pointed tips, and vein-clearing. Development of the root system was also poor. Some of the infected plants eventually recovered from the disease and produced normal foliage, as reported by El-Khadem [3]; however, most of the plants remained very stunted and chlorotic up to 40 days after sowing.

Jackson & Bell [4] coined the word 'yellow mold' for pre-emergence seed and seedling rot of peanut caused by *A. flavus* to distinguish this disease from other seed and seedling diseases incited by other soil fungi. Seed and seedling decay by *A. flavus* is most rapid when infected seed are planted where the fungus becomes active as the seed hydrate. Cotyledons of germinating seed are invaded first and, under favorable conditions, the emerging radicle and hypocotyl decay rapidly. Under unfavorable conditions, after initial infection, the fungus persists in the cotyledons and the emerged seedlings become chlo-

Table 2. Pathogenicity of *Aspergillus flavus* on peanut (cv. Tamnut 74) seedlings in the glasshouse inoculation tests.

Isolate	Method of inoculations	Seedling emergence	Disease incidence	
			Preemergence seed and seedling rot	Postemergence seedling chlorosis and stunting
		(%)	(%)	(%)
AF-S1[a]	Seed[c]	50	44	14
	Soil[d]	34	60	26
AF-S2[a]	Seed	44	50	30
	Soil	26	68	18
AF-SR[b]	Seed	54	42	20
	Soil	20	76	16
AF-I[b]	Seed	52	42	22
	Soil	24	72	18
AF-II[b]	Seed	44	52	34
	Soil	20	70	18
AF-III[b]	Seed	46	50	30
	Soil	28	62	16
Control	–	96	2	0
	–	92	0	0

[a] Isolated from peanut seed obtained from the grower.
[b] Isolated from diseased seedlings.
[c] Surface sterilized seed were inoculated with conidia of the test isolate and planted in plastic pots containing fumigated garden soil.
[d] Isolates were multiplied on rice and river-sand mixture, and inoculated to the soil.

rotic and stunted, a condition described by Chohan & Gupta [1] as 'aflaroot'. Aflatoxins produced by the fungus at the infection site (cotyledons) are translocated throughout the seedling [1]. In the present investigation aflatoxins (70 to 101 ppb) were found in cotyledons of infected seedlings; however, aflatoxins were not detected in roots, hypocotyls, or leaves. Further studies are required to understand the role of aflatoxins on yellow mold disease development.

We conclude that the yellow mold disease of peanut observed in south Texas was associated with poor seed quality. The high percentage of seed infection by *A. flavus* suggests that the seed was produced under poor agronomic conditions and not suitable for planting purposes. The seed treatment consisting of Thiram and Botran did not suppress the disease because the infection of *A. flavus* was deeply embedded. Subsequent replanting of the same field with seed from a different seed lot gave a satisfactory plant stand.

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