A STUDY OF THE POD ROT COMPLEX OF PEANUTS

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CHAPTER I

INTRODUCTION

Peanuts (*Arachis hypogaea* L.) are an important high-protein crop in many countries throughout the world. Peanuts produce well under warm, moist conditions. These conditions also favor a number of peanut diseases.

Peanut diseases are important because they reduce yield and lower peanut quality. In some cases peanut diseases may be the major limiting factor in peanut production. An important peanut disease demonstrated as early as 1931 (35, 36) was called peanut pod rot. Similar pod rots caused by various organisms have become world-wide in distribution since 1960 and a certain type of pod rot is increasing in importance in Oklahoma (45). Losses from peanut pod rots have ranged from 10 to 50% depending on the species of fungi involved.

Many microorganisms have been associated with pod rots. Some of these organisms are: *Pythium* spp., *Rhizoctonia solani* Kuehn, *Sclerotium rolfsii* Sacc., *Fusarium* spp., *Penicillium* spp., *Aspergillus* spp., *Trichoderma* spp., *Rhizopus* spp., *Verticillium* spp., *Thielaviopsis basicola* (Berk. and Br.) Ferraris, and bacterial spp.

Various control measures have been suggested for these diseases dpending upon the causal organism or organisms associated with infected pods; however, satisfactory control to date has been difficult or impossible. This may be due to the several species of organisms

reported to cause these diseases singly or together, the difficulty in determining the primary pathogens in a disease complex and confusion from the use of several different names e.g. blackhull, pod rot, or pod break down, which may or may not be manifestations of the same disease.

Because of confusion involving the causal organisms and the several different names applied to these diseases, the present study was initiated to determine the causal organism(s) of pod rot of peanuts in Oklahoma, the environmental conditions for disease development and to develop a quick varietal testing program by artificial inoculation. Hopefully, this information would lead to more effective disease control through resistance. Particularly, if a varietal screening program could be developed.

The term pod rot as referred to in these studies means pods with light brown or dark brown to black lesions, with necrotic and sometimes extensive dark, soft and water-soaked areas.

CHAPTER II

REVIEW OF LITERATURE

Peanut pod rots are serious diseases of peanuts. Reports of losses due to these diseases have long been known. In Virginia and North Carolina in 1931, (35) up to 10% of the harvested nuts were rotted or "pickouts". Losses in Georgia and Alabama were reported as high as 30% (17, 36). In 1942, Georgia, North Carolina, South Carolina, Texas, Virginia and Oklahoma reported from 2 to 12% loss (42). Later, Bell and Sobers (6) in Georgia reported peanut peg and pod rot could cause 25% loss. In North Carolina damage from pod rot costs $20 to $50 per acre (17, 30, 44). According to Jackson and Bell (30), pod rot was also recognized by Frezzi (16) in Argentina, where in some cases, 58% of the pods were destroyed. In still another country (Libya), Krang and Pucci (32) stated peanut pod rot had reduced yield sometimes as much as 50%.

In addition to direct losses, blemished pods are unfit for market or export. Further evidence of the importance of peanut pod rot was emphasized in 1955 by the Food and Agriculture Organization of the United Nations (34) in its investigation of problems arising in connection with these losses.

Frank (12, 14) observed that a dry rot of peanut geocarps, prevalent in sandy soil in Israel, was caused by Pythium spp. This dry rot is characterized by a medium to dark brown discoloration of the

shell, and by mycelial development and oospore formation within the pod cavity. The rot often terminates in mummification of the seeds and disintegration of the pods; oospores may then be found entangled in mycelia. Roots of affected plants appear healthy and no symptoms are visible on the aerial parts of the host.

Jackson et al. (30) described Pythium pod rot on both immature and mature pods. In either case the first symptoms on pods were light browning and extensive watersoaking of the tissue. Infection spread rapidly and in two to four days the entire pod appeared watery and showed a brown-black necrosis. Immature pods were usually completely destroyed.

Garren (19, 20) reported two general types of peanut pod rot in Virginia -- the more common was a general and rapid breakdown in mature fruits caused by Pythium spp. The other type was caused by Rhizoctonia sp. and involved an internal dry-rot of pods. By the use of a selective fungicide, he also found that certain fields have a definite though fluctuating potential for Pythium pod rot; whereas in other fields, pod rot was caused mainly by Rhizoctonia spp. (18, 19).

Frank (12, 13) in Israel, Frezzi (15) in Argentine, and Evans and Poole (11) in the United States, surveyed peanut fields and reported Pythium spp. were involved in peanut pod rot.

Garren (22) showed peanut pods have a rhizoplane-like flora, mainly bacteria, Fusarium spp. and Pythium spp. The latter pathogen causes most of its damage before the other prime pathogen suspect, R. solani, becomes active. R. solani and Fusarium spp. are the stable components of the whole season; molds (largely Penicillium spp. but some Aspergillus spp.) are early-season dominants in sound pods and

late-season invaders of rotted pods. Trichoderma spp., Rhizopus sp., Sclerotinia sp., Phoma sp. were also associated with peanut pods. Garren (23, 24) confirmed the infectivity of Pythium myriotylum Drechsler and demonstrated its pathogenicity to peanut pods. In one study, detached pods of Virginia Bunch 46-2 were inoculated with an isolate of the fungus from rotted pods, and all pods were decayed within seven days. In another study, attached and detached pods of Virginia Bunch 46-2 and Dixie Spanish were inoculated, and all pods were decayed within eight days. The fungus was reisolated from decayed pods in both tests. In the same report, Garren demonstrated that Pythium spp. could also be part of the quiescent endogeocarpic flora.

Jackson and Bell (30) stated that peanut fruit, from the time of soil penetration by the peg until harvest, were subject to attack by Rhizoctonia solani Kuehn. Infected pods exhibited varying degrees of discoloration, from slight superficial russeting to browning of the entire pod and decay of the contents. Rhoads (41) in Florida, reported decayed pods showed Rhizoctonia sp. and Diplodia natalensis Evans fruiting on the shell lesions as well as on stems of the same plants.

Ashworth and Langley (2) reported R. solani was the main pod-rot pathogen in Texas. Evans and Poole (11) also found R. solani to be one of the more common fungi associated with peanut pods.

Ashworth et al. (1) stated that lesions caused by Sclerotium sp. on young pods of Spanish peanut were orange-yellow to light tan, and were light brown-black and zonate on older pods. Luther and Speairs (33) reported a fruit, stem, peg and root decay of peanut by S. rolfsii, R. solani, Fusarium spp. and other fungi were very severe

in North Carolina in 1942. S. rolfsii was prominently associated with this decay late in the season. Taylor (46) and Evans et al. (11) stated rot of peanut pods was due principally to S. rolfsii. Atkinson (3) found many non-parasitic organisms associated with pod rot and suggested that damage to the pegs by S. rolfsii might predispose the pod to rot.

In Alabama, Stone and Wilson (44) showed the predominant organism associated with fruit rots was S. rolfsii, which was confined mainly to the shells and pegs. Rarely was the fungus isolated from seed.

Krantz and Pucci (32) stated that pod rot due to Fusarium solani and F. scirpi, appeared mainly in the final stages of harvest. The pod either remained intact and firm but brown to black in color, or was already in a state of decay and black or brown and friable. In the first case, the inner wall of the shell and the kernels themselves usually were covered with brownish-white-furry mycelium. When the rot was allowed to develop, the nuts and the internal mycelium disappeared eventually. Decaying pods were often empty.

Reichert and Chorin (40), as cited by Jackson and Bell (30) mentioned a violet-whitish pod color caused by Fusarium spp. According to Garren (23) symptoms of pod infection by various species of Fusarium were not usually characteristic enough to permit diagnosis by inspection.

Bell and Sobers (6) described a pod necrosis of peanuts caused by a species of Calonectria. Symptoms included chlorosis, wilting, blackening and necrosis of pegs, pods and roots. Jackson and Bell (30) described dark, brown, slightly sunken lesions caused by Cylindrocladium crotalariae (Loos) Bell and Sobers that occurred on pegs and

pods. Lesions on pods were usually discrete, but occasionally the entire pod was affected.

Frezzi (16), as cited by Jackson and Bell (30), observed that the verticillium or floury pod rot of peanut was characterized by dark, black, rotted pods which were usually sprinkled with white, powdery patches composed of masses of conidia of Verticillium sp.

Orellana and Bailey (37) reported that peanut plants attached by Botrytis cinerea Pers. ex Fr. had blighted leaves, a thick, dark-gray mycelium on the lower part of the stem and black globose to flat sclerotial masses of Botrytis up to 8 mm in size on molded stems, pegs and pods. Pods in various stages of decay were found on severely diseased or dead plants in the nurseries. Jackson and Bell (30) stated the fungus B. cinerea caused decay by attacking leaves first, and then stems, pegs and fruits, Flattened or plano-convex, black, irregular-shaped sclerotia developed on decayed stems and pods.

Another disease that is considered by some to be a pod rot is blackhull, a disease caused by Thielaviopsis basicola (Berk and Br.) Ferraris. Ciccarone (7), as cited by Jackson and Bell (30), stated that T. basicola caused black, sooty spots on pods which became irregularly confluent and involved one-half or more of the shell. Hsi (29) stated this disease occurred sporadically on Valencia peanuts in the Portales area of New Mexico but was not a problem in other peanut growing areas of the United States.

Prince (39) reported a complex of fungi, mites and nematodes associated with pod rot of peanuts.

Higgins (27) believed drought and deficiency of calcium in the soil as the primary cause of a "black pod" disorder of peanuts.

Horne (28) pointed out that root knot nematodes (Meloidogyne spp.) produced visible knots or galls on the roots and pods. The root lesion nematode (Pratylenchus spp.) caused significant losses by attacking roots, pegs and pods. The damage resulted in spots, blotches or lesions on the affected plants. Brown to black spots on peanut hulls were the most obvious symptoms.

CHAPTER III

MATERIALS AND METHODS

General Methods

The experiments involved studies to determine the possible causal organism(s) of peanut pod rot in Oklahoma and the optimum environmental conditions for disease development. Studies were made with fungi associated with peanuts in Oklahoma, including pathogenicity tests and disease development in artificially and naturally infested soils. A separate test was made to determine the effect of the fungicide Arasan 75 (Tetramethylthiuramdisulfide 75% dust) on the germination of peanut seeds for use in the soil tests.

Isolates of fungi were obtained from rotted peanut pods collected in Oklahoma. Potato-dextrose agar (PDA)[1], water agar (WA)[2] and a modified Martin's Rose-Bengal Agar (RB-M2) (5, 26) were used in all isolation work. Subsequent culture of each fungus depended on the study involved.

To obtain a pure culture of inoculum, each fungal isolate was started from a single spore or hyphal tip.

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[1]Potato-dextrose agar: Peeled and sliced potatoes, 200 g; dextrose, 20 g; agar, 17 g; distilled water to make a final volume of 1000 cc.

[2]Water agar: Agar, 17 g; distilled water to make a final volume of 1000 cc.

The Spanish peanut cultivar Starr was used in these studies. Unless otherwise specified, seeds were treated with the fungicide, Arasan 75. Teller fine sandy loam soil from the Perkins Oklahoma Agricultural Experiment Station farm was used. Peanut pods were harvested by hand. Regular fertilization and spraying for insect control were made throughout the studies involved.

Soil-test studies were conducted in growth chambers and greenhouses at several temperature ranges. A complete randomized design having a factorial arrangement of treatments was used in these studies. Peanut pods were collected when fully mature in the following way: Mature peanut plants were removed from pots and carefully washed in running tap water. A metal screen was used to hold plants and keep detached mature pods and rotted pods from being lost. The relative severity of infection for each pod was then determined.

Percent necrosis of pod surface was the criterion used in classifying pods according to the following severity index (Fig. 1): 0% = 1; 1-25% = 2; 26-50% = 3; 51-75% = 4; 76-100% = 5.

After pods from two plants of a given treatment had been examined, a disease index for each treatment was calculated. This was done by multiplying the number of pods in each disease class by the class value, adding the products obtained and dividing this total by the number of peanut pods harvested from these two plants. For example, if five pods of a given treatment were graded in class 2, three pods graded in class 3, two pods graded in class 4 and 24 pods graded in class 1 the disease index would be $\frac{(5 \times 2) + (3 \times 3) + (2 \times 4) + (24 \times 1)}{34} = 1.50$.

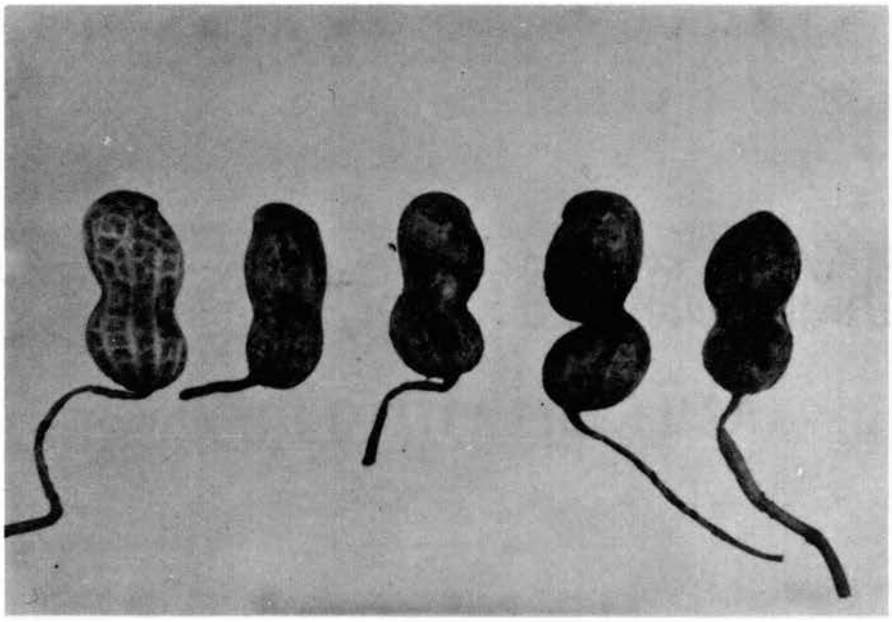

Figure 1. Peanut Pod Rot Grading Classes Based on Percentage of Rotted Pod Surface (Left to Right): 0% =1; 1-25% = 2; 26-50% = 3; 51-75% = 4; 76-100% = 5.

Fungi and Other Organisms Associated with Peanut Pods and Seedlings in Oklahoma

Peanut pods were sampled at random from fields near Atoka, Willis, Perkins, Fort Cobb and Anadarko during the harvest season in 1968. The samples were kept moist and stored in a cold room (4 C) until processed. Pods were carefully examined and only those that showed symptoms of pod rot were selected. Whole pods were washed in running tap water, surface disinfected with 30% solution of Purex (6% sodium hypochlorite) for 3 min., dipped into three series of sterile tap water and then aseptically placed on PDA and WA to detect fungal populations and isolate pathogens.

Isolations were also made from peanut seedlings obtained from seed treatment studies at Perkins and Fort Cobb, Oklahoma (48).

Fungi encountered were identified to the genus. Since the main concern was with fungi present in whole pods, no isolations were made from seeds.

Seedling Pathogenicity Test

The purpose of this test was to select the most pathogenic isolate from eight isolates of each of the four genera of fungi, Fusarium, Rhizoctonia, Pythium and Sclerotium which were isolated from rotted pods. Peanut seedlings rather than pods were used for initial pathogenicity determinations because many of the same fungi attack both seedlings and pods and because seedlings can be produced in ten or less days whereas pod production may require 90 or more days. Eight isolates of each of the four genera of fungi were aseptically grown in PDA plates at 28 C for 7-10 days. Seeds were planted in metal pans,

filled with vermiculite and then harvested after 10-12 days. Healthy and uniform-size seedlings were carefully washed in running tap water and then dipped into three series of sterile water.

Inoculations were made by covering the stems and roots of five seedlings laid on wax paper with slices of fungus and agar medium from a single isolate. All isolates were treated in the same manner. Wet cotton was then placed over the inoculum to prevent dessication and the plants wrapped with two layers of paper towels, placed in a plastic bag, and kept in incubation chambers.

Incubations were controlled at three different temperature levels. One group was incubated for 4 days at 28 C (high temperature), another was incubated for 6 days at 24 C (moderate temperature) and a third group was incubated at 20 C (low temperature) for 6 days. Uninoculated seedlings were used in each treatment for controls. Those wrapped with wet cotton only served as check I (CK_1), and those with wet cotton plus slices of the aseptic-PDA medium served as check II (CK_2).

Only one inoculation per isolate was made for each species at each temperature level. Seedling infection indices (Figure 2) were based on a one to five severity scale (1 = no infection; 2 - slight infection; 3 = moderate infection; 4 - severe infection; 5 = very severe infection).

Peanut Pod Pathogenicity Test

The most pathogenic isolates as determined from peanut seedling inoculations were further tested for their pathogenicity using newly harvested mature pods. These isolates from the genera Fusarium, Rhizoctonia, Pythium and Sclerotium rolfsii were designated F-6, R-2,

Figure 2. Seedling Disease Grading Scale (Left to Right): 5 = Very Severe Infection; 4 = Severe Infection; 3 = Moderate Infection; 2 = Slight Infection; 1 = No Infection.

P-7 and S-4 respectively. Plants were grown in the greenhouse to maturity at a temperature regime of 27-31 C day and 23-27 C at night. Healthy and uniform pods were carefully harvested, washed in running tap water, surface disinfected with 30% solution of Purex for 3 min., and then dipped into three series of sterile water. Peanut plants with healthy, uniform pods still attached were harvested also and disinfected in the same manner.

Pods which were detached as well as attached were inoculated. Inoculation was similar to that in the Seedling Pathogenicity Test except that aseptic 250 cc Erlenmeyer flasks were used instead of plastic bags, and an oat-grain medium[1] was used instead of PDA. After infection, inoculum was removed from pods and the infected pods were further incubated in the moist flasks for 6 days and then air-dried for 2-3 days prior to examination. Incubation temperatures were similar to those previously described in the Seedling Pathogenicity Test, except that the attached pods part of the Peanut Pod Pathogenicity Test was incubated only at the high temperature (4 days at 28 C). Uninoculated peanut pods served as controls.

Percentage of necrosis was the criterion used in rating pods (Figure 1).

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[1]Oat-grain medium: Oat - 515 g (approximately 1000 cc by volume); distilled water - 1300 cc (the procedure is as follows: (i) fill qt. jar with oats; (ii) sterilize dry 1 hour; (iii) fill with distilled water; (iv) sterilize 2 hours again; (v) when cool, inoculate 1 flask with spores and mycelium of one isolate from one plate.)

Artificially Infested Soil Test

The purpose of this test was to find out the influence of different amounts of inoculum and temperatures on pod rot development.

Isolates F-6, R-2, P-7 and S-4 which were the most pathogenic in the Seedling Pathogenicity Test were used in this study separately as well as in a combination of all four fungi at a ratio of 1.3:1.3:1.3:1.0 g. for *Fusarium* sp., *Rhizoctonia* sp., *Pythium* sp., and *S. rolfsii* respectively.

Soil in 15 cm pots was steam sterilized for 72 hours and aerated for 48 hours. Two peanut plants were grown in each pot in a greenhouse at 27-31 C day, and 23-27 C at night. Isolates of each of the four genera of fungi were grown on autoclaved corn meal-sand medium (corn meal, 1000 cc : washed white sand, 1000 cc : distilled water, 1500 cc). Inoculations were made in the very early pegging stage of fruit development.

Plants were inoculated by the following method: Corn meal-sand fungus inoculum, passed through a 0.99 cm screen was mixed with the surface soil in such a way as to infest the soil but not contaminate above ground parts of the plant. Peanut plants treated in the same manner with aseptic corn meal-sand medium served as controls. Both the control and the pathogen-treated pots were inoculated at two rates: 50 g and 100 g.

Forty eight pots were maintained in the greenhouse at the temperature regime previously mentioned, and 48 pots in a growth chamber at a temperature regime of 22-24 C day and 20-22 C night.

The design used was a split-split plot arrangement, with temperature regimes in the main plot as randomized blocks, amount of inoculum

as a subplot and different fungi as a sub-subplot. The ratio of 1.3 : 1.3 : 1.3 : 1.0 for Fusarium sp., Rhizoctonia sp., Pythium sp. and S. rolfsii respectively was used in the combination inoculum in the sub-subplot. This ratio was calculated in the following way:

$\frac{2.3 + 1.9 + 2.4 + 3.3}{4} = 2.5$ = mean of the total mean disease indices of Fusarium, Rhizoctonia, Pythium and Sclerotium respectively on seedlings. The fact that $\frac{2.3}{2.5} : \frac{1.9}{2.5} : \frac{2.4}{2.5} : \frac{3.3}{2.5} = 1.0 : 1.0 : 1.0 : 1.3$, determines the hypothetical ratio 1.3 : 1.3 : 1.3 : 1.0 since 1.0 x 1.3= 1.0 x 1.3 = 1.0 x 1.3 = 1.3 x 1.0 = 1.3 for each of them.

Naturally Infested Soil Test

The purpose of this test was to find out if peanut pod rot would develop in naturally infested soil which had produced pod rot and which also contained nematodes, at temperatures and soil moisture levels believed to favor pod rot development. Cobb fine sandy loam soil was collected from Fort Cobb and a Dougherty-Teller-Yahola fine sandy loam was collected from Willis. Steam sterlized soil from each locality served as controls.

Soil moisture levels were varied by watering half of the plants at a given temperature once a day (low) and the other half received twice the water volume but in two applications (high).

Temperature treatment was conducted under four regimes:

T_1 = 27-31 C day and 23-27 C night

T_2 = 22-24 C day and 20-22 C night

T_3 = 26-32 C day and 20-26 C night

T_4 = 19-24 C day and 15-19 C night

T_2 and T_4 were maintained in growth chambers while T_1 and T_3 were maintained in greenhouses.

This study was a factorial arrangement of treatments in the greenhouses and in the growth chambers. There were two soil types, four temperatures and two moisture levels. Five pots of each moisture level for each soil were maintained at each temperature regime.

The soil was examined for nematodes after harvest. Nematode extraction was done by a combination of Cobb's gravity-sifting and Baermann funnel techniques (4, 8).

CHAPTER IV

RESULTS

Fungi and Other Organisms Associated with Peanut Pods and Seedlings in Oklahoma

Frequency of fungi and other organisms isolated from roots and stems of peanut seedlings and discolored pods is listed in Table I. Most of the organisms were soil-inhabiting and soil-invading fungi. The genera of fungi isolated were very similar to those reported by early workers (20, 22, 38). A number of fungal isolates were classified as unknown due to absence of spores for identification.

Seedling Pathogenicity Test

Data in Tables II, III, IV and V show that some isolates of each of the four genera of fungi Fusarium, Rhizoctonia, Pythium and Sclerotium were more pathogenic than others. The highest mean disease index among the eight isolates of each of the four genera of fungi designated as F-6, R-2, P-7 and S-4 were 3.2, 2.6, 3.4 and 4.6 respectively. The total mean disease indices of all isolates for each of the four genera of fungi at three different temperatures were as follows:

Genus of Fungus	Total Mean Disease Index
Fusarium spp.	2.3
Rhizoctonia spp.	1.9
Pythium spp.	2.4
Sclerotium spp.	3.3

TABLE I

FREQUENCY OF FUNGI AND OTHER ORGANISMS ISOLATED FROM ROOTS AND STEMS OF PEANUT SEEDLINGS AND DISCOLORED PODS IN OKLAHOMA DURING 1968

Organisms[1]	Number of Organisms Isolated		Percent of Total Isolates	
	Seedlings[2]	Pods[3]	Seedlings	Pods
Fusarium spp.	87	167	22	31
Rhizoctonia spp.	25	83	6	15
Pythium spp.	23	68	6	13
S. rolfsii	6	20	2	4
Penicillium spp.	43	36	11	7
Alternaria spp.	78	20	20	4
Rhizopus spp.	36	14	9	3
Aspergillus spp.	0	13	0	2
Trichoderma spp.	0	18	0	3
Helminthosporium spp.	0	7	0	1
Nematodes	9	41	2	8
Bacteria	11	18	3	3
Unknown	70	31	18	6

[1]Media of PDA, WA and a modified Martin's Rose Bengal Agar (RB-M2) were used for isolation work.

[2]Isolations were made from infected roots and stems.

[3]Isolations were made from margins of pod infections.

TABLE II

PATHOGENICITY RATINGS OF EIGHT ISOLATES OF FUSARIUM SPP. OBTAINED FROM MATURE DISCOLORED PEANUT PODS IN OKLAHOMA DURING 1968 ACCORDING TO DISEASE DEVELOPMENT ON INOCULATED SEEDLINGS[1]

Temperature of Incubation	Isolate Number										
	1	2	3	4	5	6	7	8	CK_1[2]	CK_2[3]	Mean
High	2.2	1.4	1.6	2.4	2.2	3.2	1.8	3.4	1.8	3.0	2.3
Moderate	2.2	1.6	3.2	2.8	1.8	3.6	3.4	3.4	1.4	2.2	2.6
Low	2.0	1.2	2.2	3.6	2.0	2.8	2.8	1.8	1.4	2.2	2.2
Mean	2.1	1.4	2.3	2.9	2.0	3.2	2.7	2.8	1.5	2.4	2.3

[1]Each value is the mean severity of disease development of five seedlings at each temperature according to the following severity index:
1 = no infection
2 = slight infection
3 = moderate infection
4 = severe infection
5 = very severe infection

[2]Treated with wet cotton only, no PDA

[3]Treated with sterile PDA and wet cotton

TABLE III

PATHOGENICITY RATINGS OF EIGHT ISOLATES OF RHIZOCTONIA SPP. OBTAINED FROM MATURE DISCOLORED PEANUT PODS IN OKLAHOMA DURING 1968 ACCORDING TO DISEASE DEVELOPMENT ON INOCULATED SEEDLINGS[1]

Temperature of Incubation	Isolate Number										
	1	2	3	4	5	6	7	8	CK_1^2	CK_2^3	Mean
High	1.2	3.0	2.8	1.6	1.8	2.2	1.8	3.4	1.8	3.0	2.3
Moderate	1.8	2.6	1.2	2.0	1.4	1.8	2.2	2.4	1.4	2.2	1.9
Low	1.0	2.2	1.2	2.0	1.4	1.4	1.4	1.8	1.4	2.2	1.6
Mean	1.3	2.6	1.7	1.9	1.5	1.8	1.8	2.5	1.5	2.4	1.9

[1]Each value is the mean severity of disease development of five seedlings at each temperature according to the following severity index:
1 = no infection
2 = slight infection
3 = moderate infection
4 = severe infection
5 = very severe infection

[2]Treated with wet cotton only, no PDA

[3]Treated with sterile PDA and wet cotton

TABLE IV

PATHOGENICITY RATINGS OF EIGHT ISOLATES OF PYTHIUM SPP. OBTAINED FROM MATURE DISCOLORED PEANUT PODS IN OKLAHOMA DURING 1968 ACCORDING TO DISEASE DEVELOPMENT ON INOCULATED SEEDLINGS[1]

Temperature of Incubation	Isolate Number								CK_1^2	CK_2^3	Mean
	1	2	3	4	5	6	7	8			
High	2.6	1.4	1.4	3.0	2.4	2.0	3.2	2.8	1.8	3.0	2.4
Moderate	1.8	2.2	1.4	3.4	3.4	2.2	3.4	3.4	1.4	2.2	2.5
Low	2.2	1.4	1.2	3.4	2.8	2.6	3.6	3.4	1.4	2.2	2.4
Mean	2.2	1.6	1.3	3.3	2.9	2.3	3.4	3.3	1.5	2.4	2.4

[1]Each value is the mean severity of disease development of five seedlings at each temperature according to the following severity index:
1 = no infection
2 = slight infection
3 = moderate infection
4 = severe infection
5 = very severe infection

[2]Treated with wet cotton only, no PDA

[3]Treated with sterile PDA and wet cotton

TABLE V

PATHOGENICITY RATINGS OF EIGHT ISOLATES OF S. ROLFSII OBTAINED FROM MATURE DISCOLORED PEANUT PODS IN OKLAHOMA DURING 1968 ACCORDING TO DISEASE DEVELOPMENT ON INOCULATED SEEDLINGS[1]

Temperature of Incubation	Isolate Number 1	2	3	4	5	6	7	8	CK_1^2	CK_2^3	Mean
High	3.4	2.8	3.8	4.8	5.0	5.0	4.6	4.8	1.8	3.0	3.9
Moderate	2.6	3.2	3.6	4.8	5.0	5.0	2.4	4.2	1.4	2.2	3.4
Low	1.8	1.6	2.2	4.2	3.4	3.4	3.2	3.8	1.4	2.2	2.7
Mean	2.6	2.5	3.2	4.6	4.5	4.5	3.4	4.3	1.5	2.4	3.3

[1]Each value is the mean severity of disease development of five seedlings at each temperature according to the following severity index:
1 = no infection
2 = slight infection
3 = moderate infection
4 = severe infection
5 = very severe infection

[2]Treated with wet cotton only, no PDA

[3]Treated with sterile PDA and wet cotton

Results in Tables II, III, IV and V are summarized in Figure 3 to show the effects of temperature on the pathogenicity of four genera of fungi on peanut seedlings.

Peanut Pod Pathogenicity Test

Data in Table VI show a higher mean disease index was observed for the non surface disinfected treatment in each of the four genera of fungi in the newly harvested attached pods method. In decreasing order, the total mean disease indices of S. rolfsii, Pythium sp., Fusarium sp. and Rhizoctonia sp. were 2.9, 2.6, 2.5, 2.3 respectively as compared with the check which was 2.1.

Disease data for the newly harvested, mature, detached pod method are shown in Table VII. In decreasing order, the total mean disease indices of the combination treatment and the single pathogen treatments with Pythium sp., Fusarium sp., Rhizoctonia sp., and S. rolfsii were 3.1, 3.0, 2.9, 2.8 and 2.8 respectively as compared with the check which was 1.8.

Data in Table VII and Figure 4 show generally that the higher the incubation temperature the larger the mean disease index in the newly harvested mature detached pods method. However, the Fusarium sp. treatment showed the highest reading at the moderate incubation temperature.

Artificially Infested Soil Test

Symptoms of fungus infection were not visible on aerial parts of peanut plants in the two temperature regimes T_1 (27-31 C day, 23-27 C night in the greenhouse) and T_2 (22-24 C day, 20-22 C night in a

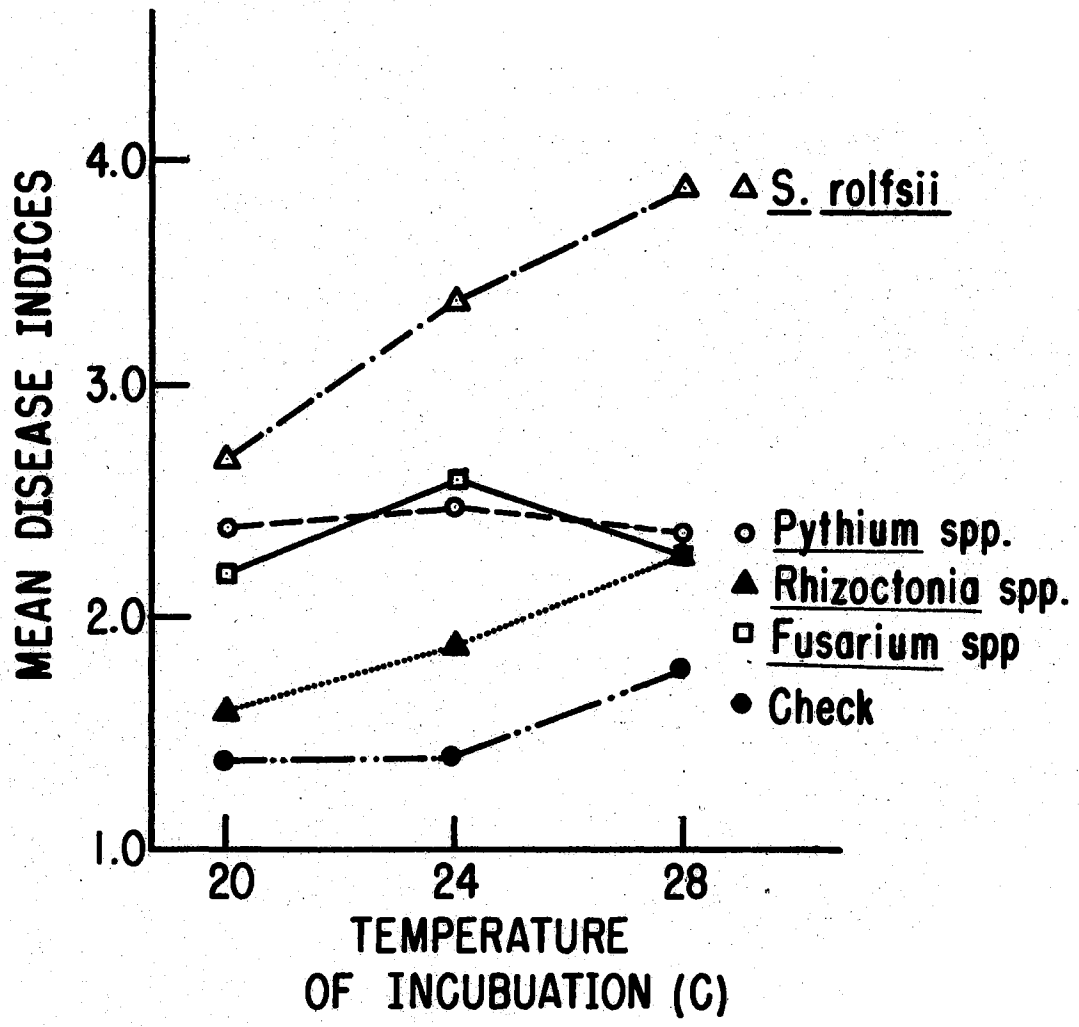


Figure 3. Effects of Temperature on the Pathogenicity of Eight Isolates of each of Four Genera of Fungi on Peanut Seedlings.

TABLE VI

PATHOGENICITY RATINGS OF ISOLATES F-6, R-2, P-7 AND S-4 OF *FUSARIUM* SP., *RHIZOCTONIA* SP., *PYTHIUM* SP. AND *S. ROLFSII* RESPECTIVELY ACCORDING TO DISEASE DEVELOPMENT ON HARVESTED PLANTS WITH ATTACHED PODS INCUBATED AT 28 C FOR FOUR DAYS[1]

Treatment	Isolate Number				
	F-6	R-2	P-7	S-4	CK
Surface disinfected	2.4	2.1	2.6	2.7	2.0
Surface non-disinfected	2.6	2.6	2.7	3.2	2.2
Mean	2.5	2.3	2.6	2.9	2.1

[1]Mean severity of disease development of four peanut plants, each with four pods attached and rated according to the following severity index:
1 = 0% rotted
2 = 1-25% rotted
3 = 26-50% rotted
4 = 51-75% rotted
5 = 26-100% rotted

TABLE VII

PATHOGENICITY RATINGS OF ISOLATES F-6, R-2, P-7 AND S-4 OF *FUSARIUM* SP., *RHIZOCTONIA* SP., *PYTHIUM* SP. AND S. *ROLFSII* RESPECTIVELY AND A COMBINATION OF ALL FOUR ACCORDING TO DISEASE DEVELOPMENT ON NEWLY HARVESTED, MATURE DETACHED PODS[1]

Temperature of Incubation	Isolate Number					
	F-6	R-2	P-7	S-4	Combination[2]	CK
High	2.8	3.0	3.1	2.9	3.4	2.0
Moderate	3.0	2.8	2.9	2.9	3.1	2.0
Low	2.7	2.7	2.9	2.5	2.8	1.6
Mean	2.9	2.8	3.0	2.8	3.1	1.8

[1]Each value is the mean severity of disease development of 99 pods according to the following severity index:
1 = 0% rotted
2 = 1-25% rotted
3 = 26-50% rotted
4 = 51-75% rotted
5 = 76-100% rotted

[2]The combination of isolates of the four genera consisted of one part S. *rolfsii* to 1.3 for each of the other three fungi.

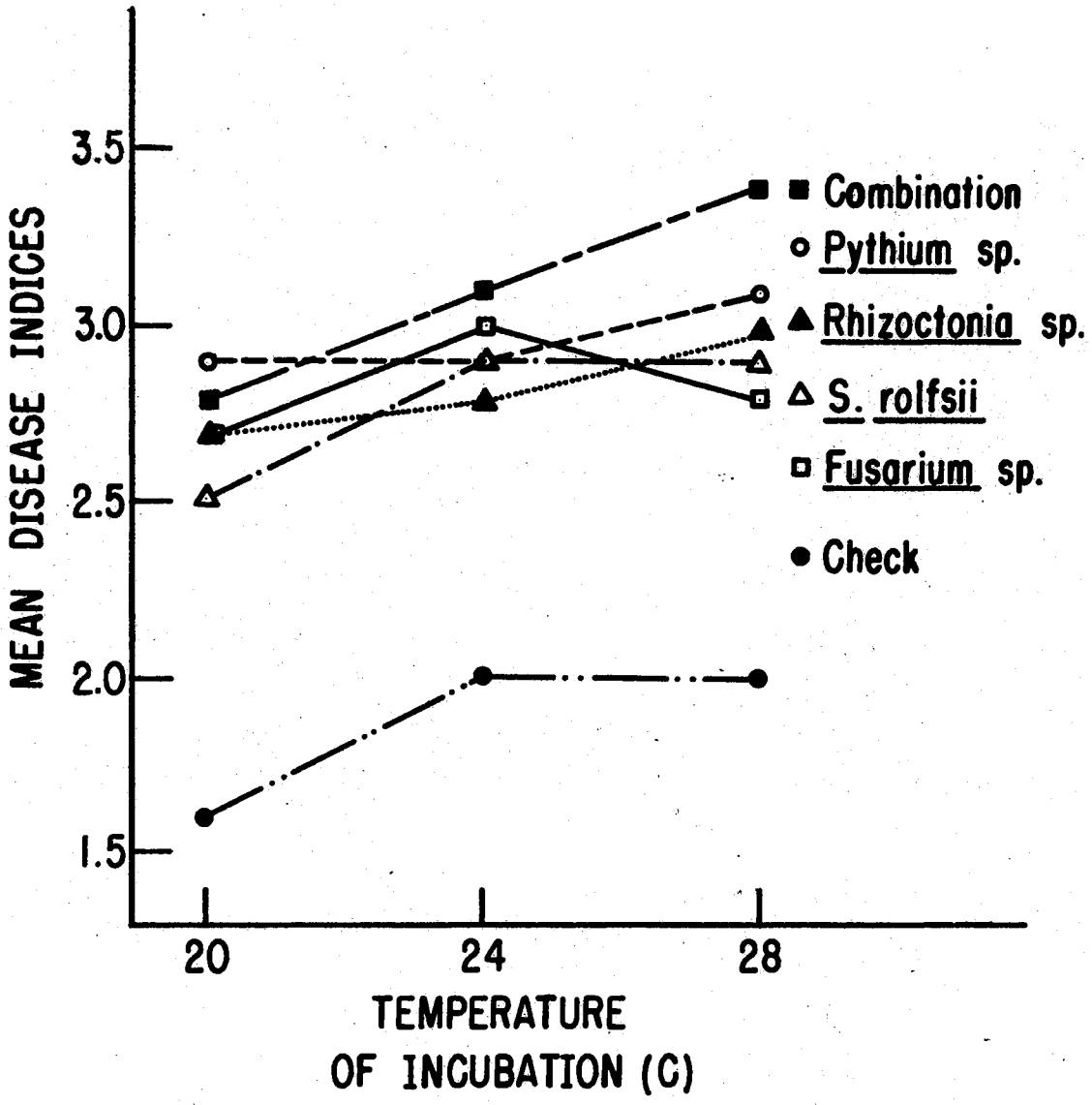


Figure 4. Effects of Temperature on the Pathogenicity of Fusarium sp., Rhizoctonia sp., Pythium sp., and S. rolfsii and on the Combination Treatment of All Four on Detached Mature Peanut Pods.

growth chamber) (see Figure 5). However, out of 16 pots in both temperature regimes inoculated with S. rolfsii, three plants were dead. Another plant was dead in the combination of four fungi at T_2 temperature. Roots of the plants with infected pods appeared healthy, and controls appeared healthy.

However, all plants grown in soil infested with each of the four fungi and the combination developed rotted pods. The average disease ratings are shown in Table VIII. The lesions on rotted pods induced by these four fungi and the combination were similar, and were light brown or dark brown to black in color. Some decaying pods were empty. Pods with extensive dark, soft and water soaked areas also occurred (Figures 6, 7 and 8). The control plants on the other hand, mostly produced healthy pods. However, some became diseased because treatment with the aseptic corn meal-sand medium ultimately favored contamination in such a long range test.

Although pod symptoms at the two temperatures were similar, plants grown at the warm temperature (T_1) were much larger. The average heights in T_1 and T_2 were 73.4 and 42.9 cm, respectively.

Results of statistical analysis of the Artificially Infested Soil Test are as follows:

(a) Effect of interaction: Because the results in Table XV indicated interaction between Temperature x Pathogen (TP) and Inoculum rate x Pathogen (IP), these interactions were further analyzed by Duncan's Multiple Range Tests and are shown in Table IX to indicate these differences. These differences are also exhibited in Figures 9 and 10.

(b) Effect of temperature level: At the 1% level of confidence,

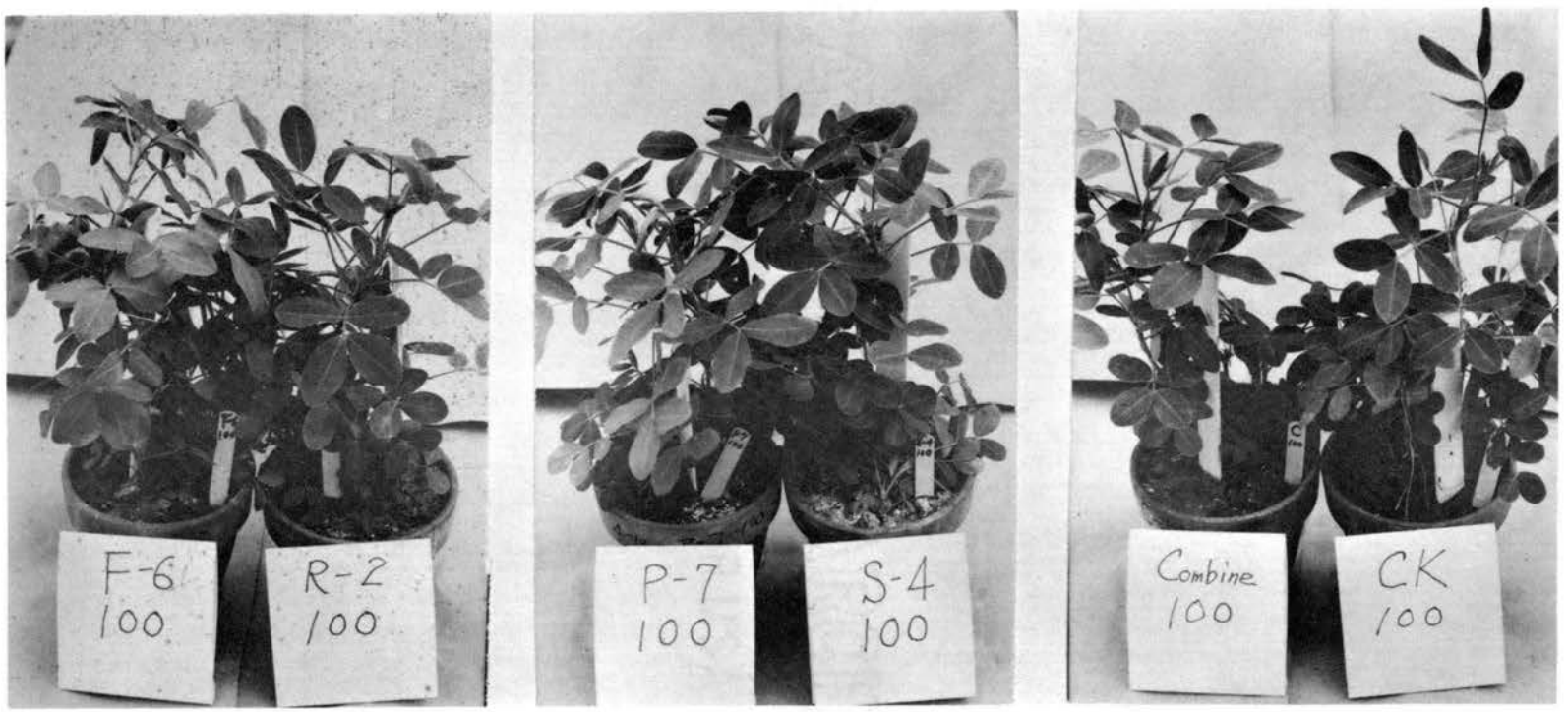


Figure 5. Growth of Peanut Plants in Sterilized Soil Infested Separately with 100 g of Fusarium sp. (F-6), Rhizoctonia sp. (R-2), Pythium sp. (P-7), S. rolfsii (S-4), and a Combination of all Four. Check (CK) was treated with a sterile medium. Plants were maintained in a greenhouse.

TABLE VIII

AVERAGE MEAN DISEASE RATINGS FOR POD ROT PRODUCED IN ARTIFICIALLY INFESTED SOILS UNDER DIFFERENT CONDITIONS[1]

Pathogens	Amount of Inoculum[2]	Location	
		Greenhouse[3]	Growth Chamber[4]
Fusarium sp.	Low	1.4	2.9
	High	1.6	3.0
Rhizoctonia sp.	Low	1.4	2.4
	High	1.8	2.9
Pythium sp.	Low	1.7	3.1
	High	1.7	2.6
S. rolfsii	Low	2.1	2.8
	High	2.9	3.8
Combination[5]	Low	1.4	3.1
	High	1.7	3.2
Check[6]	Low	1.1	1.4
	High	1.2	2.2

[1]Each value is the average of four pots according to the following severity index: 1 = 0% rotted; 2 = 1-25% rotted; 3 = 26-50% rotted; 4 = 51-75% rotted; 5 = 76-100% rotted

[2]Low amount of inoculum was 50 g. per pot; high amount was 100 g per pot.

[3]27-31 C day and 23-27 C night

[4]22-24 C day and 20-22 C night

[5]The combination of isolates of the four genera consisted of one part *S. rolfsii* to 1.3 for each of the other three fungi.

[6]Checks were inoculated with aseptic corn meal-sand medium.

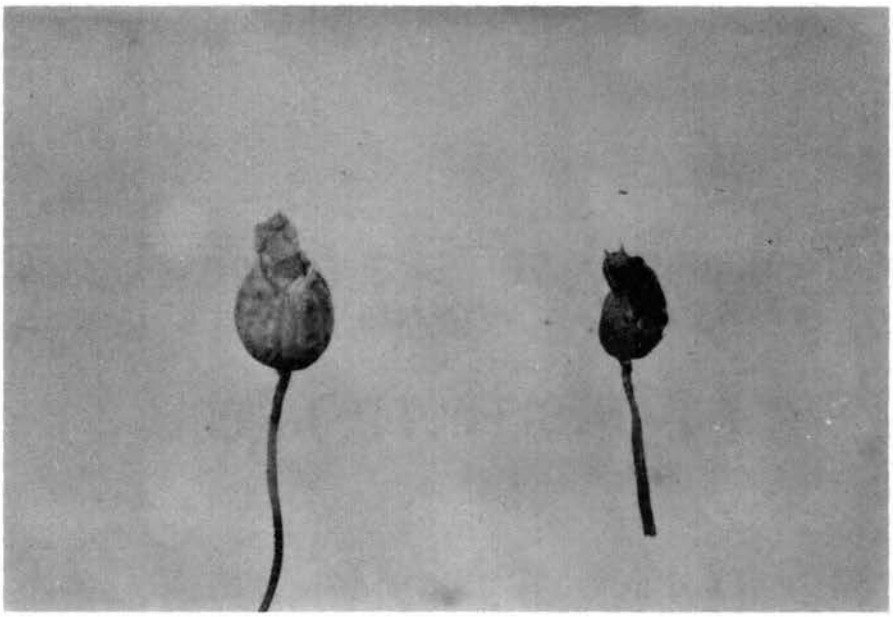


Figure 6. Peanuts from the Artificially Infested Soil Test in 1968 Showing (Left) a Healthy Pod Broken Open, and (Right) a Decaying Empty Pod.

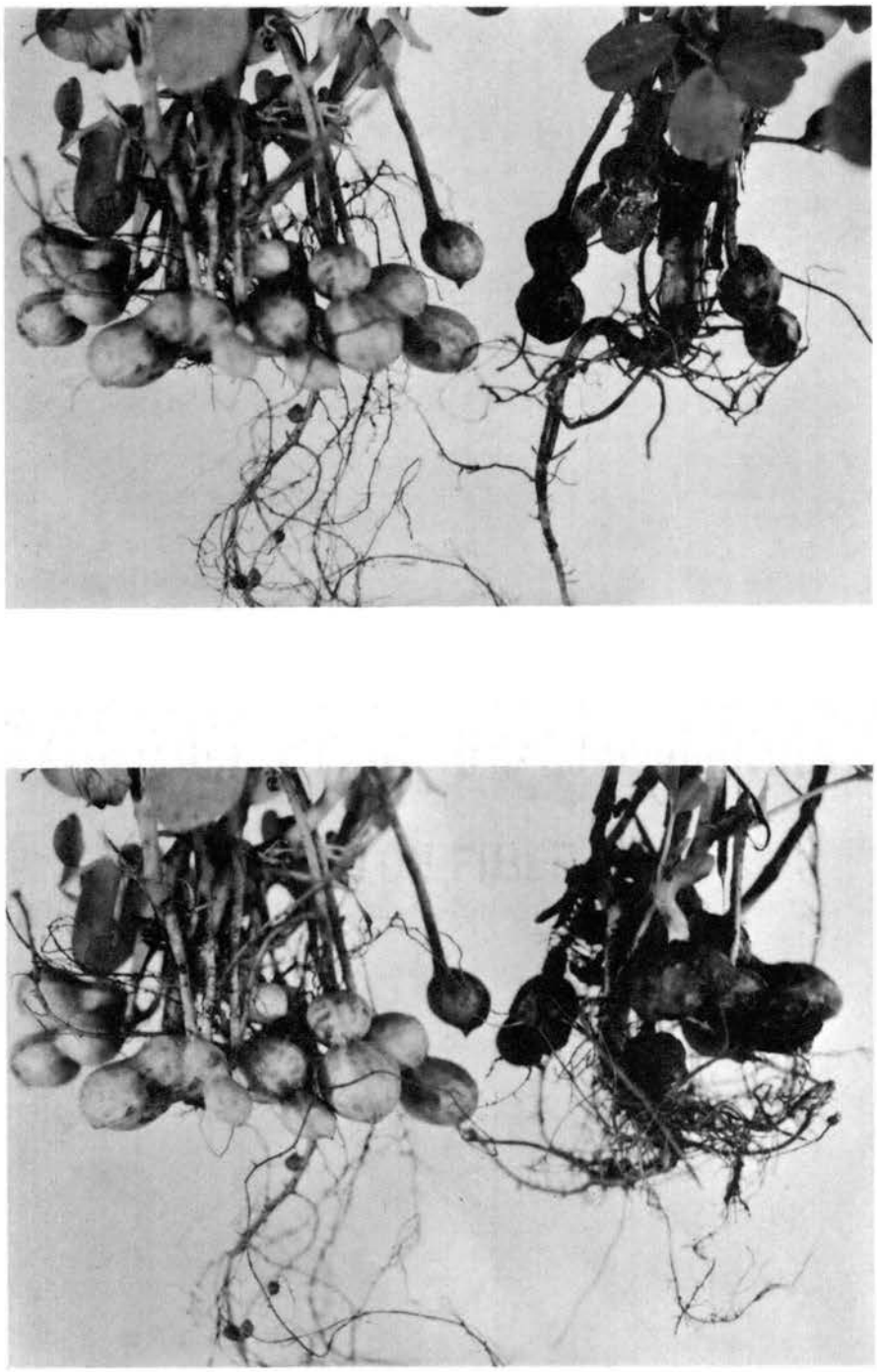


Figure 7. Peanut Pod Rot Produced with a Pythium sp. (Above, Right) and with S. rolfsii (Below, Right). Healthy Controls Shown on the Left.

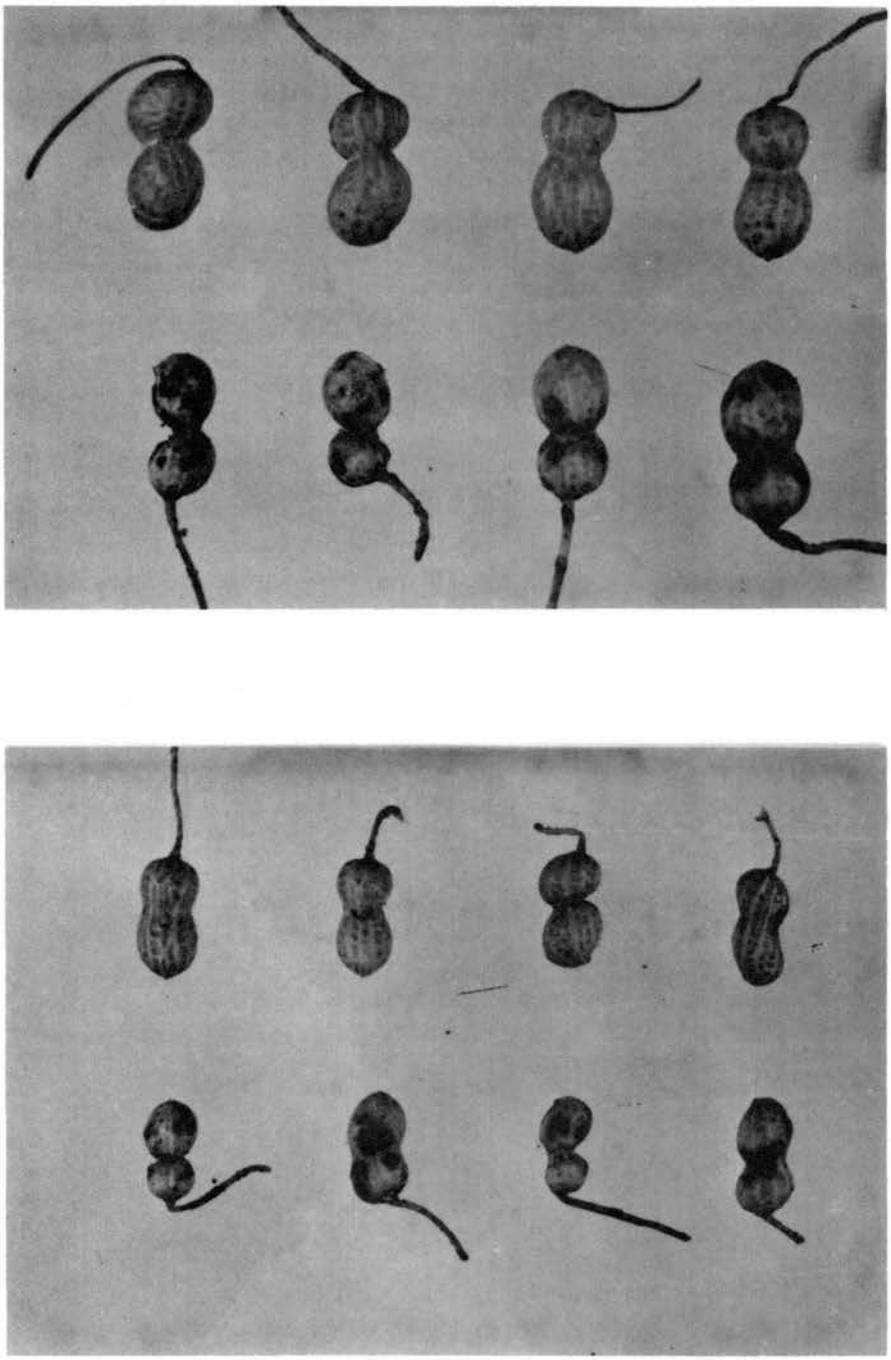


Figure 8. Effect on Peanut Pods of Inoculation with S. rolfsii in Sterilized Soil at Two Temperatures. (Above) at T_1. (Below) at T_2: Top Row, Normal Pods; Bottom Row, Infected Pods.

TABLE IX

DUNCAN'S MULTIPLE RANGE TEST FOR THE INTERACTION OF TEMPERATURE x PATHOGEN AND INOCULUM RATE x PATHOGEN OF DIFFERENT FUNGI ON DEVELOPMENT OF PEANUT POD ROT AT 0.05 LEVEL[1]

	Temperature[2]		Inoculum[3]	
	Low(T_2)	High(T_1)	Low	High
Fusarium sp.	2.98[a]	1.53[g]	2.18[k]	2.33[p]
Rhizoctonia sp.	2.64[b]	1.57[g]	1.90[l]	2.32[p]
Pythium sp.	2.81[c]	1.69[h]	2.36[m]	2.14[q]
S. rolfsii	3.32[d]	2.53[i]	2.46[n]	3.39[r]
Combination[4]	3.16[e]	1.59[g]	2.28[m]	2.47[s]
Check[5]	1.84[f]	1.21[j]	1.31[o]	1.73[t]

[1]Within each column means having the same superscript letters are not significantly different

[2]Temperature: Low = 22-24 C day and 20-22 C night (T_2). High = 27-31 C day and 23-27 C night (T_1)

[3]Inoculum: Low = 50 g. High = 100 g.

[4]The combination of isolates of the four genera consisted of one part *S. rolfsii* to 1.3 for each of the other three fungi.

[5]Checks were inoculated with aseptic corn meal-sand medium

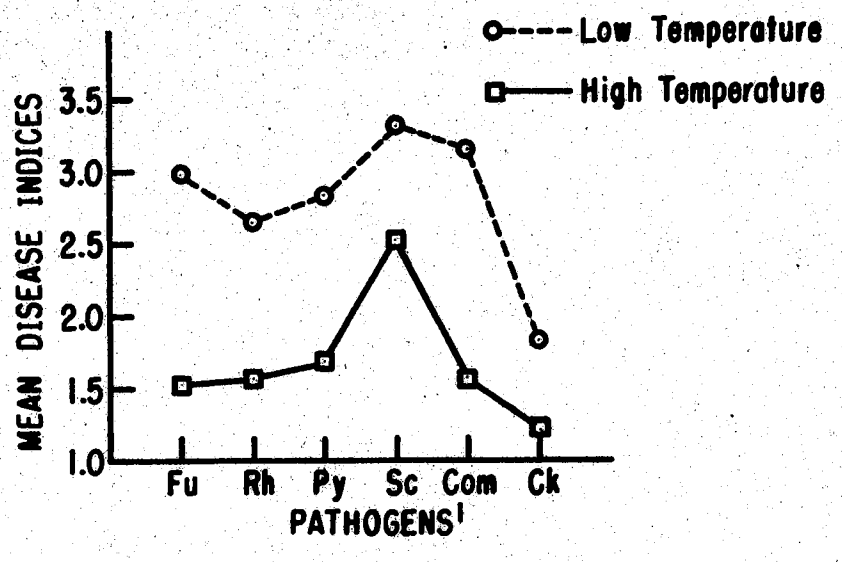


Figure 9. Interaction of Temperature and Pathogens on Peanut Pod Rot in Artificially Infested Soil.

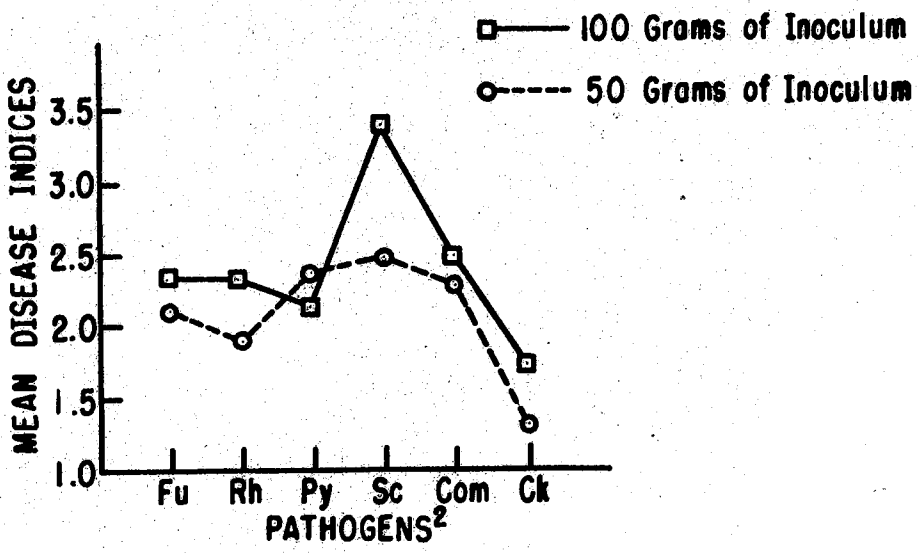


Figure 10. Interaction of Amount of Inoculum and Pathogens on Peanut Pod Rot in Artificially Infested Soil.

[1&2]Abbreviations: Fu, Fusarium sp.; Rh, Rhizoctonia sp.; Py, Pythium sp.; Sc, S. rolfsii; Com, combination of all four fungi at a ratio of Sc:Fr:Rh:Py = 1.0:1.3:1.3:1.3; CK, uninoculated control.

a statistically significant difference in peanut pod rot development was observed between the two temperature regimes (Appendix Table XV). The disease was more severe in the lower temperature regime than in the higher temperature regime (Table IX).

(c) Effect of inoculum levels: At the 1% level of confidence, a statistically significant increase in peanut pod rot was observed between the two inoculum levels (Appendix Table XV). The more the inoculum the more severe was the disease development (Table IX).

(d) Effect of different fungi: At a 1% level of confidence, a statistically significant difference in peanut pod rot development was observed among the four fungi Fusarium, Rhizoctonia, Pythium, S. rolfsii, their combination and the control (Appendix Table XV). Duncan's Multiple Range Test was used to indicate where such differences might be. Results are shown in Table X.

The results of Duncan's Multiple Range Test showed a significant difference between the uninoculated control (CK) and S. rolfsii but no differences among the other fungal treatments.

Results of the Seedling Pathogenicity Test, Peanut Pod Pathogenicity Test and pod rot development in the Artificially Infested Soil Test are summarized in Figure 11.

Naturally Infested Soil Test

The peanut plants were very well developed at the high temperature regime of the greenhouse (27-31 C day and 23-27 C night), moderately developed at the temperature regime of a growth chamber (22-24 C day and 20-22 C night), and the low temperature regime of the greenhouse (26-32 C day and 20-26 C night), but badly stunted at the low

TABLE X

DUNCAN'S MULTIPLE RANGE TEST SHOWING THE INFLUENCE OF DIFFERENT FUNGI ON DEVELOPMENT OF PEANUT POD ROT IN ARTIFICIALLY INFESTED SOIL

Fungus[1]	CK	Rh	Py	Fu	Com	Sc
	1.52	2.11	2.25	2.26	2.38	2.93
Mean disease indices[2]						

[1]Abbreviations: CK, uninoculated control; Rh, Rhizoctonia sp.; Py, Pythium sp.; Fu, Fusarium sp.; Com, combination of all four fungi at a ratio of Sc.:Fu.:Py.:Rh. = 1.0:1.3:1.3:1.3; Sc, S. rolfsii.

[2]Underscoring for nonsignificance

Figure 11. Pathogenicity of Four Isolates of the Four Genera of Fungi, S. rolfsii, Pythium sp., Rhizoctonia sp., and Fusarium sp., and a Combination of the Four Summarized According to the Various Parts of the Host Affected by Artificial Inoculation.

[1]Abbreviations; Sc., S. rolfsii, Py., Pythium sp.; Fu., Fusarium sp., Rhizoctonia sp., Com., Combination of all four fungi at a ratio of Sc.: Py.: Fu.: Rh. = 1.0: 1.3: 1.3: 1.3.

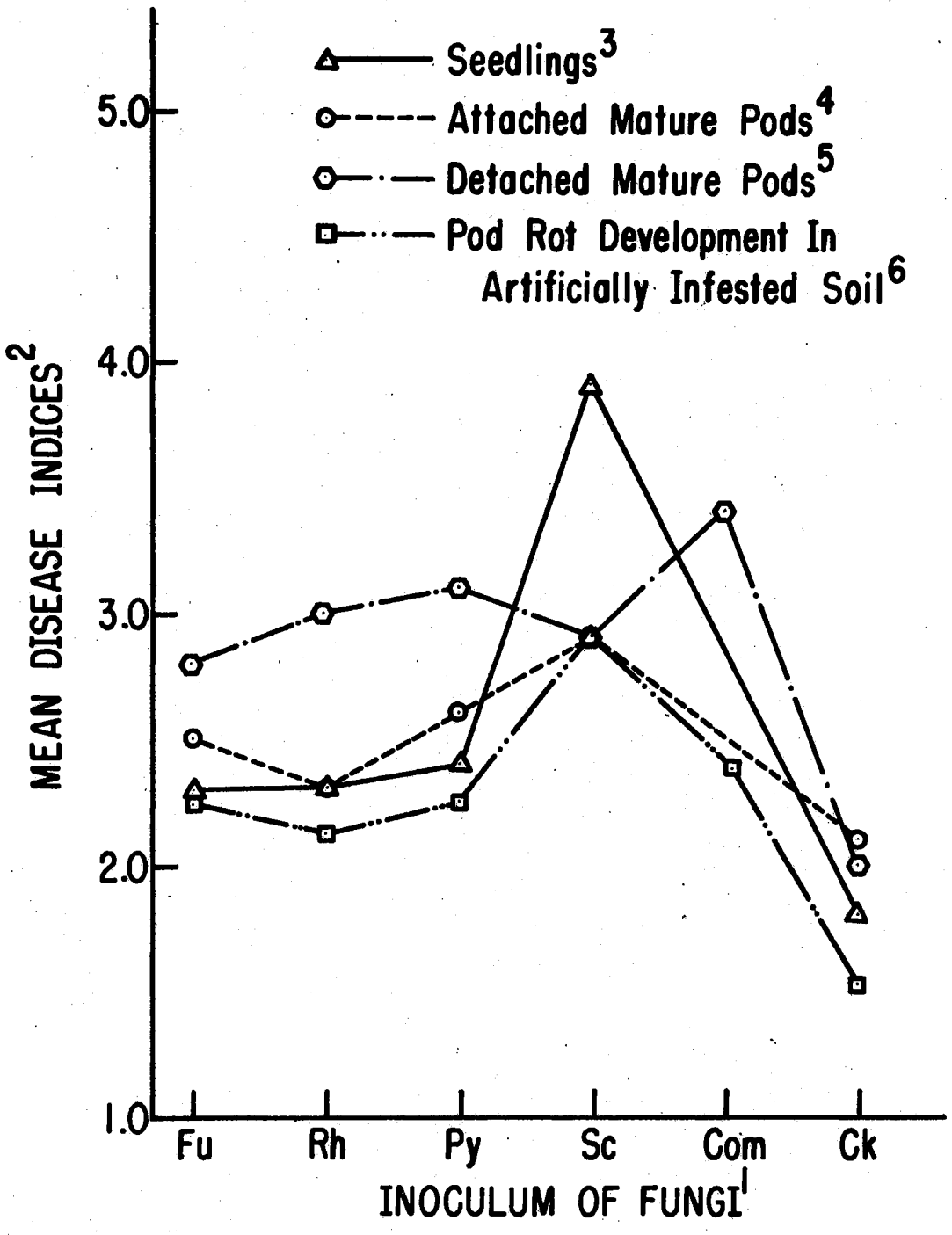
[2]Disease indices at high incubation temperature (28 C).

[3]Each value is the mean severity of disease development of eight isolates, each isolate evaluated on five seedlings at the high incubation temperature (28 C) as shown in Tables II, III, IV and V.

[4]Each value is the mean of the mean severity of disease development of surface disinfected and non surface disinfected treatments as shown in Table VI.

[5]Each value is the mean severity of disease development of 99 pods at the high incubation temperature as shown in Table VII.

[6]Each value is the mean disease index of 32 peanut plants in the Artificially Infested Soil Test as shown in Table X.


Seedlings[3]
Attached Mature Pods[4]
Detached Mature Pods[5]
Pod Rot Development In Artificially Infested Soil[6]
MEAN DISEASE INDICES[2]
5.0
4.0
3.0
2.0
1.0
Fu
Rh
Py
Sc
Com
Ck
INOCULUM OF FUNGI[1]

temperature regime of a growth chamber (19-24 C day and 15-19 C night). The foliage produced in these four temperature regimes was mostly green and healthy. However, some chlorotic leaves were observed in the latter three regimes. The average heights of plants in these four regimes were 26.0, 12.0, 12.2 and 9.1 cm respectively.

No difference in symptoms of the aerial parts of the plants was observed between the two levels of soil moisture at the high temperature regimes of the greenhouse. However, at the high temperature regime of a growth chamber some yellowing of foliage was found in five heavily watered pots. At the low temperature regime of the greenhouse, all peanut plants had spider mite infestations with most damage occurring in the five heavily watered pots.

There was no visible difference of the aerial parts of the peanuts grown in the two soil types at each temperature. Rotted pods (Figures 12 and 14) and seeds (Figure 13) were found in both soil types.

The average disease ratings are shown in Table XI and the statistical analysis for the Naturally Infested Soil Test is shown in Appendix Table XVI.

The number of plant parasitic nematodes per 100 cc of soil in the Naturally Infested Soil Test was about three and one-half times greater in Willis soil than in Fort Cobb soil (Table XII). A small number of nematodes was found in the sterile soil.

The analysis of pod rot development in naturally infested soil (Appendix Table XVI) showed both soils significantly different from the controls at the 5% level in the greenhouses and significant at 1% level in the growth chambers; and also showed the naturally infested soils of Fort Cobb and Willis significantly different from the sterile soils

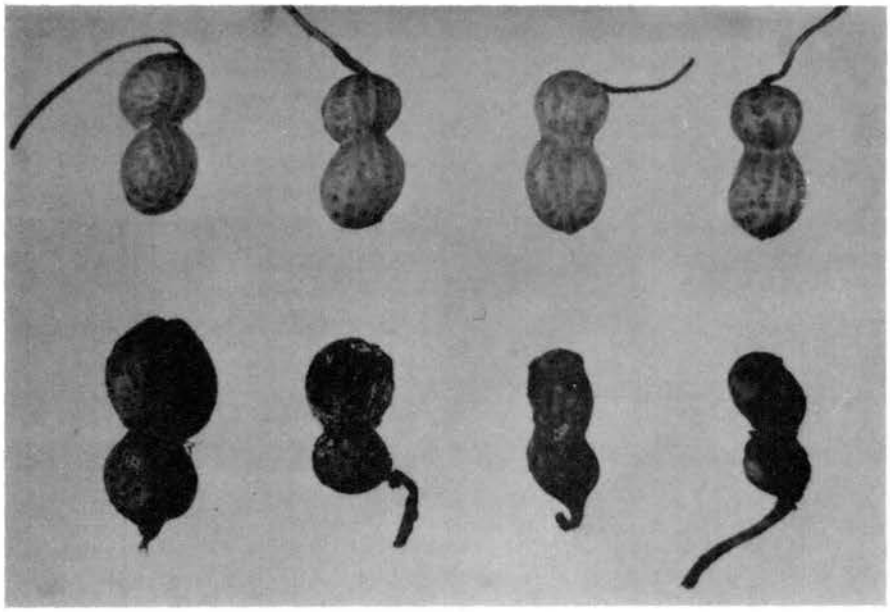


Figure 12. Pods of Starr Peanuts Grown in the Naturally Infested Soil Test. (Above) Healthy, and (Below) Rotted Pods.

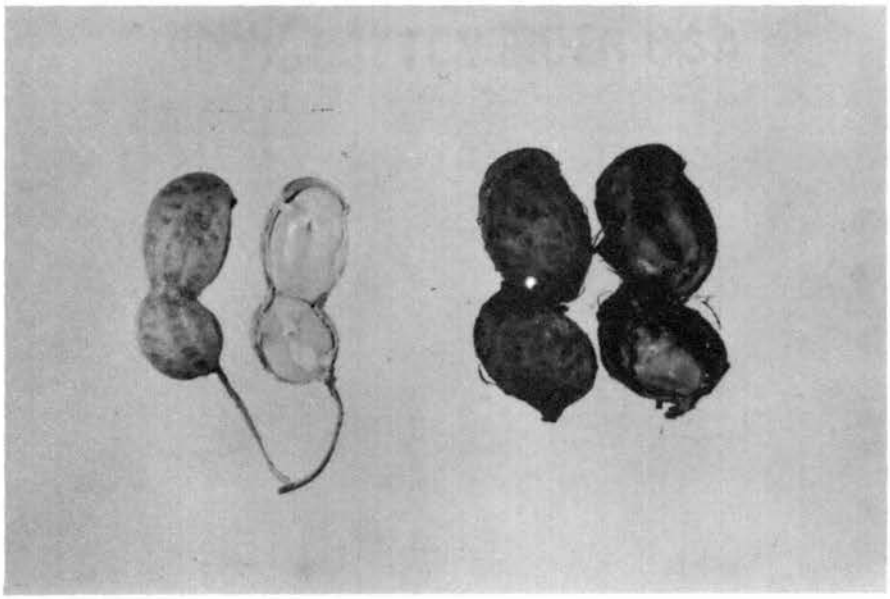


Figure 13. Internal View of Peanut Pods. Left: Healthy. Right: Rotted.

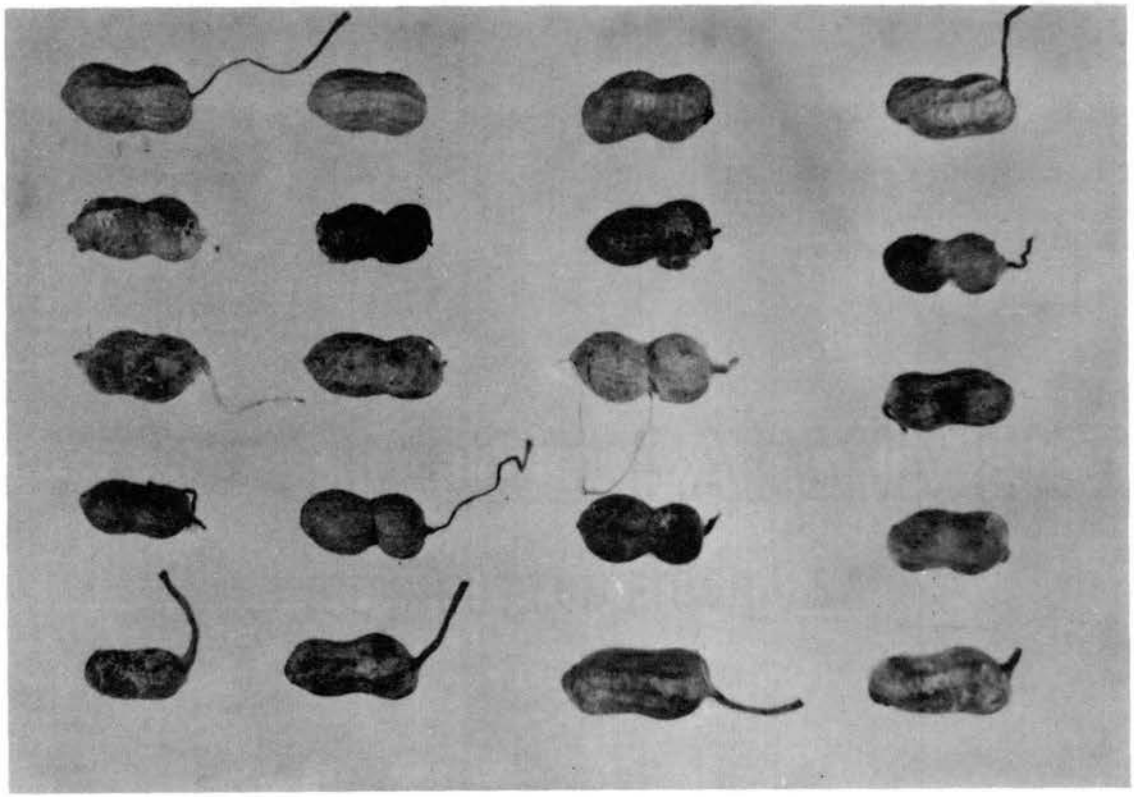


Figure 14: Peanut Pods Showing Various Degrees of Rot from the Naturally Infested Soil Test: Top Row, Normal Pods. Lower Rows, Infected Pods.

TABLE XI

AVERAGE MEAN DISEASE RATINGS FOR POD ROT PRODUCED IN NATURALLY INFESTED SOILS UNDER DIFFERENT CONDITIONS[1]

Watering[2]	Soil Type	Sterile Control and Naturally Infested Soil	Location: Greenhouse Temperature[3] Low(T_3)	Location: Greenhouse Temperature[3] High(T_1)	Location: Growth Chamber Temperature[3] Low(T_4)	Location: Growth Chamber Temperature[3] High(T_2)
Low	Ft. Cobb	Sterile	1.8	2.5	2.6	2.8
Low	Ft. Cobb	Infested	2.7	2.5	2.9	2.9
Low	Willis	Sterile	2.7	2.4	3.2	2.9
Low	Willis	Infested	3.1	2.7	3.4	3.1
High	Ft. Cobb	Sterile	2.4	2.4	2.6	2.5
High	Ft. Cobb	Infested	2.7	2.6	2.9	3.2
High	Willis	Sterile	2.4	2.4	3.4	2.7
High	Willis	Infested	3.1	2.9	3.5	3.6

[1]Each value is the average of five pots according to the following severity index: 1 = 0% rotted; 2 = 1-25% rotted; 3 = 26-50% rotted; 4 = 51-75% rotted; 5 = 76-100% rotted.

[2]Low level of watering was once a day to keep the soil in good moist condition; high level was watered twice a day and double the amount of water in total volume.

[3]Low = (T_3) 26-32 C day and 20-26 C night; High = (T_1) 27-31 C day and 23-27 C night.

[4]Low = (T_4) 19-24 C day and 15-19 C night; high = (T_2) 22-24 C day and 20-22 C night.

TABLE XII

NUMBER OF PLANT PARASITIC NEMATODES PER 100 CC OF SOIL IN THE VARIOUS TREATMENTS OF THE NATURALLY INFESTED SOIL TEST[1]

Temperature[3]	Nematodes	No. of Nematodes Recovered[2] F-S	F-N	W-S	W-N
T_1 (27-31 C D, 23-27 C N)	Criconemoides sp.	6	82	44	636
	Meloidogyne sp.	-	160	6	20
	Tylenchus sp.	-	4	-	2
	Pratylenchus sp.	-	-	-	4
	Total	6	246	50	662
T_2 (22-24 C D, 20-22 C N)	Criconemoides sp.	-	21	14	321
	Meloidogyne sp.	-	12	2	5
	Tylenchus sp.	-	1	1	6
	Pratylenchus sp.	-	-	-	-
	Total	0	34	17	332
T_3 (26-32 C D, 20-26 C N)	Criconemoides sp.	-	2	8	154
	Meloidogyne sp.	-	68	-	6
	Tylenchus sp.	-	1	-	4
	Pratylenchus sp.	-	-	-	7
	Total	0	71	8	171
T_4 (19-24 C D, 15-19 C N)	Criconemoides sp.	-	-	4	14
	Meloidogyne sp.	-	-	-	9
	Tylenchus sp.	1	2	1	1
	Pratylenchus sp.	-	-	-	-
	Total	1	2	5	24
	Main Total	7	353	80	1189

[1]Nematodes were identified by C. G. Goseco, nematologist, former graduate student of Department of Botany and Plant Pathology, Oklahoma State University

[2]Abbreviations: F-S, Fort Cobb soil sterilized; F-N, Fort Cobb soil non sterilized; W-S Willis soil sterilized; W-N, Willis soil non sterilized.

[3]Abbreviations: C D, Day temperature in centigrade; C N, Night temperature in centigrade.

(control) at the 1% level in greenhouse, and significant at 5% level in the growth chamber.

The average disease ratings of two soil types of Fort Cobb and Willis are shown in Table XIII, which indicated that the Willis soil gave higher disease ratings in both the greenhouse and growth chambers.

Data in Table XIV showing the sterile soil (control) gave lower ratings in both places of greenhouse and growth chamber.

TABLE XIII

PATHOGENICITY RATINGS BASED ON POD ROT DEVELOPMENT IN NATURALLY INFESTED FORT COBB AND WILLIS SOIL IN TWO LOCATIONS[1]

Locations	Mean Disease Indices of Pod Rot	
	Soil Types	
	Fort Cobb	Willis
Greenhouse	2.6	2.9
Chamber	3.0	3.4
Average	2.8	3.2

[1]Each value is the mean severity of disease development according to the following severity indices: 1 = 0% rotted; 2 = 1-25% rotted; 3 = 26-50% rotted; 4 = 51-75% rotted; 5 = 76-100% rotted.

TABLE XIV

PATHOGENICITY RATINGS BASED ON POD ROT DEVELOPMENT IN STERILE (CONTROL) AND NON STERILE SOILS (NATURALLY INFESTED) FOR FORT COBB AND WILLIS SOIL IN TWO LOCATIONS

	Mean disease indices of pod rot	
	Soil Treatments	
Locations	Sterile (Control)	Non sterile (Naturally Infested)
Greenhouse	2.4	2.8
Chamber	2.8	3.2
Average	2.6	3.0

[1]Each value is the mean severity of disease development according to the following severity indices: 1=0% rotted; 2=1-25% rotted; 3=26-50% rotted; 4=51-75% rotted; 5=76-100% rotted

CHAPTER V

DISCUSSION

Results of studies of fungi associated with peanut pods in Oklahoma showed that Fusarium spp., Rhizoctonia spp., Pythium spp. and S. rolfsii were isolated with a frequency of 31, 15, 13 and 4% respectively. Since these four genera of fungi constituted 63% of all isolates from mature discolored pods, it was possible they caused peanut pod rot either separately or in combination.

In this study 8% of the total isolates from mature discolored pods were pathogenic nematodes. This supports references that in peanut pod rot development, nematodes may play a relatively important role (17, 39, 43).

The microfloras in the rhizosphere of peanut seedlings were similar to those in the geocarposphere of peanut pods. Differing primarily in that the number of nematodes in the latter were four times greater than the former; and Fusarium spp., Rhizoctonia spp., Pythium spp. and S. rolfsii were isolated somewhat less frequently from seedlings than from pods.

The effect of temperature on the pathogenicity of the four genera of fungi on peanut seedlings shown in Tables II, III, IV and V are summarized in Figure 3. This figure shows the highest mean disease index for Fusarium spp. and Pythium spp. at the moderate incubation temperature (24 C) while the highest mean disease index for

Rhizoctonia spp. and S. rolfsii occurred at the high incubation temperature.

In these seedling tests the total mean disease indices showed Sclerotium to be the most pathogenic of the four fungal genera. The other genera in decreasing order of pathogenicity were Pythium, Fusarium and Rhizoctonia. The same order of pathogenicity in reproducing pod rot is shown in Table VI and somewhat similar order in Table VII. These results confirmed the validity of using seedlings for initial pathogenicity evaluations in order to select the most pathogenic isolate from a total of eight isolates for each genus. The most pathogenic isolates which were used for further tests would probably be considered as moderately pathogenic. More pathogenic isolates could probably have been selected had the initial evaluations involved more than the 32 isolates used in this study. However, as previously shown, the severity of pathogenicity varied with the pathogen and the incubation temperatures used. Therefore, the presentation of total disease indices as an average from two or more temperatures necessarily indicates less pathogenicity than could be shown for a pathogen at the optimum temperature for maximum disease development. Despite this limitation, the use of total disease indices does reflect a valid rating for the comparison in these tests. Also, in tests with soil fungi which extend over long periods of time, disease ratings for the experimental controls frequently are higher than desired because some contamination of the control is inevitable. As a result, statistical analyses may fail to show significance because data could not be obtained before some contamination of the controls occurred.

The disease of peanut caused by S. rolfsii is commonly called stem

rot, and stem infection is usually the most frequent and serious manifestation of this disease (30). However, S. rolfsii showed the highest total mean disease index in the Seedling Pathogenicity Test, the attached pods part of the Peanut Pod Pathogenicity Test and pod rot development in the Artificially Infested Soil Test (Figure 11). Conversely, S. rolfsii showed next to the lowest total mean disease index on detached mature pods. This may suggest the presence of a factor in the mature detached pods that acts as a natural resistance barrier to the fungus of S. rolfsii.

Ashworth et al. (1) reported stems and pegs of peanut were more frequently attacked than pods or kernels. This might be the reason why S. rolfsii showed the lower total mean disease index in the detached mature pods parts of the Peanut Pod Pathogenicity Test, and the highest total mean disease index in the other three tests.

Results in the detached pods method showed that the combination of all four fungi produced the highest total disease index while Pythium sp. showed the next to the highest rating (Figure 11). Frank (12, 14) reported that Pythium spp. were essential to the development of peanut pod rot, but they were not the sole causal organisms. He also concluded that peanut pod rot is caused by a pathogenic complex of Pythium spp. and of other organisms. Results in Table VII and Figure 4 support the observation of Frank (14). Garren (19, 20, 21) stated Pythium myriotylum was the prime pod-rot pathogen and Rhizoctonia solani was a sporadically important pod-rot pathogen. Garren's observations were only partially supported in these studies with pathogens occurring in Oklahoma.

Results of the studies of pathogenicity and pod rot development

in the Artificially Infested Soil Test are also summarized in Figure 11. This figure indicates that S. rolfsii showed its highest total mean disease index whenever the stem of the peanut plant was involved as in the Seedling Pathogenicity Test, the attached pods part of the Peanut Pod Pathogenicity Test and pod rot development in the Artificially Infested Soil Test, while combination of all four fungi showed its highest mean disease index in the detached mature pods part of the Peanut Pod Pathogenicity Test. However, Pythium sp. showed the next to the highest rating in the Seedling Pathogenicity Test, attached and detached pods part of the Peanut Pod Pathogenicity Test and showed the fourth highest rating in pod rot development in the Artificially Infested Soil Test. Fusarium sp. showed the third highest rating in pod rot development in the Artificially Infested Soil Test, the Seedling Pathogenicity Test, attached pods part of the Pod Pathogenicity Test, and showed the lowest rating in detached pods part of the Pod Pathogenicity Test. Rhizoctonia sp. showed the third highest rating in the detached pods part of the Pod Pathogenicity Test; and showed the lowest rating in the Seedling Pathogenicity Test, attached pods part of Pod Pathogenicity Test and pod rot development in the Artificially Infested Soil Test.

No symptoms of disease were visible on aerial parts or roots of peanut plants at different temperatures or in various soil types or different moistures. Similar observations were also reported by Frank (14).

The effects of temperature varied greatly and interacted with other factors. Garrett (25) stated that temperature affects antagonistic organisms as well as pathogens. Temperature also affects

biochemical composition of the host.

Temperature not only affected growth of both the pathogen and the host but also affected subsequent disease development. Further, results from the Artificially Infested Soil Test, summarized in Figure 9, indicated that the disease was more severe at the lower temperature than at the higher level. The same was true for the tests with naturally infested soils from Fort Cobb and Willis, in spite of the fact that the statistical analysis of these tests showed only slight interaction with temperature and was not of significant proportions.

Results in Appendix Table XV showed the important role of temperature in peanut pod rot development because a statistical difference at the 1% level was found between the two temperature regimes. The following figures obtained from the raw data for Appendix Table XV showed that lower temperature provided favorable conditions for disease development resulting in greater mean disease indices than at the higher temperature:

Temperature regime	Mean disease index
27-31 C day and 23-27 C night	1.7
22-24 C day and 20-22 C night	2.8

The amount of inoculum was also of importance in the artificially infested soils. The higher amount of inoculum gave higher responses for all pathogens except Pythium sp. This is shown in Figure 10 and the analysis (Appendix Table XV) showed disease development at the 100 g level to be highly significant over the lower level.

The mean disease indices of inoculum levels were as follows:

Inoculum level	Mean disease index
50 g	2.1
100 g	2.4

The mean disease indices of soil moisture levels of the Naturally Infested Soil Test were as follows:

Soil moisture level	Mean disease index
Low	2.7
High	2.8

It was apparent that the soil moisture levels affected the plant growth more than disease development in peanut plants. No statistical difference in disease indices was found between the low and high soil moisture levels. However, visible observation indicated poorer plant growth in the latter than in the former. Frank (12), Vaartaja (47) and Wright (49) reported that the tendency of increased disease with increased moisture was marked in certain cases; especially with the virulent natural soil flora, mainly Pythium spp. and with heavy inoculum of Phytophthora cactorum. Rhizoctonia spp. are less exacting and are reported to be favored sometimes by high moisture and sometimes by low, perhaps because their strains vary in water and oxygen requirement (9, 10, 31).

Effect of different fungi (Appendix Table XV) was highly significant in pod rot development. Further statistical analysis showed that each of the four fungi, Fusarium sp., Rhizoctonia sp., Pythium sp., S. Rolfsii and their combination were significantly different from the uninoculated control (Table X).

Vaartaja et al. (47) pointed out different soils, other than sterilized ones, were associated with slight differences in disease.

In the present study, results (Appendix Table XVI) also indicated that the flora associated with different types of soil could cause various degrees of severity of peanut pod rot in both the greenhouse and the growth chamber.

It was seen (Table XIII) in the Naturally Infested Soil Test that there appeared to be a larger difference between the disease indices when plants were grown in the growth chamber than when grown in the greenhouse. Since the temperatures were not the same in the greenhouse as in the growth chamber, it is difficult to say whether the temperature or the location caused this effect. One could conclude that these effects were due to the differences in temperatures. Since the lower temperatures tended to give slightly higher responses in both places.

Data in Table XIV shows the flora coming into the sterile soil (control) gave lower disease ratings than the flora of the naturally infested non-sterile soil in both the greenhouse and growth chamber. These results confirmed the assumptions that the naturally infested soil of Fort Cobb and Willis both contained significant amounts of causal organisms for peanut pod rot.

The analysis for naturally infested soils (Appendix Table XVI) showed no significant effects due to watering or temperature and there appeared to be very little interaction between watering, types of soil, temperature or soil sterilization.

From these observations it was concluded that low temperatures (19-24 C day and 15-19 C night) favored pod rot development in these tests also, and that naturally infested Willis soil produced a greater disease severity than Fort Cobb soil.

Data in Table XII showing the number of plant parasitic nematodes

per 100 cc of soil in the Naturally Infested Soil Test. The number of plant parasitic nematodes in the non-sterile soil of Willis was about three and one-half times as much as in Fort Cobb soil, thus showing the variation in these pathogen propagules in the two different soils.

Nematodes were important in peanut pod rot development as shown in Table XII where more nematodes in the soil were associated with higher mean disease indices; however, nematodes were not essential for pod rot development.

Results and observations in the present study support these conclusions:

(1) Peanut seedlings are satisfactory for initial pathogenicity determination of certain peanut pathogens because many of the same fungi attack both seedlings and pods, and because seedlings can be produced in fewer days. Thus, seedling tests can be repeated under controlled conditions as needed.

(2) Newly harvested detached mature pods are satisfactory for pod rot reproduction in the laboratory or greenhouse and possibly could be used for varietal evaluations for resistance. Once mature pods are harvested, varietal testing under controlled conditions could be repeated as frequently as needed but pod production requires at least 90 days.

(3) Peanut plants with attached mature pods, when inoculated, more nearly simulate natural disease development in the field. But this method requires more labor and is subject to more possibility of contamination with other organisms since more plant parts are involved. It was rather difficult to run the test under controlled conditions.

(4) Results of the pathogenicity tests as well as field

observation have confirmed the fact that S. rolfsii causes more severe pod rot than the other three genera of fungi. Hence, less S. rolfsii was used in the combination inoculum than with the other three genera.

(5) Isolates F-6, R-2, P-7 and S-4 which were the most pathogenic in the Seedling Pathogenicity Test were chosen only from eight isolates of the four genera of fungi. More pathogenic isolates might be found if more isolates were tested, but a total of 32 isolates was the maximum number that could be used in this study.

(6) The possibility of contamination and the coarseness of the scale grade could have contributed to some fairly high check values. PDA was used in part of the controls and labeled Check II. As a result, Check II had higher disease ratings than Check I (CK_1 without PDA) in all three incubation temperatures and caused higher check values than desired. Reisolation from Check II for identification of the causal organism(s) was not carried out because seedlings were badly rotted. In addition, some controls were inoculated with a sterile corn meal-sand medium which also contributed to higher disease severity ratings in these treatments than might otherwise have occurred. A scale grade of 1 to 50 rather than 1.0, 2.0, 3.0, 4.0, 5.0 would have given better separation of disease severity and provided more accurate rating of the amount of disease in the control. Such a rating would have reduced the severity in the controls also.

CHAPTER VI

SUMMARY

Results of experiments conducted to study the soil fungi associated with peanuts in Oklahoma, and pod rot disease development in the laboratory and greenhouse indicated the following:

(1) Of several fungi found associated with rotted peanut pods Fusarium, Rhizoctonia, Pythium and S. rolfsii were predominant and were demonstrated to cause symptoms of the disease when inoculated either singly or in combination. These four fungi constituted 63% of the total isolates obtained from mature diseased pods.

(2) A seedling pathogenicity test was developed to detect the most pathogenic isolates of the four genera above. These isolates were studied in peanut pod pathogenicity tests with newly harvested detached, mature pods and peanut plants with attached mature pods. The same isolates were used to reproduce pod rot in artificially infested soils. Studies were also conducted with naturally infested soil which contained in addition to fungi, pathogenic nematodes. S. rolfsii was the most pathogenic of the four causal organisms tested by artificial inoculations in producing seedling stem infections and rotted pods which remained attached to parent plants. S. rolfsii was also the most pathogenic fungus in producing pod rot in artificially infested soils, but the combination of four fungi produced the greatest severity of pod rot when mature detached pods were artificially

inoculated. The combination of four fungi was second in pathogenicity in producing pod rot in artificially infested soils. Pythium spp. ranked second in pathogenicity on seedlings and attached and detached pods.

(3) In seedling infection Fusarium spp. and Pythium spp. were most pathogenic when incubated at moderate temperature (24 C), while Rhizoctonia spp. and S. rolfsii were most pathogenic at high temperature (28 C). In pod rot development of mature detached pods, the combination of four fungi S. rolfsii, Pythium sp. and Rhizoctonia sp. were most pathogenic at 28 C, but Fusarium sp. was favored by the moderate temperature (24 C). In artificially infested soil, the combination of four fungi S. rolfsii, Pythium sp., Rhizoctonia sp. and Fusarium sp. was most pathogenic at the low temperature range of 22-24 C day and 20-22 C night. In naturally infested soils and artificially infested soils, pod rot was favored by the lower temperatures. The temperature affected growth of both the pathogen and the host and consequently affected the infection by the pathogen and the subsequent disease development.

(4) The microfloras in the rhizosphere of peanut seedlings were similar to those in the geocarposphere of peanut pods differing slightly in that pathogenic nematodes were approximately four times greater than in the former.

(5) No symptoms of fungus infection were visible on aerial parts or roots of peanuts infected with pod rot at different temperature levels, or with various amounts of inoculum, or at two levels of soil moisture, or in two different naturally infested soil types.

(6) Symptom expression on pods caused by the different test

fungi were similar. Generally, they caused the pods to turn brown, or dark brown to black in color. Some decaying pods were necrotic and empty. Pods with advanced infection became very dark, soft and moist.

(7) A high inoculum level (100 g/pod) caused more severe pod rot development than in a low inoculum level (50 g/pot).

(8) Pod rot was significantly different in naturally infested and sterilized control soils from Fort Cobb and Willis both in the greenhouse and growth chamber. These soils contained both pathogenic fungi and nematodes.

(9) Peanut pod rot was more severe when soils contained nematodes, therefore, nematodes are believed to play an important role in disease development possibly by providing points of entrance for primary pathogens or secondary invaders. However, pod rot was reproduced in the absence of nematodes, but reciprocal tests were not made.

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APPENDIX

TABLE XV

ANALYSIS OF VARIANCE FOR PEANUT POD ROT PRODUCED IN THE ARTIFICIALLY INFESTED SOIL TEST

Source of Variation	d.f.	M.S.
Temperature (T)	1	29.35**
Inoculation level (I)	1	2.38**
T x I	1	0.00
Pathogens (P)	5	3.28**
T x P	5	0.54*
I x P	5	0.58**
T x I x P	5	0.19
Pots in (TIP)	72	0.17

*Significantly different at the 5% level

**Significantly different at the 1% level

TABLE XVI

ANALYSIS OF VARIANCE FOR PEANUT POD ROT DEVELOPMENT IN THE NATURALLY INFESTED SOIL TEST

Source of Variation	d.f.	Mean squares for Fort Cobb and Willis soil in Greenhouse	Growth Chamber
Watering (R)	1	0.1170	0.0732
Soil type (A)	1	1.2201*	3.5280**
RA	1	0.1566	0.0192
Sterility (B)	1	3.1047**	2.2512*
RB	1	0.0000	0.4061
AB	1	0.1065	0.0105
RAB	1	0.2354	0.0016
Temperature (C)	1	0.1110	0.2553
RC	1	0.0145	0.0045
AC	1	0.6956	0.7880
RAC	1	0.6336	0.0000
BC	1	0.4651	0.3645
RBC	1	0.1843	0.5916
ABC	1	0.2020	0.1602
RABC	1	0.1620	0.0076
Error	64	0.2107	0.3578
Overall mean		2.58	3.01
C.V.		18%	20%

*Significantly different at the 5% level

**Significantly different at the 1% level

VITA

2

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