REACTION OF PEANUT CULTIVARS TO POD ROT AND THEIR INFLUENCE ON THE MAINTENANCE OF *PYTHIUM* SPP. ON PODS, ROOTS AND IN SOIL

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1984

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 1989

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AND THEIR INFLUENCE ON THE

MAINTENANCE OF PYTHIUM

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ACKNOWLEDGEMENTS

I wish to thank my thesis advisor, Dr. Alexander Filonow, for his guidance and support throughout the course of my master's thesis. His support while I was a student at Oklahoma State University was helpful in enabling me to complete the master's program successfully. I would also like to thank my committee members, Dr. Hassan Melouk, Dr. Ron Sholar and Dr. Wolfgang Schuh for their assistance throughout the course of my research.

I would also like to thank Mr. Ken Jackson for the assistance he provided me from the planting to the harvest of my peanut plots. The many trips to the field with him gave me a nice "short course" into the history of Oklahoma. Thanks are also in order for Phil Gibson, Dave Wiggins, Troy McGill and Bryan Fields for all the laboratory work they assisted me in from assaying soil samples to harvesting peanut plants. I would like to thank Dr. Helen Fagbenele and Tom Clemente for their assistance throughout the course of my research. Financial support by the Oklahoma Peanut Commission to conduct my research is also appreciated.

Finally, I would like to thank my parents and sister, Lori, for their encouragement and support throughout my

graduate studies at Oklahoma State University. Their patience and support was critical in enabling me to reach my professional goals.

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CHAPTER I

INTRODUCTION

Pod rot of peanut (*Arachis hypogaea*, L.) is a soilborne disease found in many peanut growing regions of the United States and abroad (12,16,47). In Oklahoma, 43% of 37 peanut fields sampled in 1983 had pod rot, and disease incidence in these fields was 5.0 - 36.7% (12). Pod rot cost Oklahoma peanut growers about $3.4 million in lost yield in 1985 (A.B. Filonow, personal communication). Symptoms of the disease include various degrees of discoloration, from superficial russetting to complete blackening of the hulls, usually accompanied by various stages of hull and kernel decay (48). The junction between pegs and pods can be weakened by the disease, resulting in substantial loss of pods upon digging.

Pod rot can be caused by one of several fungal pathogens acting alone or in combination. Fungi that have been reported to cause pod rot are *Pythium myriotylum* (13,16), *Rhizoctonia solani* anastomosis group 4(13,48), and *Fusarium solani* (18). Other organisms that have been implicated in the epidemiology of the disease are nematodes (22) mites (50) and the lesser southern corn rootworm (48).

Pythium myriotylum is considered a major cause of pod rot in Oklahoma (13) and elsewhere (48). Pythium irregulare and Pythium aphanidermatum have been reported to be pathogenic to peanut, but little is known of their ability to cause pod rot (48) or of their prevalence in Oklahoma peanut soils.

Control of Pythium pod rot in Oklahoma has proven difficult. The fungicide metalaxyl which is specific for oomyceteous fungi such as Pythium spp. has been used with variable success in reducing pod rot and increasing pod yield (14,27). Although Pythium-active fungicides will likely remain an important component of Pythium pod rot management programs for some time, they would most likely be more effective in combination with peanut cultivars exhibiting less susceptibility to Pythium pod rot. Genotypes derived from parents of spanish market types have been considered less susceptible to pod rot (20,46). Pronto and Spanco are cultivars with spanish type pedigree that are frequently grown in Oklahoma; however the field performance of these cultivars in reducing pod rot has not been very high or consistent (14; Filonow personal communication). Moreover, there is little information as to how Pronto and Spanco compare with other peanut cultivars for Pythium pod rot susceptibility under Oklahoma growing conditions.

Peanut roots (49) and pods (28) leak energy-rich components that are most likely utilized by Pythium spp. for

growth in soil. The peanut plant, therefore play a role in regulating soil populations of Pythium spp. and in maintaining Pythium spp. on roots and pods. Peanut cultivars may differ in their abilities to harbor Pythium spp. on roots, pods, or in surrounding soil. Cultivars suppressing growth and maintenance of Pythium spp. could be helpful over several years in reducing the seasonal carryover of the fungus and if used for several years could reduce the disease potential in fields with a high risk of Pythium pod rot. No study comparing peanut cultivars for their influence on Pythium maintenance has been reported, however.

The objectives of my research were to:

1. Compare several peanut cultivars adapted to Oklahoma for their susceptibility to pod rot in field, greenhouse and microplot experiments.
2. Compare cultivars for their influence on population of Pythium spp. in field soil. The effects of soil moisture and temperature on these populations were also determined.
3. Determine if the cultivars differ in the relative frequency of P. myriotylum isolated from roots and pods.

CHAPTER II

LITERATURE REVIEW

Etiology of Pod Rot of Peanut

The etiology of pod rot has been a subject of debate. Although fungal pathogens such as Pythium myriotylum Drechs. (14,33) or Rhizoctonia solani Kuhn (anastomosis group 4) (13,23) have been shown to be sole incitants in greenhouse or microplot studies, the disease is most often considered to be of complex etiology in the field. Other fungi (18,21,22), plant-parasitic nematodes (22) and soil mites (50) have been implicated in the disease.

In addition to biotic components, a deficiency of calcium has also been reported to play a role in causing the disease (10). High rates of calcium in gypsum (hydrated calcium sulfate) effectively reduced pod rot (10). Hallock and Garren (29) suggested that pods containing >0.20% calcium would have less pod rot than those containing <0.15% calcium. Moore and Wills (42), however, found no correlation between calcium rates applied to soil and pod rot caused by P. myriotylum and/or R. solani. Later, Csinos and Gaines (9) and Csinos et al. (10) suggested that pod rot

is primarily an abiotic disease involving a calcium imbalance in the pod and is similar to blossom-end rot of other fruits. According to this view, phytopathogenic fungi are secondary to a calcium deficiency in pod rot etiology. Filonow et al. (13) found evidence that supports the role of phytopathogenic fungi such as P. myriotylum or R. solani (AG4) as primary determinants in the disease. In Oklahoma, P. myriotylum is frequently found in peanut fields and has been isolated from rotted pods with high frequency (12).

Characteristics of P. myriotylum

P. myriotylum is a fungus belonging to the Oomycetes. It grows by coenocytic hyphae, releases biflagellate zoospore from filamentous sporangia, and forms aplerotic oospores (55). There are usually 3-6 hook-necked antheridia (sometimes up to 10) per oogonium. Antheridia are usually diclinous. Appressoria are formed in clusters of 4-8. Cardinal temperatures for growth listed by Van Der Plaats-Niterink (55) are a minimum of 5 C, an optimum of 37 C and a maximum above 40 C on potato-carrot agar. Vigorous growth at 37 C on corn meal agar, the ready formation of appressoria and oospores, and the 3-6 antheridia enveloping oogonia are cultural characteristics which aid in the identification of this species in fungal isolates obtained from peanut.

Several agar media have been used to isolate Pythium species from soil and plants (7,13,53,54). All have their

advantages and disadvantages and choice of one is often dependent on a number of factors including the matrix (soil or plant) assayed, cost of ingredients, toxicity of ingredients to unwanted fungi, bacteria and other microbes.

Factors Affecting the Epidemiology of *Pythium* spp. in Pod Rot

Other species of *Pythium* have been reported to be pathogenic to peanut roots (48), but little is known of their pathogenicity to pods. *Pythium myriotylum*, however, has received the greater study as a pod rotting organism.

Soil moisture has a pronounced effect on the growth and survival of *Pythium* spp. in soil (31). Soil moisture sufficient for the release and taxis of zoospore is needed by the fungus in soil, particularly since for most *Pythium* species, zoospores are an infective propagule. Little has been reported regarding the role soil moisture plays in *Pythium* pod rot, except for a study by Frank (15,19). He showed pod rot caused by *P. myriotylum* was aggravated in sandy soils by frequent applications of less water than fewer irrigations of more water. He suggested that good aeration in soil plus readily available water are necessary for good growth and infectivity of *P. myriotylum*.

Soil temperature is another factor greatly influencing *P. myriotylum* growth and infectivity (36,40). Littrell and McCarter (36) showed that *P. myriotylum* caused root and

crown rot on rye at 23 C, but that disease severity increased as soil temperatures increased to 35 C. In vitro studies (40) showed that zoospores of P. myriotylum were produced most rapidly at 28-31 C, but were produced at lower temperatures only after longer periods of incubation in standing water. Jones (33) found that zoospores and hyphae of P. myriotylum penetrated pods within 2 hr after inoculation at 30-34 C. No penetration by zoospores occurred below 25 C. Zoospores penetrating the epidermis of pods did not cause infection. Pod rot was caused only by hyphae and only at 25-35 C.

P. myriotylum isolates have been reported to vary in their virulence to peanut roots. Jones (34) found that two isolates from Texas differed in their ability to cause root rot and stunting. Fourteen isolates of P. myriotylum obtained from various hosts and from different regions of the United States were found to differ considerably in their ability to cause root rot on twelve different hosts (39). These results agree with the findings of Wills and Moore (57) who showed that P. myriotylum isolates from rotted pods varied in their ability to cause death of peanut seedlings. There are no reports of difference in virulence among isolates of P. myriotylum to pods.

Fluctuations in Soil Populations of *Pythium* species

The population dynamics of *Pythium* spp. in soil have been reported in several studies (1,2,17,30,38,44). Populations of *Pythium* spp in soil are influenced for the most part by temperature and moisture (30,31), microbial antagonism (8) and the presence and nature of a host (6).

Little is known about the dynamics of *Pythium* spp. in soil planted with peanut, although warmer soil temperatures would be expected to influence growth of *P. myriotylum* in peanut soils of the southern United States. *Pythium aphanidermatum* a warm temperature species very similar to *P. myriotylum* showed population peaks during January and February and in late August in field soils in Israel (1). The rise in *Pythium* populations detected in August was attributed to higher soil temperatures at that time. Frank (17) concluded that quantitative changes in *Pythium* populations appeared to be characteristic of individual fields and that cropping history had little effect on population fluctuations. Lumsden et al. (38) reported that *P. aphanidermatum* populations in a Maryland vegetable field were highest in winter at the start of the study and dropped to a lower population in the spring. The population remained at the low level for two years, regardless of bean and rye rotation or incorporation of plant residue. *P. myriotylum*

populations in this study were not directly monitored because of the low germinability of its oospores on agar media (37). Results from baiting with bean seedlings suggested that P. myriotylum population did not show any significant fluctuations under bean or rye; however total Pythium spp. in this study were highest in the winter and declined considerably in the spring. The researchers suggested germination of oospores followed by microbial lysis may have accounted for the decline.

The relationship between populations of P. myriotylum and pod rot of peanut has received little attention, except for the work of Garcia and Mitchell (21). They showed that 10 p/g soil caused 19.2% pod rot incidence. Root rot of peanut caused by P. myriotylum resulted from inoculum densities of 15-43 oospores/g soil (41). Other researchers (31) who have related inoculum densities of Pythium to disease have reported that populations of about 100-1,000 propagules/g soil may be commonly needed for high levels of disease.

Control of Pod Rot

Chemical control of pod rot has proven difficult due to the several interacting biotic and abiotic factors in field soils that influence the etiology and epidemiology of the disease. Metalaxyl, a fungicide specific for Pythium spp. and other oomycetes has been used to control Pythium pod rot

with variable success (14,27). Application of metalaxyl at appropriate times during peanut growth for maximum effectiveness against pod rot development is a problem that needs study. However little is known about the effect of peanut development on fluctuations of Pythium populations and their contributions to pod rot severity or incidence. Apart from this, the cost of fungicides such as metalaxyl and possible nontarget effects may diminish the attractiveness of their use.

Cultural practices such as crop rotation and soil moisture management for control of Pythium pod rot have received little attention. This may be due to the broad host range of P. myriotylum (rye, wheat, bean tomato, oat, cucumber, tobacco and sorghum) (39), and the difficulty with managing soil moisture in the irrigated, sandy soils where peanut is grown in Oklahoma.

Genetic resistance to pod rot has been studied by several researchers (5,20,25,26,35,43,46,51,52). Porter et al (46) compared 13 commonly grown Virginia Bunch type peanut cultivars for their susceptibility to pod rot. They showed that genotypes derived from a cross with a spanish type peanut were less susceptible to pod rot compared to those not having a spanish pedigree. Frank (20) also found spanish type peanut less susceptible to pod rot in field studies, and suggested that pod rot susceptibility with spanish type peanut may be due to their short gynophores

which form pods near to the pod surface where conditions are less favorable for pathogens. Anatomical differences among three peanut genotypes were suggested by Petit et al. (43) to account for pod rot resistance. They found that the less susceptible genotypes had a more uniform sclerenchyma band in their pods in addition to higher levels of tannin and lignin compounds. Later, Godoy et al. (25,26) found that resistant genotypes had a more compact arrangement of palisade mesophyll. They also found that genotypes showing less susceptibility to pod rot had more lignified walls in the epicarp and sclerenchymatous mesocarp compared to less susceptible cultivars.

Presently, there is no commercially grown cultivar that has a high degree of resistance to Pythium pod rot. In Oklahoma, the spanish market type cultivars Spanco and Pronto have yet to significantly reduce the importance of pod rot as a factor limiting peanut production. Other cultivars are grown or are agronomically suitable for production in Oklahoma, yet little is known of their pod rot reactions compared to Spanco or Pronto under Oklahoma field conditions.

CHAPTER III

MATERIAL AND METHODS

Reaction of Cultivars To Pod Rot

Field Experiments

In 1987, the cultivars Pronto, Spanco, Florunner, Okrun, Langley and GK7 were grown in fields at Ft. Cobb and Madill, Oklahoma. The Ft. Cobb field was situated in the west-central region of Oklahoma. The soil was a fine sandy loam (62% sand, 24% silt and 14% clay). The field at Madill was situated in the southeastern region of Oklahoma within 10 miles of the Texas border. Soil at Madill was 65% sand, 19% silt and 16% clay. Plots consisted of four rows, 6.0 meters long and with 0.91 meter spacing, arranged in a randomized, complete block design. There were four replicate plots per cultivar. The two center rows were used for yield determinations and the two outer rows were used for plant and soil sampling.

The peanut cultivars Pronto and Spanco (spanish market types); Florunner, Okrun, Langley and GK-7 (runner market types); and Florigiant (Bunch market type) were treated with Gramox PMF at 6 oz/ 100 lb and planted at ca. 17 seeds per

meter for the spanish types and 14 seeds per meter for the others. Cultivars were planted on May 12, 1987 at Madill and on May 13, 1987 at Ft. Cobb. Plots at Madill and Ft. Cobb were irrigated every five and seven days, respectively, with ca. 5 cm of water per irrigation.

Plant samples were taken at three random locations from the outside rows of plots at harvest and at two to three interim times prior to harvest to assess pod rot severity. Three plants were randomly dug from each border row of a plot, placed in large plastic bags and transferred to 5 C within 24 hours of collection. Pods were removed from the plants, washed under water and rated for pod rot severity using a rating scale of 1-5, where 1 = no lesions on pod surface, 2 = 1-25% of pod surface with lesions, 3 = 26-50% of surface with lesions, 4 = 51-75% of surface with lesions and 5 = > 75% of surface with lesions. A weighted mean was used for the pod rot severity rating. The number of pods in each disease class was multiplied by the disease class and these values were summed. The final number was divided by the total number of pods rated. Data were subjected to a Student-Newman-Keuls multiple comparison test for mean separation at the P = 0.05 level. Pieces of hulls were plated on a Pythium selective medium (PSM) (11) to determine the presence of P. myriotylum in infected pods.

At Madill, Spanco and Pronto were harvested on October 2 and the other cultivars were harvested on October 16. At

Ft. Cobb, Spanco and Pronto were harvested on October 5 and the other cultivars were harvested on October 26.

At harvest, plots were dug with a peanut digger-invertor and threshed. Peanut pods were sacked, dried to ca. 10% moisture, cleaned, and weighed for pod yield.

The same cultivars that were planted in 1987 plus NC-7, another bunch type, were evaluated in 1988 at the Ft. Cobb location and at a grower's field in Enos, Oklahoma. The soil at the Enos location was a loamy sand (85% sand, 14% silt and 1% clay). The experimental design and replication was the same as 1987. Plant samplings, isolations from roots and pods for Pythium spp., pod rot ratings and yield determinations were conducted as in 1987. At Ft. Cobb, the cultivars were planted on May 11. Spanish type cultivars were harvested on October 11, whereas the other cultivars were harvested on October 24. At Enos the cultivars were planted on June 6 and all eight cultivars were harvested on October 28.

Greenhouse Experiments

Prior to conducting greenhouse experiments, several isolates of P. myriotylum in storage were assessed for their virulence to peanut. These isolates came from rotted pods collected from growers fields in Oklahoma (Filonow, unpublished). Peanut seed of the seven cultivars used in the 1987 field experiments were surfaced disinfested with

1.05% sodium hypochlorite for 3 minutes and washed five times with sterile distilled water. Seeds were incubated at 25 C in sterile vermiculite in a covered 44 X 33 X 6.6 cm aluminum foil pan for 48 hours. Three 1.5-cm-dia. plugs of two day-old cornmeal agar (CMA) cultures of P. myriotylum were placed on sterile, moist vermiculite in 310 cc styrofoam cups. A radicle (5-10 mm long) of a germinated seed was gently pushed into a plug. There were three seedlings per cup and three cups per isolate. Germinated seeds were covered with 2 cm of vermiculite and the cups randomly placed on a laboratory bench. All cups were incubated at 22 C under fluorescent lights (12 hours at 300 $uE/m^2/sec$, 30 cm above cup surface) and draped with a plastic tarp. Cups were watered every two days and the seedlings harvested after 14 days.

At harvest, plants were rated for their degree of seedling root rot on a scale of 1 to 6 where 1 = no root rot, 2 = less that 25% root rot, 3 = greater that 25%, less than 50% root rot and no stunted growth, 4 = greater than 50% root rot and no stunted growth, 5 = greater than 50% root rot and stunted growth, and 6 = no seedling emergence. Following harvest, pieces of roots were plated on PSM and isolates of P. myriotylum were transferred to CMA and stored in sterile water.

Experiment with oospores as inoculum. Based on results from the virulence assessment above, isolate PM 6 from Caddo

County was used in this experiment. Oospores were produced in V8 broth containing 50 mg/L of cholesterol (3). Fifty ml cultures in 250 ml flasks were incubated in darkness for three weeks at 25 C. Three cultures were then composited in a Sorvall Omni Mixer, and the volume brought to 200 ml. The mixing container was packed in ice and the mycelia comminuted for 10 minutes at 8000 rpm to break up hyphae and release oospores. The macerate was centrifuged at 3,100 X g, the supernatant decanted and sterile water added. Oospores were resuspended on a vortex mixer. The oospore concentration was estimated microscopically using a hemacytometer. Recovery was 18,000 oospores/ml and the preparation was predominately oospores.

Oospores were mixed into steam-pasteurized soil mixture (3 parts soil: 1 part sand, w/w) to obtain the following inoculum densities of oospores per gram of soil: 0, 10, 100, 250 and 500. Spanish type peanuts were planted in 23 cm dia. pots. The remaining cultures (the bunch and runner types) were planted in 23 cm pots, which were then placed inside 30.5 cm dia. pots and the spaces between the pots were filled with soil. This arrangement provided greater soil volume for the runner type cultivars to peg into, while still restricting root growth similar to that of the spanish type cultivars. There were 4 replicates of each cultivar arranged in a completely randomized design. Plants were watered daily and fertilized with Hoagland's (32) solution

once per week. Plants were harvested after four months and pods rated for disease severity.

Experiment with Pythium inoculum from cornmeal-sand cultures. Pathogenic isolates PM 3 and PM 7 from the peanut seedlings experiment plus an isolate of P. myriotylum from the roots of a plant from the 1987 Ft. Cobb study were used to inoculate aluminum oven pans (42 X 31 X 17 cm) containing about 2 kg of autoclaved 5% (w/w) cornmeal in coarse sand medium (13). The medium had been premoistened with water containing 50 mg cholesterol/L. The inoculated medium was incubated aseptically in the dark for three weeks. After incubation, pan cultures were hand mixed into steam-pasteurized soil (2 parts sand: 1 part soil: 1 part peat moss, w/w), and assayed for inoculum density using soil dilutions plated on PSM (11). Further dilutions of this soil were made with steam pasteurized soil to give the needed inoculum densities for the experiments. In the first experiment, inoculum densities of 0, 50, 250, and 357 propagules p/g soil were used.

Peanut cultivars were grown in 30.5 cm dia. plastic pots containing steam pasteurized soil. At flowering, about 1300 g of soil was removed from the pegging zone and replaced with a similar amount of soil infested with P. myriotylum. There were four replicates per inoculum density per cultivar, arranged in a randomized complete block

design. All plants were watered every 1-2 days and fertilized weekly with Hoagland's solution. Pod rot severity at harvest was assessed using the rating described above.

Microplot Experiments

In 1987, microplot experiments were conducted at the Plant Pathology Farm, Stillwater, OK. The microplots were wooden boxes 2.4 X 2.4 X 0.3 cm in dimension. They were filled with a sandy loam soil (62% sand, 34% silt and 4% clay). Microplots were rototilled and fumigated with 0.9 Kg Bromogas (methylbromide) per microplot under a plastic tarp for two days. The tarps were removed and the soil was allowed to aerate for one week prior to planting. The seven cultivars used in the 1987 field experiments were evaluated. In each microplot four peanut cultivars were grown in 1 meter rows with ten plants per row. The cultivar Krinkle Leaf was used as a filler. The experiment was an incomplete unbalanced design with two adjoining blocks considered as an experimental unit. Seeds were planted 0.6 meters in from the wall of the box and 0.3 meters separated the four cultivars. All boxes were hoed periodically to control the weeds and sprinkler-irrigated (2 cm/irrigation) on a five day schedule.

At flowering, microplots were inoculated with P. myriotylum propagules. Soil was removed from both sides of the rows to form trenchs (10 cm wide X 10 cm deep). Care was

taken not to injure the plant root system. Steam pasteurized soil, artificially-infested (13) with P. myriotylum was put into the trenches. Inoculum densities were: 0, 100, 250 p/g soil.

In 1988 two consecutive experiments were conducted at different microplot locations at the Plant Pathology Farm. One location was the same as the previous year and the other consisted of microplots filled with a sandy loam soil (76% sand 22% silt and 2% clay). Following rototilling of the boxes, each was fumigated with 0.9 kg of Bromogas and allowed to aerate for at least one week. The cultivar NC-7 replaced Krinkle Leaf in these experiments. Planting dates of the two experiments were separated by five days. Infestation of the microplots with P. myriotylum, experimental design and treatment replication were the same as in the 1987 experiment. At harvest, pod rot severity was determined and isolations from pod pieces for P. myriotylum were made.

Populations of Pythium spp. In soil Planted to Cultivars

During the experiments comparing peanut cultivars for reaction to pod rot, the effect of the cultivars on populations of Pythium spp in soil was also assessed. Soil from the pegging zones of three randomly chosen plants per row was collected with a garden trowel to a depth of 10-12

cm. The soil samples were composited in a plastic bag. All bags were temporarily kept in a styrofoam chest while in the field and transferred to 5 C within 24 hours. Ten grams of soil were placed in 250 ml flasks containing 90.0 ml of 0.2% agar in water (w/v). Flasks were shaken for 30 min. on a reciprocating shaker. Populations of Pythium in soil were estimated by plating 0.2 ml of 1/10 or 1/50 dilutions of soil in 0.2% water agar on PSM. There were five plates per dilution and plates were incubated at 24-26 C for 24 - 36 hours. Population data were expressed as propagules of Pythium per gram soil and 0.01 propagules per gram was added to each datum prior to statistical analysis. Data was analyzed by analysis of variance and differences between means determined by multiple comparison tests using Student-Newman-Keuls test (P = 0.05).

Soil Temperature and Matric Potential Measurements

The water matric potential of soil samples was determined from soil moisture release curves constructed from readings using a soil moisture pressure plate apparatus (Soil Moisture Equipment Corporation, Santa Barbara, CA). Soil samples obtained during the population samplings were used. Saturated soil samples were subjected to increasing nitrogen pressure from 10 K Pa (.1 bar) to 200 K Pa for 48 hours. Moist soil were weighted. Soil moisture content of

soils after equilibration was determined after drying at 80 C for 72 hours and weighted. In 1987 soil temperature was monitored only at the Ft. Cobb field using a Campbell CR7 micrologger. In 1988, soil temperatures were monitored at both the Ft. Cobb and Enos fields using homemade, inexpensive thermistors that allowed an increased sampling of soil temperatures in the plots. There was one thermistor per plot, arranged randomly along the rows.

Isolation of Pythium spp. from Roots and Pods

In 1987, roots were cut from plants at the crown, washed under running water and cut with scissors into 2 cm pieces. The pieces were composited in a pile and ten randomly selected pieces were placed on two Petri dishes of PSM modified from Eckert and Tsao (11). There were two dishes per replicate and four replication per cultivar. Dishes were incubated at 24-26 for 24 hours. All Pythium spp. growing from roots were reisolated on PSM and observed for the following characteristics of P. myriotylum: (1) growth at 37 C, (2) appressoria development within 48 hours, (3) lobulate sporangia,and (4) three to six antheridia surrounding the oogonium. The remaining Pythium spp. were transferred to cornmeal agar slants and agar pieces of the isolates were put into sterile distilled water storage.

In 1988, roots and pods from Ft.Cobb and pods from the

Enos location were assayed for frequency of isolation of Pythium spp. Root pieces were assayed as in 1987. Hulls were removed from pods, cut into about 1 cm sections, and composited in a pile. Five randomly selected hull pieces were placed on modified PSM (11). There were three plates per replicate and four replicates per cultivar.

CHAPTER IV

RESULTS AND DISCUSSION

Reaction Of Cultivars To Pod Rot

Field Experiment

In 1987 at Ft. Cobb (Table 1), Florigiant had the greatest amount of pod rot at each sampling period compared to the other cultivars and was significantly (P = 0.05) more susceptible than Pronto, Spanco, Okrun and GK-7. Generally, there were no significant (P = 0.05) differences between Pronto, Spanco, Florunner or Langley as to pod rot susceptibility. For most cultivars, pod rot severity was found to be lower at harvest than earlier in the season with the greatest pod rot found at 97 days after planting (DAP). Only Pronto, the lowest yielding cultivar (2159 kg/ha) and GK-7 the highest (3100 kg/ha), were significantly (P = 0.05) different in yield. Mean yield for all cultivars at Ft. Cobb in 1987 was 2762 kg/ha.

In 1987, no significant (P = 0.05) differences were seen in pod rot susceptibility among cultivars grown were seen (Table 2) at Madill. These results may be attributed to less virulent isolates of P. myriotylum

TABLE 1

POD ROT SEVERITY AND YIELD OF PEANUT CULTIVARS AT FT. COBB, OKLAHOMA IN 1987

Cultivar	Severity rating at DAP[z] 97	131	147[y]	167[x]	Yield kg/ha
Pronto	1.90b	1.68b	1.58b		2159a
Spanco	1.85bc	1.75bc	1.57b		2845ab
Florunner	1.96bc	1.81ab	1.97ab	1.76ab	2744ab
GK7	2.17bc	1.62b	1.84bc	1.57b	3100b
Langley	2.46b	1.75b	1.81bc	1.82ab	2922ab
Okrun	2.26bc	1.63b	1.73c	1.68c	2922ab
Florigiant	3.35a	1.99a	2.07a	1.97a	2642ab

[z]DAP = days after planting. The mean pod rot severity of 6 plants per replicate and 4 replicates per sampling was determined. Pod rot severity was rated on a scale of 1-5, where 1 = no lesions on pod and 5 = >75% of pod surface with lesions. Means with the same letter are not significantly (P = 0.05) different according to Student-Newman-Keuls test.

[y]Pronto and Spanco harvested.

[x]All other cultivars harvested.

TABLE 2

POD ROT SEVERITY AND YIELD OF PEANUT CULTIVARS AT MADILL, OKLAHOMA IN 1987

Cultivar	Severity rating at DAP[z] 128	157[y]	Yield kg/ha
Pronto	1.44a	1.44a	2843a
Spanco	1.38a	1.38a	3074a
Florunner	1.51a	1.23a	4595b
GK7	1.40a	1.31a	4848b
Langley	1.38a	1.24a	5054b
Okrun	1.47a	1.22a	4900b
Florigiant	1.45a	1.24a	4774b

[z]DAP = days after planting. The mean pod rot severity rating of 6 plants per replicate and 4 replicates per cultivar per sampling. Pod rot severity was rated on a scale of 1-5, where 1 = no lesions on pod and 5 = >75% of pod surface with lesions. Means with the same letter are not significantly (P = 0.05) different according to the Student-Newman-Keuls test.

[y]Harvest.

(34,40) indigenous to the field and/or to insufficient inoculum potential to cause pod rot severe enough to reveal differences in pod rot reaction. Except for Pronto and Spanco which had significantly (P = 0.05) lower yields than other cultivars, no significant differences in yield between the other cultivars were found. Mean yield for all cultivars at Madill in 1987 was 4301 kg/ha.

In 1988 at Ft. Cobb (Table 3), Florigiant and NC-7 had more pod rot than the other cultivars at all sampling periods. However, only at 146 DAP and harvest samplings were these differences significant (P = 0.05). Pronto and Spanco had significantly (P = 0.05) less pod rot than all other cultivars at harvest, but at earlier samplings Pronto or Spanco were not consistently different (P = 0.05) from the other cultivars except for Florigiant and NC-7. Generally, pod rot for all cultivars increased slightly from 89 DAP to harvest. There were no differences (P = 0.05) in yield for the cultivars. Mean yield for all cultivars at Ft. Cobb in 1988 was 3071 kg/ha.

As the growing season progressed, pod rot generally increased at Enos in 1988 (Table 4). No cultivar appeared to show a consistent and significant (P = 0.05) difference as to pod rot reaction. Although NC-7 and Florigiant had more pod rot than other cultivars at all samplings, only NC-7 in 90 DAP and Florigiant at 122 DAP were distinctive in pod rot reaction (P = 0.05) from the other cultivars.

TABLE 3

POD ROT SEVERITY AND YIELD OF PEANUT CULTIVARS AT FT. COBB, OKLAHOMA IN 1988

Cultivar	Severity rating at DAP[z] 89	118	146[y]	166[x]	Yield kg/ha
Pronto	1.59abc	1.61c	1.47c		3051a
Spanco	1.34c	1.49c	1.56c		3179a
Florunner	1.69abc	1.47c	1.55b	1.80b	2975a
GK7	1.56bc	1.69bc	1.69b	1.83b	3306a
Langley	1.63abc	1.69bc	1.63b	1.81b	2848a
Okrun	1.47bc	1.57c	1.57b	1.89b	3306a
Florigiant	1.78ab	1.97a	1.98a	2.32a	2899a
NC7	1.92a	1.88ab	2.02a	2.24a	3306a

[z]DAP = days after planting. The mean pod rot severity of 6 plants per replicate and 4 replicates per cultivar per sampling was determined. Pod rot severity was rated on a scale of 1-5, where 1 = no lesions on pod and 5 =>75% of pod surface with lesions. Means with the same letter are not significantly (P = 0.05) different according to the Student-Newman-Keuls test.

[y]Pronto and Spanco harvested.

[x]All other cultivars harvested.

TABLE 4

POD ROT SEVERITY AND YIELD OF PEANUT CULTIVARS AT ENOS, OKLAHOMA IN 1988

Cultivar	Severity rating at DAP[z] 90	122	140[y]	Yield kg/ha
Pronto	1.24bc	1.45c	1.65b	3255b
Spanco	1.16c	1.54c	1.69b	3051b
Florunner	1.24bc	1.58bc	1.77b	3026b
GK7	1.23bc	1.56c	1.81ab	3382b
Langley	1.28bc	1.61bc	1.75b	2238a
Okrun	1.18c	1.51c	1.65b	2899b
Florigiant	1.53ab	1.93a	1.92a	2238a
NC7	1.74a	1.82ab	1.82ab	3128b

[z]DAP = days after planting. The mean pod rot severity of 6 plants per replicate and 4 replicates per cultivar per sampling was determined. Pod rot severity was rated on a scale of 1-5, where 1 = no lesions on pod and 5 = > 75% of pod surface with lesions. Means with the same letter are not significantly (P = 0.05) different according to the Student-Newman-Keuls test

[y]Harvest

Pronto and Spanco did not have significantly (P = 0.05) less pod rot than the runner types. Langley and Florigiant had significantly (P = 0.05) lower yields than the other cultivars. The mean yield for all cultivars at Enos in 1988 was 2902 kg/ha.

The results from this study did not support those of Porter et al (46) who reported that Florunner and Florigiant and two other cultivars were more resistant to pod rot than several other cultivars, plant introductions and breeding lines. They suggested that resistance in these cultivars was derived from a cross between a Florida small white spanish-type peanut (A. hypogaea var. vulgaris) and Dixie Giant (A. hypogaea var. hypogaea) a large seeded Virginia-type peanut. They claimed that pod rot resistant cultivars were related to this cross, whereas susceptible cultivars lack these parental types. In the present study, Pronto and Spanco, which are also spanish type cultivars were not more resistant to pod rot than Florunner. In Texas, Florunner is considered to be susceptible to pod rot in the field (35) but in greenhouse tests (25) it was shown not to differ in pod rot resistance from Starr (a spanish type cultivar), Toalson, Goldin I or a plant introduction. Results with Florunner agreed with their greenhouse evaluation rather than their field assessments. In Georgia, Walker and Csinos (56) reported that Florunner had significantly less pod rot than Early Bunch or Florigiant.

Results at the Ft. Cobb and Enos locations demonstrated that the Virginia-type cultivars were generally more susceptible to pod rot, and in many sampling periods were significantly different from (P = 0.05) from many of the other cultivars. However, at these locations and Madill, the runner and spanish types were not significantly different (P = 0.05) from each other. Therefore, based on comparative pod rot reactions and yield performances, Florigiant and NC7 are not recommended for Oklahoma peanut growers with a history of pod rot in their fields.

Okrun, a cultivar developed at the Oklahoma Experiment Station was not significantly less susceptible to pod rot than the other runner or spanish market type cultivars. It is doubtful that this cultivar will be of great benefit to growers as far as pod rot resistance is concerned.

Greenhouse Experiments

The experiment using oospores as a source of inoculum did not produce any pod rot on any of the cultivars at any of the inoculum densities. This was an unexpected result, as Garcia and Mitchell (22,41) were able to incite pod rot at 10 p/g soil. However they assumed in their work that the propagules were oospores, and it is possible that they assayed hyphal fragments rather than oospores. Lumsden et al. (37,38) showed that oospores of P. myriotylum had very low germinability on some popular agar media selective for

Pythium.

Following the experiment using oospores, P. myriotylum propagules (hyphae plus oospores) were used as the source of inoculum for future greenhouse experiments. Five different greenhouse experiments were attempted but only one experiment was completed with data taken. Poor temperature regulation in the plastic greenhouse at the Plant Pathology Farm and the continued problem of mites and thrips forced the termination of several experiments.

Cultivars planted in noninfested soil had healthy roots and showed no pod rot. However root and pod rot were observed in infested soils. At 50 p/g soil, few pods were produced by the cultivars, except for Spanco and Pronto and these have some degree of pod rot. At inoculum densities greater than 50 p/g, no cultivar produced any significant yield of pods compared to the noninfested controls. The severe disease pressure even at the lowest inoculum density, therefore did not allow a valid comparison of pod rot reactions among the cultivars. Severe root rot and wilting of most cultivars was extensive even at 50 p/g soil, although it did have root rot. Only Pronto did not show wilting at 50 p/g soil. At this inoculum density, and particularly at greater densities, plants were killed or severely debilitated. The results from this experiment substantiated a report (45) that P. myriotylum is an aggressive root infecting pathogen and established a maximum

inoculum density (50 p/g) for future experiments. This experiment was not repeated because of continued problems with the greenhouse.

Microplot Experiments

Approximately three weeks after planting the 1987 microplot experiment and before infestation with P. myriotylum propagules, many of the seedlings mysteriously began to wilt and die. Isolations of root and foliar pieces of dying and dead plants on agar media showed no pathogen recovery consistent with the presence of symptoms. Throughout the season, plants showed a burning or bronzing of the leaves, later leading to death of the plants. By the end of the growing season no more than two replicates of each inoculum density were present, therefore no data were collected in 1987.

In 1988, at the same microplot location, a similar situation occurred. Within one month after planting, peanut seedlings began to wilt and some died. Again, no more than two replications were left at harvest for each inoculum density, therefore data were not collected for this experiment, as well.

At the second microplot location in 1988 wilt symptoms were not as severe and the experiment was harvested with sufficient replication (Table 5). There was no significant ($P = 0.05$) effect of inoculum density on pod rot for any

TABLE 5

POD ROT SEVERITY RATING AND YIELD OF CULTIVARS FROM 1988 MICROPLOT EXPERIMENT

Cultivar	Severity[z]	Yield kg/ha
Pronto	1.35 d	1325 ab
Spanco	1.29 d	1679 a
Florunner	1.39 cd	1571 ab
GK7	1.55 bc	1589 ab
Langley	1.44 cd	1142 ab
Okrun	1.41 cd	1572 ab
Florgiant	1.71 ab	1046 b
NC7	1.73 a	1565 ab

[z]Average of the disease severity ratings for all replicates of a cultivar in all infested microplots. Disease severity was rated from 1-5, where 1 = no disease and 5 = > 75% discolored pods. Means with the same letter are not significantly (P = 0.05) different according to the Student-Newman-Keuls test.

cultivar, therefore for each cultivar an average pod rot severity rating for all inoculum densities was calculated. Florigiant and NC 7 had significantly (P = 0.05) more pod rot than Pronto, Spanco, Okrun, Langley, and Florunner. Yields were not different except for the yield of Spanco, which was significantly (P = 0.05) greater than Florigiant. The microplot results agreed with the results from the Ft. Cobb and Enos field experiments, in that Florgiant and NC7 were more susceptible to pod rot than the other cultivars.

Population of *Pythium* spp. in soil planted with cultivars

In 1987 at the Ft. Cobb location, the mean population of *Pythium* spp. for all cultivars (Figure 1) was relatively stable at planting in May and 35 DAP with an average of 7 and 3 p/g of soil, respectively. At 67 DAP the mean population rose to 657 p/g, followed by a decline at 96 DAP and 128 DAP with a final population at harvest of 32 p/g. Except at 96 DAP, there were no significant (P = 0.05) differences in soil populations for any cultivar at any sampling period. In addition, when monthly populations for cultivars were averaged over the entire season, there were no differences (P = 0.05) among the cultivars. There was no significant (P = 0.05) interaction of soil moisture and peanut cultivars on populations of *Pythium* spp.

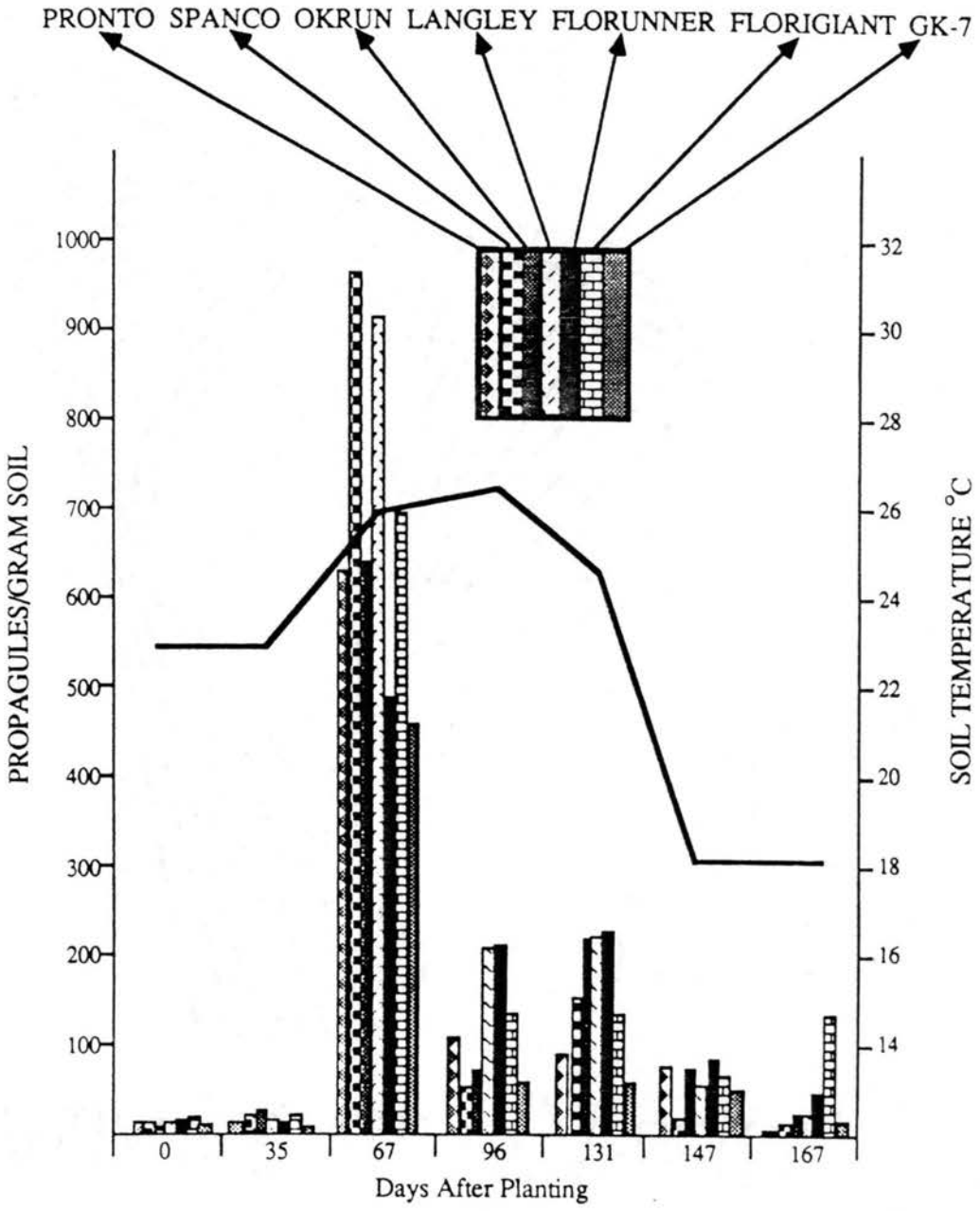

Figure 1. Populations of Pythium spp. in soil planted to peanut cultivars and monthly mean soil temperatures at Ft. Cobb, Oklahoma in 1987. Each bar is the mean of 4 replicates.

At Madill (Figure 2) populations of Pythium spp. in 1987 proliferated and declined similar to populations at Ft. Cobb. The mean populations for all cultivars at planting in May and at 36 DAP were 12 and 44 p/g soil, respectively. At 67 DAP the population rose to 741 p/g and then declined for the remaining months, with a final population at harvest of 68 p/g. Except at 67 DAP and at harvest, there were no significant (P = 0.05) differences in soil populations for any cultivar at any sampling period. When averaged over the season, populations means for cultivars were not different (P = 0.05). As at the Ft. Cobb experiment, there was no significant interaction of soil moisture and peanut cultivar on population of Pythium spp. population.

In 1988 at Ft. Cobb (Figure 3), mean populations of Pythium spp. for all cultivars were 8, 5, 13 p/g soil at planting 30, and 62 respectively. At 89 DAP the population climbed to 346, and then declined the following sampling period. At harvest the population was 28 p/g soil. Except at 89 and at harvest, cultivars had no effect on populations of Pythium in soil. Averaged over the season, population means for cultivars were not different (P = 0.05). Interactions of either soil moisture or soil temperature and peanut cultivar on population of Pythium spp. were not significant (p = 0.05).

Mean populations for all cultivars at Enos (Figure 4) averaged 1 p/g at planting and 31 DAP. At 62 DAP the

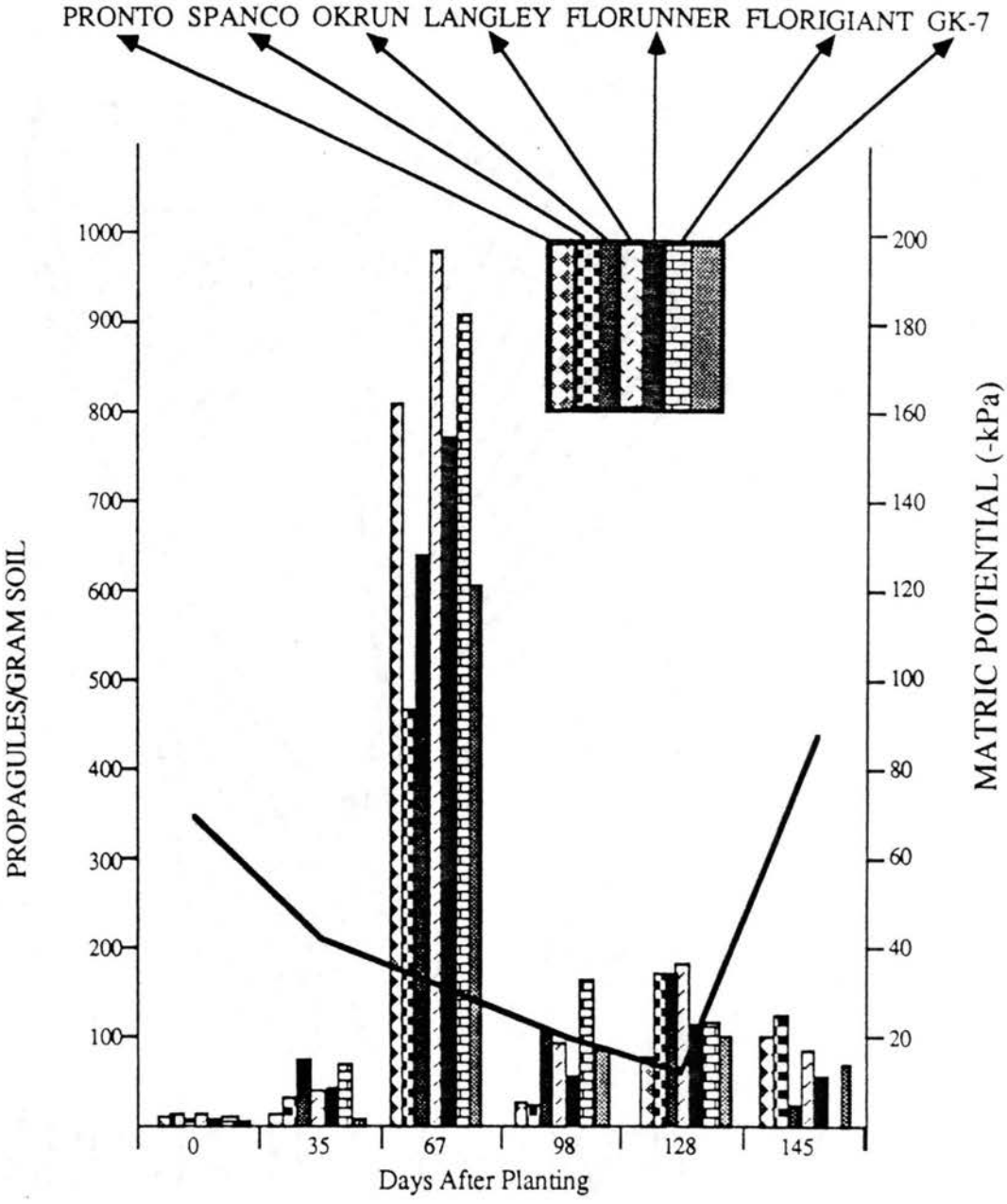

Figure 2. Populations of Pythium spp. in soils planted to peanut cultivars and monthly mean matric potential of soil at Madill, Oklahoma in 1987. Each bar is the mean of 4 replicates.

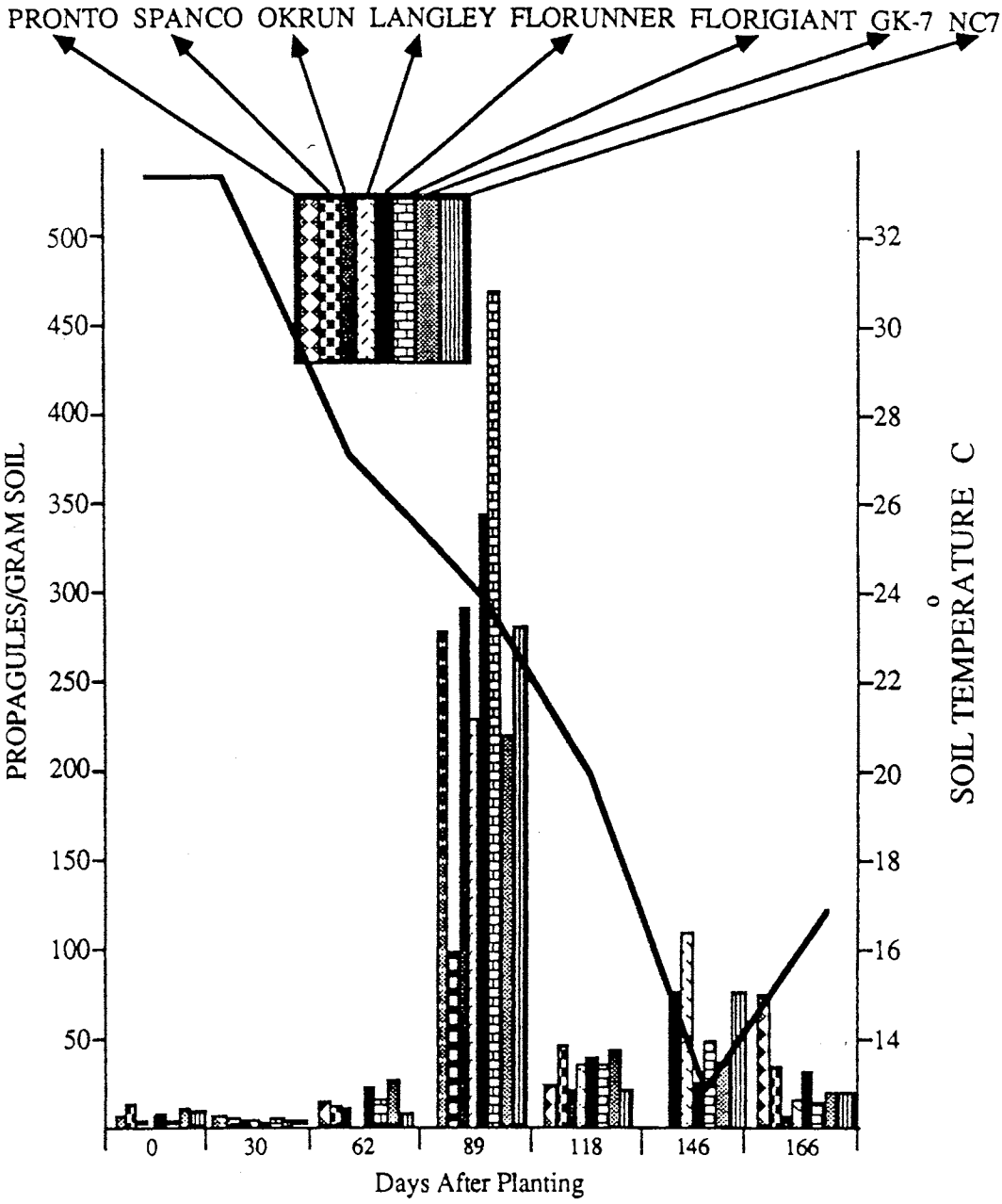

Figure 3. Populations of Pythium spp. in soil planted to peanut cultivars and monthly mean soil temperatures at Ft. Cobb. Oklahoma in 1988. Each bar is the mean of 4 replicates.

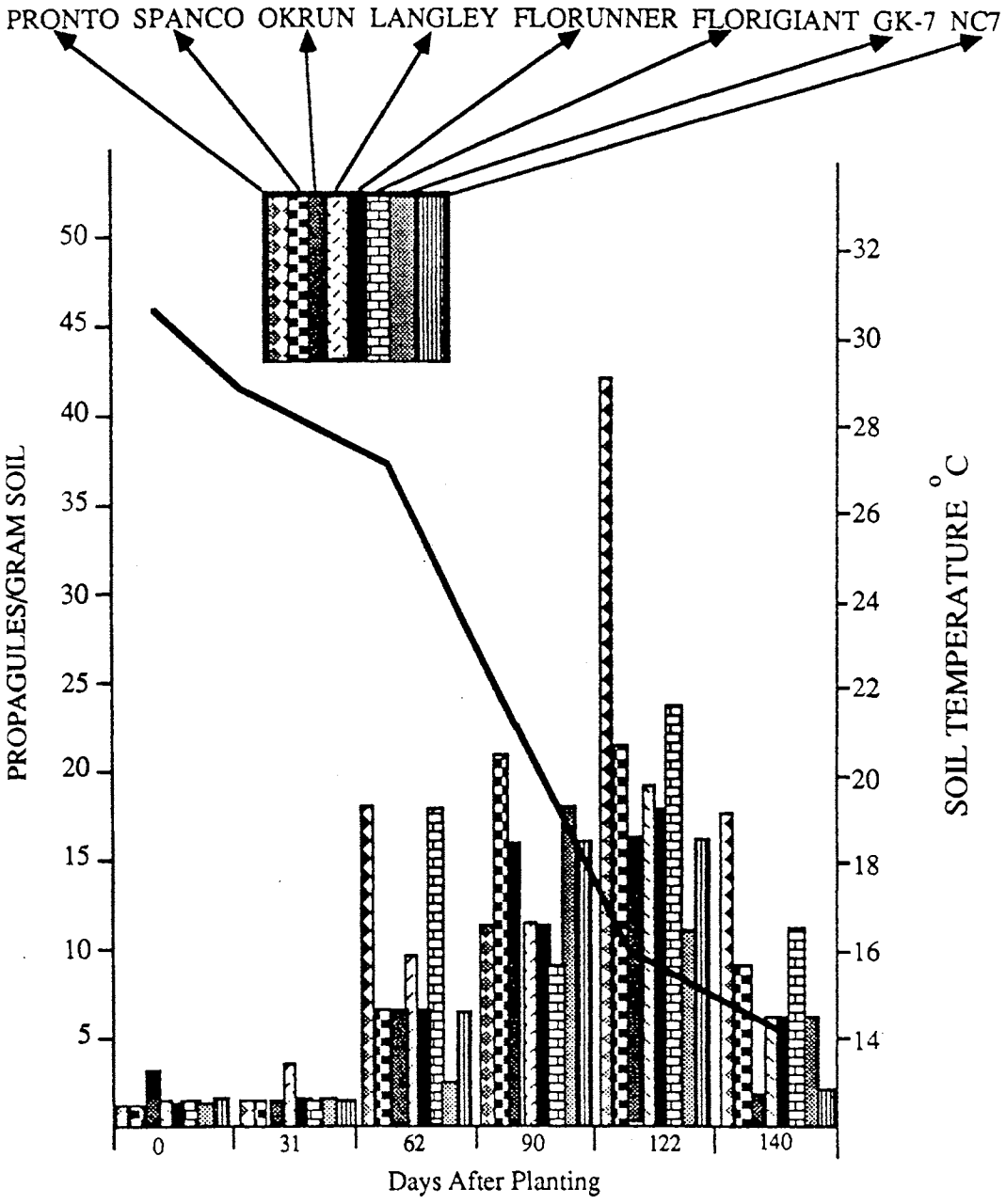

Figure 4. Populations of Pythium spp. in soil planted to peanut cultivars and monthly mean soil temperatures at Enos, Oklahoma in 1988. Each bar is the mean of 4 replicates.

populations began to increase, reaching a peak of 21 p/g soil at 127 DAP. Populations then declined by harvest to an average of 13 p/g. No significant (P = 0.05) differences occurred among the cultivars at any sampling period or when comparing averaged monthly populations of a cultivar for the growing season.

At Ft. Cobb in 1987 (Figure 1) the mean monthly soil temperature reached a maximum of 27 C at 96 DAP whereas the Pythium population peak occurred at 67 DAP. In 1988, mean monthly soil temperatures (Figure 1) peaked at 34.3 C in 30, but populations of Pythium spp. were highest 89 DAP . At Enos in 1988, (Figure 4) mean monthly soil temperature was highest at planting (30.9 C) whereas populations were greatest in 122 DAP. Soil water potential fluctuated considerably from location to location and from year to year (Figure 2 and 5-7). No discernible relationship between soil matric potential and fluctuations in populations of Pythium spp. were observed.

In fungicide/pod rot control studies at Ft. Cobb in 1986 and 1987, Filonow and Jackson (14) observed similar proliferations of Pythium in soils at 60 DAP and 75 DAP, respectively. They did not monitor soil temperature or moisture, however. The results of these studies confirmed their observations. The proliferation occurred at all three locations and it appeared not to be cultivar dependent. Also it appeared that proliferations of Pythium at Ft. Cobb,

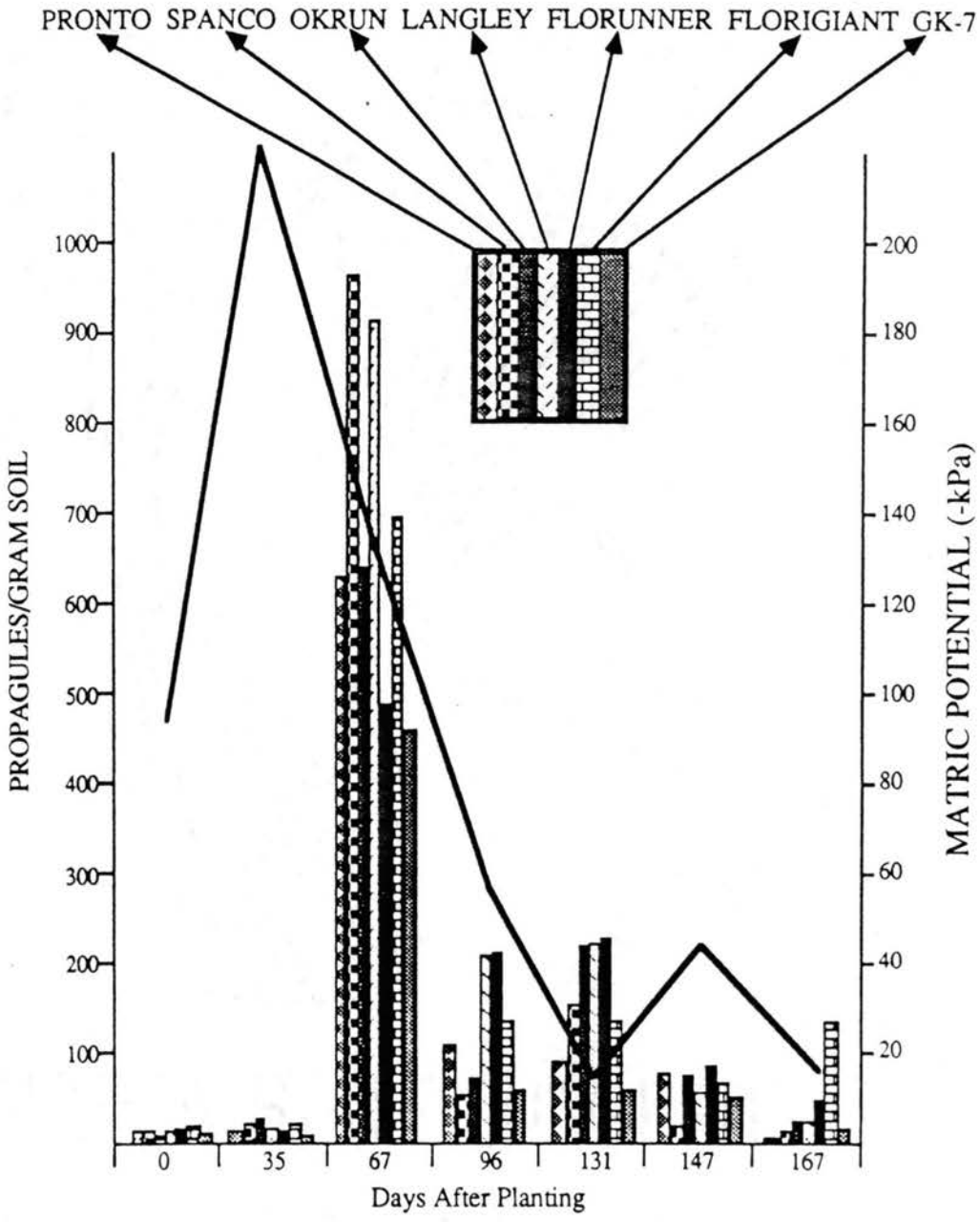

Figure 5. Populations of Pythium spp. in soil planted to peanut cultivars and monthly mean matric potential of soil at Ft. Cobb in 1987. Each bar is the mean of 4 replicates.

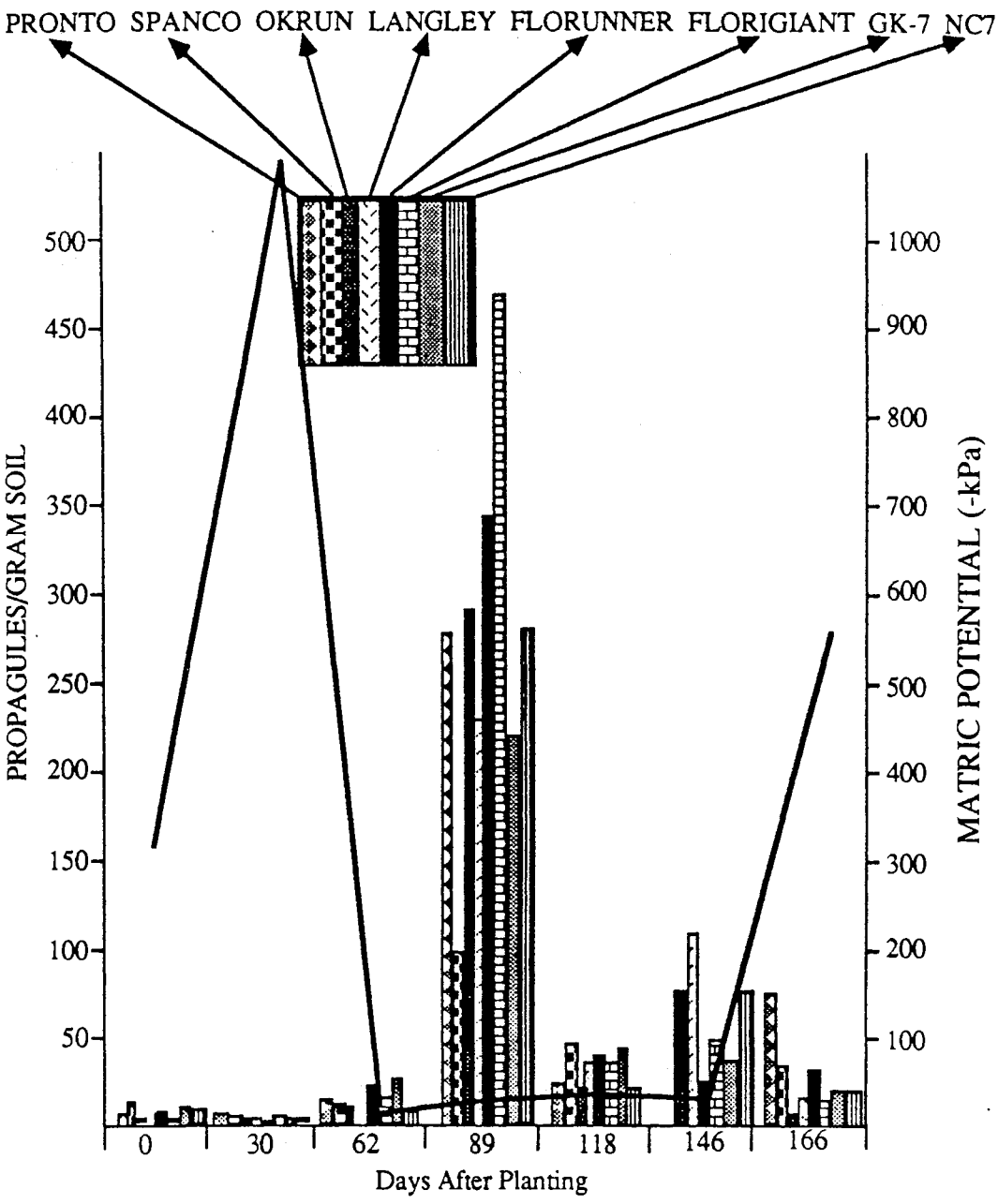

Figure 6. Populations of Pythium spp. in soil planted to peanut cultivars and monthly mean matric potential of soil at Ft. Cobb in 1988. Each bar is the mean of 4 replicates.

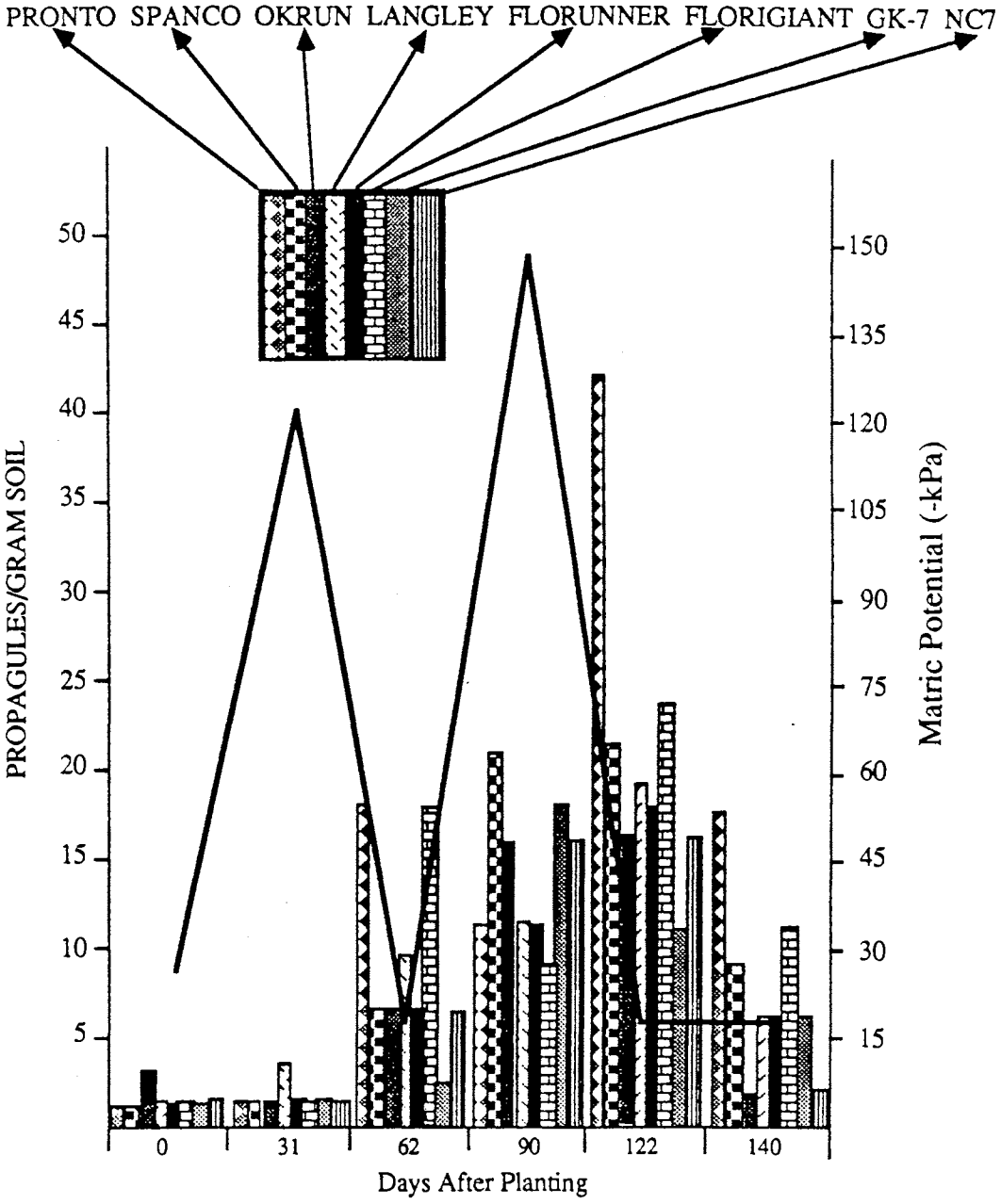

Figure 7. Populations of Pythium spp. in soil planted to peanut cultivars and monthly mean matric potential of soil at Enos in 1988. Each bar is the mean of 4 replicates.

Madill and Enos were not directly related to temperature or water potential of the soils at these sites.

It is suggested then, that the proliferation and decline of Pythium in soils at these sites responded to the development and maturation of pods in soils. At Ft. Cobb and Madill, the timing of the population peaks, (July and August) was similar to the R4 - R6 reproductive growth stages for peanut (4) during which plants have added significant pod numbers and weight. At Enos cultivars were planted four weeks later than the other locations due to unusually dry and cool spring in 1988. Peanut plants at this site pegged later and produced pods slowly and later into the season. As suggested by work of Hale (28) nutrients released by pods forming in soil may be greatest during early development and decrease considerably as pods mature. Growth of Pythium spp. may have proliferated in response to nutrients from developing pods. Following subsidence of nutrient exudation as pods mature, populations of Pythium may have declined due to microbe-induced lysis of hyphae (24,38). Possibly, growth of Pythium spp. in soil may be attuned to peanut phenology. More research is needed to determine the factors that influence the dynamics of Pythium populations in soils planted to peanut, as this knowledge may benefit the scheduling of fungicides targeted against Pythium.

Isolation of *Pythium* spp. from roots and pods

The precent of *Pythium* spp. recovered from roots of cultivars grown at Ft. Cobb in 1987 (Table 6) or 1988 (Table 7) were 0-75% or 0-100%, respectively. In 1987, but not in 1988, recovery of *Pythium* was generally greater in August than in September. *P. myriotylum* was rarely recovered from the roots (maximum of 10% isolation in 1988). There were no significant ($P = 0.05$) differences in the frequency of *Pythium* isolations from roots among cultivars.

Pythium spp. (Table 7) were recovered from rotted pods in 1988 with frequencies of 0-93% at Ft. Cobb and 0 - 74% at Enos. Cultivar or time of sampling did not appear to influence the occurrence of *Pythium* spp. on rotted pods in any consistent way. Of the *Pythium* spp. recovered from pods, *P. myriotylum* appeared to be the dominant species. These results suggested that *P. myriotylum* prefers colonization of pods compared to roots and that species of Pythium other than *P. myriotylum* were more frequent colonists of peanut roots.

TABLE 6

Isolation of *Pythium myriotylum* spp. from roots of peanut cultivars from Ft.Cobb, Oklahoma in 1987

Cultivar	DAP	% [z] *P. myriotylum*	% [z] *P.* spp.
Pronto	96	5	50
	128	1	20
Spanco	96	0	0
	128	1	9
Florunner	96	0	0
	128	4	25
GK7	96	1	20
	128	0	0
Langley	96	1	20
	128	0	0
Okrun	96	8	75
	128	0	0
Florigiant	96	3	50
	128	1	8

[z]Number of Pythium colonies growing on a selective medium after 24 hours expressed as a percent of the pod or root pieces plated. There were 5 pod pieces and 10 root pieces per plate and 2 plates per replicate. Values given are the means of 4 replicates.

TABLE 7

ISOLATION OF *PYTHIUM MYRIOTYLUM* OR *PYTHIUM* SPP. FROM PODS OR ROOTS OF PEANUT CULTIVARS FROM FT. COBB OR ENOS, OKLAHOMA IN 1988.

		FT. COBB				ENOS	
		%[z]					
		P. myriotylum		*P.* spp.		*P. myriotylum*	*P.* spp.
Cultivar	Sampling period	pods	roots	pods	roots	pods	pods
Pronto	August	53	0	73	0	30	25
	September	07	9	07	47	13	20
	October	19		23		05	20
Spanco	August	07	0	13	0	33	43
	September	46	1	46	50	03	13
	October	11		20		07	07
Florunner	August	07	3	13	66	25	28
	September	52	1	57	53	07	07
	October	34		40		00	12
GK7	August	11	1	47	100	23	46
	September	11	3	13	20	07	16
	October	30		35		10	23

Langley	August	08	1	15	25	00	40
	September	29	3	37	8	15	42
	October	10		30		00	00
Okrun	August	93	0	93	0	23	47
	September	29	5	37	11	15	42
	October	22		39		04	17
Florigiant	August	00	0	07	0	62	74
	September	11	1	13	1	07	16
	October	35		48		04	25
NC7	August	31	5	36	33	35	50
	September	37	10	42	89	13	20
	October	33		47		20	27

[z] Number of Pythium colonies growing on a selective medium after 24 hours, expressed as a percent of the pod or root pieces plated. There were 5 pod and 10 root pieces per plate and two plates per replicate. Values given are the means of 4 replicates.

CHAPTER V

SUMMARY AND CONCLUSIONS

Results from four field experiments at three sites and from a microplot experiment showed that the peanut cultivars Florigiant and NC-7 were more susceptible to pod rot than Pronto, Spanco, Florunner, Okrun, Langley or GK-7. These latter cultivars did not differ significantly from each other in pod rot reaction. No cultivar tested was consistently and significantly better as to yield.

In regard to the second objective, results showed that no cultivar significantly influenced populations of Pythium spp. in the pegging zones of soil. In 1987 at Ft. Cobb and Madill, Oklahoma populations of Pythium spp. in soil proliferated several fold at 67 DAP and then rapidly declined at 96 and 98 DAP respectively by August. This proliferation and decline occurred regardless of the cultivar planted. In 1988, a similar proliferation of Pythium spp. occurred at 89 DAP at Ft. Cobb and at 127 DAP at Enos, but to a lesser extent. The proliferation and decline of Pythium spp. appeared not to be directly related to fluctuations in temperature or matric potential of soil. Possibly, the dynamics of Pythium spp. in soil planted to peanut follows the

development and maturation of pods in soil.

In regard to the third objective, it was found that P. myriotylum appeared not to be a common colonist of peanut roots, as it was less frequently isolated from roots than other, as yet unidentified, Pythium spp. On the other hand P. myriotylum was a common colonist of pods with symptoms of pod rot.

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