

INFLUENCE OF THE PEANUT HOST ON
FLUCTUATIONS OF PYTHIUM
SPP POPULATIONS
IN SOIL

BY

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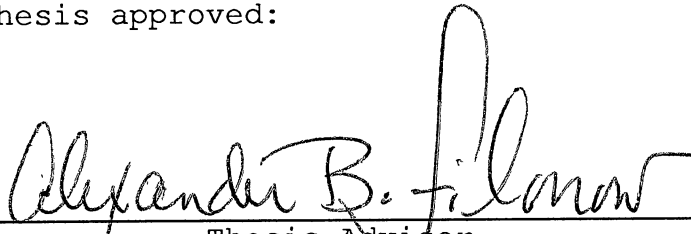
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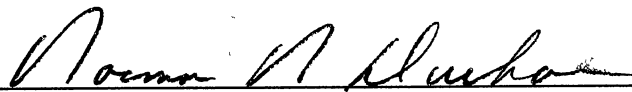
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TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION.....	1
II. LITERATURE REVIEW.....	4
Losses Due to Pod Rot.....	4
Pod Rot Etiology.....	4
Characteristics of <u>P. myriotylum</u>	6
Control of Pod Rot.....	6
Factors Affecting Populations of <u>Pythium</u> spp. in Soil.....	8
Relationship Between Populations of <u>Pythium</u> spp. in Soil and Disease.....	17
III. MATERIALS AND METHODS.....	18
Field Experiments.....	18
Ft. Cobb.....	18
Other Fields in 1990.....	21
Growth Chamber Experiments.....	22
Box Experiments.....	22
Pod Training Experiments.....	25
Pathogenicity of <u>Pythium</u> spp. to peanut pods.....	27
Statistical Analysis.....	29
IV. RESULTS AND DISCUSSION.....	30
Field Experiments.....	30
Ft. Cobb 1989.....	30
Ft. Cobb 1990.....	39
Other Fields 1990.....	49
Growth Chamber Experiments.....	69
Box Experiments.....	69
Pod Training Experiments.....	73
Pathogenicity of <u>Pythium</u> spp. to peanut pods.....	79

Chapter	Page
V. SUMMARY AND CONCLUSIONS.....	84
LITERATURE CITED.....	87
APPENDIX.....	93

LIST OF TABLES

Table	Page
1. Plant height, numbers of pegs and pods and pod rot index of peanut plants grown in steam pasteurized soil artificially infested with different species of <u>Pythium</u> : First Growth Chamber Experiment.....	81
2. Plant height, numbers of pegs and pods and pod rot index of peanut plants grown in steam pastuerized soil artificially infested with different species of <u>Pythium</u> : Second Growth Chamber Experiment.....	82

LIST OF FIGURES

Figure	Page
1. Peanut, proximal and peripheral zones for soil sampling in the box experiments.....	23
2. Nested pot arrangements for the pod training experiments.....	26
3. Populations of <u>Pythium</u> spp. in fallowed soil or in soils planted with peanut or soybean at Ft. Cobb, Oklahoma in 1989.....	31
4. Populations of <u>Pythium</u> spp. in fallowed soil as related to soil temperatures at Ft. Cobb, Oklahoma in 1989.....	32
5. Populations of <u>Pythium</u> spp. in fallowed soil as related to soil matric potentials at Ft. Cobb, Oklahoma in 1989.....	33
6. Populations of <u>Pythium</u> spp. in soil planted with soybean as related to soil temperatures at Ft. Cobb, Oklahoma in 1989.....	34
7. Populations of <u>Pythium</u> spp. in soil planted with soybean as related to soil matric potentials at Ft. Cobb, Oklahoma in 1989.....	36
8. Populations of <u>Pythium</u> spp. in soil planted with peanut as related to soil temperatures at Ft. Cobb, Oklahoma in 1989.....	37
9. Populations of <u>Pythium</u> spp. in soil planted with peanut as related to soil matric potentials at Ft. Cobb, Oklahoma in 1989.....	38
10. Populations of <u>Pythium</u> spp. in fallowed soil or in soils planted with peanut or soybean at Ft. Cobb, Oklahoma in 1990.....	40

11. Populations of <u>Pythium</u> spp. in fallowed soil as related to soil temperatures at Ft. Cobb, Oklahoma in 1990.....	41
12. Populations of <u>Pythium</u> spp. in fallowed soil as related to soil matric potentials at Ft. Cobb, Oklahoma in 1990.....	42
13. Populations of <u>Pythium</u> spp. in soil planted with soybean as related to soil temperatures at Ft. Cobb, Oklahoma in 1990.....	44
14. Populations of <u>Pythium</u> spp. in soil planted with soybean as related to soil matric potentials at Ft. Cobb, Oklahoma in 1990.....	45
15. Populations of <u>Pythium</u> spp. in soil planted with peanut as related to soil temperatures at Ft. Cobb, Oklahoma in 1990.....	46
16. Populations of <u>Pythium</u> spp. in soil planted with peanut as related to soil matric potentials at Ft. Cobb, Oklahoma in 1990.....	47
17. Populations of <u>Pythium</u> spp. in soil planted with peanut as related to soil temperatures at site C4 in Caddo County, Oklahoma in 1990.....	50
18. Populations of <u>Pythium</u> spp. in soil planted with peanut as related to soil moisture contents at site C4 in Caddo County, Oklahoma in 1990.....	51
19. Populations of <u>Pythium</u> spp. in soil planted with peanut as related to soil temperatures at site C6 in Caddo County, Oklahoma in 1990.....	52
20. Populations of <u>Pythium</u> spp. in soil planted with peanut as related to soil moisture contents at site C6 in Caddo County, Oklahoma in 1990.....	53

21. Populations of <u>Pythium</u> spp. in soil planted with peanut as related to soil temperatures at site C10 in Caddo County, Oklahoma in 1990.....	55
22. Populations of <u>Pythium</u> spp. in soil planted with peanut as related to soil moisture contents at site C10 in Caddo County, Oklahoma in 1990.....	56
23. Populations of <u>Pythium</u> spp. in soil planted with peanut as related to soil temperatures at site G1 in Garvin County, Oklahoma in 1990.....	57
24. Populations of <u>Pythium</u> spp. in soil planted with peanut as related to soil temperatures at site G2 in Garvin County, Oklahoma in 1990.....	58
25. Populations of <u>Pythium</u> spp. in soil planted with peanut as related to soil moisture contents at site G1 in Garvin County, Oklahoma in 1990.....	59
26. Populations of <u>Pythium</u> spp. in soil planted with peanut as related to soil moisture contents at site G2 in Garvin County, Oklahoma in 1990.....	60
27. Populations of <u>Pythium</u> spp. in soil planted with peanut as related to soil temperatures at site M1 in Marshall County, Oklahoma in 1990.....	61
28. Populations of <u>Pythium</u> spp. in soil planted with peanut as related to soil temperatures at site M2 in Marshall County, Oklahoma in 1990.....	62
29. Populations of <u>Pythium</u> spp. in soil planted with peanut as related to soil moisture contents at site M1 in Marshall County, Oklahoma in 1990.....	64
30. Populations of <u>Pythium</u> spp. in soil planted with peanut as related to soil moisture contents at site M2 in Marshall County, Oklahoma in 1990.....	65

31. Populations of <u>Pythium</u> spp. in the peanut, proximal and peripheral zones of soil in boxes planted with peanut: First experiment.....	70
32. Populations of <u>Pythium</u> spp. in the peanut, proximal and peripheral zones of soil in boxes planted with peanut: Second experiment.....	72
33. First pod training experiment.....	74
34. Second pod training experiment.....	76
35. Pathogenicity of <u>P. myriotylum</u> and <u>P. irregulare</u> to Pronto peanut as compared to a noninoculated control.....	82

CHAPTER I

INTRODUCTION

Pod rot of peanut (Arachis hypogaea L.) is a soilborne disease of worldwide importance. In Oklahoma, pod rot cost growers \$3.9 million in 1985 (A.B. Filonow, personal communication). Symptoms include various degrees of pod discoloration plus several stages of hull and kernel decay. The junction between pegs and pods can be weakened by the disease, resulting in substantial loss at harvest (61, 62).

The etiology of pod rot is a matter of controversy. The availability of calcium, applied to soil as gypsum ($\text{CaSO}_4 \cdot \text{H}_2\text{O}$), has been related to pod rot incidence and severity (13, 14, 15, 35, 36, 53). Pod rot has also been reported to have a biotic etiology. Principal causal agents include fungi such as: Pythium myriotylum Drechs. (19, 21, 23, 27, 28), Rhizoctonia solani Kühn (Anastomosis Group IV) (19, 21, 28), Fusarium solani (Mart.) App. & Wr. emend. Syn. and Hans. (24), and Sclerotium rolfsii Sacc. (54, 61, 62). In addition to P. myriotylum, other Pythium species such as P. irregulare have been implicated as causal agents of pod rot. The etiology of pod rot also involves soilborne mites (64), plant parasitic nematodes (21, 27), and insects (61).

In addition to P. myriotylum, other Pythium species such as P. irregulare Busiman have been implicated as causal agents of pod rot (61, 62). However, no information is available regarding the pathogenicity of these Pythium spp. to peanut pods. Pod rot is usually considered to be a disease complex involving one or more fungal pathogens interacting with other organisms (21, 27, 61, 62).

Effective control of pod rot has proven to be difficult, most likely due to the various organisms and complex interactions that may be involved in pod rot. Fungicides, e.g. metalaxyl for Pythium spp., are available for control of pod rotting fungi; however, they are not always efficacious. Efficacy of control might be improved by knowing more about the epidemiology of pod rotting organisms, particularly at the population level.

Little was known about the population dynamics of pod rotting fungi in field soil, until a few years ago, when Filonow and Jackson (19) reported a rise and fall in Pythium spp. populations after pegging in peanut soil at Ft. Cobb, Oklahoma. Later, Lewis and Filonow (46) observed similar patterns in Pythium spp. populations between 60-90 days after planting. These patterns were observed at two other fields sites in addition to Ft. Cobb and the phenomenon occurred regardless of the peanut cultivar planted. The commonality of the population pattern in other peanut fields in Oklahoma is not known. Soil temperature or matric

potential appeared to have no effect on the population fluctuations; however, only limited measurements of these environmental parameters were made (46). Their results suggested that fluctuations of Pythium spp. populations in soil may be related to peanut phenology. They hypothesized that populations of Pythium spp. may have increased in response to nutrients exuded from peanut roots and pods; whereas populations in soil declined when pods matured, and nutrients released into soil became too low to support continued hyphal growth. Hyphae of Pythium spp. may then be lysed by other microorganisms in the geocarposphere or they may have moved from the soil to colonize pods.

Therefore the objectives of my research were the following:

- (1) to determine whether or not Pythium spp. populations in peanut fields at Ft. Cobb, fluctuated over time according to previously observed patterns, and to determine if similar patterns exist in fields in other peanut growing areas of the state.
- (2) to further elucidate the role that the peanut host has on fluctuations of Pythium spp. populations in soil over time, and
- (3) to compare other species of Pythium to P. myriotylum for their pathogenicity to peanut pods.

CHAPTER II

LITERATURE REVIEW

Losses Due To Pod Rot

Pod rot of peanut is a soilborne disease found in several peanut producing states of the U.S.A. and in other countries. Major symptoms are pod discoloration with dark brown to black lesions, followed by pod decay. The junction between peg and pod is also weakened by this disease (61, 62). Pod quality can be severely reduced by the disease. Yield is reduced due to pod decay or to pods left in the soil after digging.

Losses to pod rot can be substantial. In Oklahoma, 42% of 36 peanut fields that were sampled in a 1983 survey had pod rot, and mean pod rot incidence was 6.1% (21). In 1985, Oklahoma's peanut growers lost an estimated 3.9 million dollars to pod rot (A.B.Filonow, personal communication)

Pod Rot Etiology

Pod rot etiology is a matter of controversy. Calcium availability and its relation to pod rot incidence and severity have been studied (13, 14, 15, 35, 36, 53). High

levels of calcium applied as gypsum ($\text{CaSO}_4 \cdot \text{H}_2\text{O}$) to soil have been reported to reduce pod rot (13, 15, 36). Pods with less than 0.15% calcium in the hulls had more pod rot than those with more than 0.20% calcium (36). It was suggested that a decrease in calcium in the cell walls of the hull results in a pod which is more susceptible to plant pathogens. Another hypothesis was offered by Csinos and his colleagues in Georgia. They have concluded that pod rot of peanut is similar to blossom end rot of tomato, and is primarily caused by a calcium deficiency (13, 14, 15). According to this view, fungal pathogens are of secondary importance to pod rot initiation.

Pod rot has been reported to have a biotic etiology. Some researchers have not found significant correlations between levels of applied calcium and pod rot (22, 53). Filonow et al. (18, 22) have shown that pod rot in Oklahoma is caused by Pythium myriotylum Drechs. and /or Rhizoctonia solani Kühn (Anastomosis Group IV). In addition to P. myriotylum (19, 21, 23, 27, 28), other species, e.g. P. irregulare Busiman have been implicated as causal agents of pod rot (61, 62). Rhizoctonia solani (19, 21, 28), Fusarium solani (Mart.), App. & Wr. emend. Snyder & Hans. (24) and Sclerotium rolfsii Sacc. (56, 61, 62), are other fungi reported to cause pod rot. Pod rot is usually considered to be a disease complex involving combinations of fungal pathogens. The etiology of pod rot also involves soilborne

mites (64), plant parasitic nematodes such as Meloidogyne arenaria (Neal) Chitwood and M. hapla Chitwood (21, 27), and insects such as the southern corn root worm (61).

Characteristics of P. myriotylum

Pythium myriotylum is recognized by coenocytic hyphae, filamentous sporangia and oogonia with typically 3-6 hooked shaped, diclinous antheridia (68). Oospores are aplerotic. Appresoria are easily formed on surfaces, usually in clusters of 4-8. Cardinal temperatures for growth are a minimum of 5 C. and an optimum of 37 C. (68). The fungus does not survive temperatures in excess of 42-45 C.

Control of Pod Rot

Reports of effective fungicidal control of pod rot are few. In Georgia, PCNB and metalaxyl were generally ineffective (13). Filonow and Jackson (19) had variable success with metalaxyl plus PCNB or metalaxyl plus tolclofos-methyl. Metham sodium (trade name: Vapam) applied preplant by sprinkler irrigation to soil significantly reduced pod rot incidence; however, it was not effective in reducing oospore populations in soil (44). The difficulty in the chemical control of pod rot may be attributed to the diverse array of fungi and other organisms that may be present in peanut soil.

Crop rotation for control of pod rot may have some value (61), depending on what fungi are present in the soil. Pythium myriotylum has a wide host range which limits the choice of a rotation crop (10). In Oklahoma, crop rotation as a means of reducing pod rot is not normally practiced.

Peanut cultivars have been evaluated for resistance to pod rot (8, 29, 30, 46, 59, 60). Resistant peanut lines may have higher levels of lignin and tannin compounds in addition to a more uniform sclerenchyma layer in their pods (59). More lignified walls in the epicarp and mesocarp were associated with lines less susceptible to pod rot (29, 30). Lewis and Filonow (46) showed that Florigiant and other Virginia bunch market types were more susceptible to pod rot than runner or spanish market types. However, there is no commercial cultivar that exhibits a high degree of resistance to Pythium spp., or other pod-rotting fungal pathogens.

Presently, there is no biological control for pod rot. Biological control of Pythium-induced diseases using microorganisms have been reported by several workers (1, 6, 11, 40, 41, 42, 47, 48, 50, 54). Mechanisms of control included antibiosis (40, 41, 42), competition (6, 11) and mycoparasitism (1, 20, 47, 48, 50) of oospores or hyphae.

Factors Affecting Populations of
Pythium spp. in Soil

The biology of phytopathogenic Pythium spp. was reviewed nearly twenty years ago by Hendrix and Campbell (39). In general, populations of Pythium spp. in soil are affected by abiotic and biotic factors. Principal abiotic factors include soil temperature, moisture, pH and soil fertility.

Populations of Pythium spp. showed seasonal fluctuations in several fields in the West Bank of Jordan and in the Gaza Strip (2). Eighteen fields had the highest Pythium spp. populations during the winter and early spring and the lowest during summer. Populations in winter and spring appeared to be related to high soil moisture and low temperature, whereas in summer, populations may have been reduced by high soil temperature and low moisture, Ali-Shtayeh (2) speculated that population increases in the winter and spring may have resulted from the germination of dormant propagules due to the increased moisture and decreased activity of antagonistic microorganisms at these times. Also, in this study, P. aphanidermatum (Edson) Fitz. which is typically a warm-temperature pathogen had a different population pattern with a peak in the late summer and low population in the winter. In addition, P. aphanidermatum was found only in irrigated fields (2).

Soil temperature and rainfall were considered to be prime factors influencing short-term fluctuations in soil populations of Pythium spp. in a study conducted in a rough grass meadow in Reading, England (38). In this study, populations of Pythium spp. in 1983 peaked at 26,360 propagules (p)/g soil and then rapidly declined to a few thousand p/g. In April, another proliferation (34,410 p/g) and decline was noticed. In 1984, no peaks were noticed in the same plots. Multiple regression analysis suggested that soil temperature was more important than rainfall in accounting for variations in Pythium spp. populations (38).

In studies of longer duration at Reading, Ali-Shtayeh et al. (3) observed a winter peak and a summer trough in populations of total Pythium spp. in soil. A sine curve model best explained the variations in populations. Predominant species such as P. intermedium also followed the same periodicity. Multiple regression indicated that soil moisture was more important than soil temperature in improving the fit of the periodic curve to observed data (3). However, the authors suggested that low populations in the summer may have been related to low soil water content and higher soil temperature.

Soil populations of P. ultimum Trow in cotton fields were highest in the cooler months than in August or early September, when they were the lowest (37). Seven of 10 fields exhibited this seasonal pattern of Pythium

populations. Temperatures, (30-37 C), were not favorable for survival of P. ultimum during the summer months, but were favorable (<28 C) in the cooler months of the season. In contrast to the majority of fields, one field in this study (37) had the greatest populations of P. ultimum during the late summer. It was suggested that extensive defoliation caused by Verticillium wilt in late August followed by a late irrigation provided considerable substrate for colonization that may have enhanced P. ultimum survival.

Growth of P. myriotylum and P. aphanidermatum in soil should be favored by warm temperatures (68). However, Lumsden et al. (49) reported that populations of P. aphanidermatum in a vegetable field were greatest in winter at the beginning of the study and were lower in the spring. It was suggested that germination of oospores of P. aphanidermatum followed by microbial lysis may have accounted for the lower population in the spring. Populations then remained low for two years, regardless of bean or rye rotation.

Similarly, no general pattern in populations of Pythium spp. in snap bean fields were noticed by Pieczarka and Abawi (58). In Brazil, Decarvalho and Milanez (16) found that populations of P. splendens in sterile soil were not affected by temperature.

Pythium myriotylum (19, 21, 46, 61, 62) and possibly other species (61, 62) are considered to be important pod-rotting fungi found in soil planted with peanut. Little was known about the fluctuations of Pythium spp. in peanut soil until the report of Filonow and Jackson (19), who observed an 8-10 fold increase in populations of Pythium spp. in an Oklahoma peanut field. Populations peaked at 60 after planting (DAP) in one year and at 75 (DAP) in another. These peaks occurred after pods had entered soil. Populations rapidly declined after these peaks and stayed low until harvest. This proliferation and decline of Pythium spp. in the same peanut field was later verified by Lewis and Filonow (46). Pythium myriotylum was frequently isolated from rotted pods in their study. These workers observed similar population peaks and declines in two other fields and reported no significant correlations between soil temperature or matric potential and population fluctuations. Lewis and Filonow (46) speculated that the increase and decline of Pythium spp. in soils observed in their study was attuned to the development and maturation of the pods. Their results suggested the involvement of peanut plant in the dynamics of Pythium spp. in soil.

Soil moisture is a critical factor in the epidemiology of any Pythium sp. (9, 39). High levels of soil moisture are needed for sporangial germination and zoospore dispersal. Frank (26) reported a positive relationship

between moisture in the top soil and pod rot infection. Hardman and Dick (38) found a positive correlation between soil moisture and fluctuations of Pythium spp. populations in soil. On the other hand, significant correlations were not observed between soil matric potential and fluctuations of Pythium spp. populations in three fields in Oklahoma (46).

The direct effect of soil fertility on populations of Pythium spp. in soil has received little attention. More work had been directed toward the role of inorganic nutrients, particularly calcium and nitrogen in disease incited by Pythium spp. Kao and Ko (43) reported that Hawaiian soils suppressive to the germination of P. splendens sporangia had high calcium content and high total microbial populations. Soils that favored sporangial germination were low in calcium and microbial populations. Effects due to pH and to formation of ammonia (45), which can be found in soils amended with calcium were ruled out. It was suggested that calcium enhanced microbial activity in suppressive soils leading to greater levels of fungistasis. On the other hand, calcium may enhance the survival of Pythium spp. Yang and Mitchell (69) showed that calcium aided the formation of Pythium oospores in a synthetic medium. Calcium is also needed in plant tissue for conversion of pectin to calcium pectate which helps cell

walls resist attack by polygalacturonase enzymes which may be produced by Pythium spp (17).

Pythium spp. are generally able to tolerate a wide range of pH (68). Pythium myriotylum grew on corn meal agar adjusted to pH 3-9, with an optimum of pH 6 (A. B. Filonow; personal communication). Thus, the effect of pH on predisposition of a host to infection by Pythium spp. is probably more important than direct effects on the fungus (39).

Principal biotic factors that have been reported to influence populations of Pythium spp. in soil are the host and antagonistic organisms.

The influence of living roots (rhizosphere), and seed (spermosphere) on microbial activity in soil is well known (4, 9, 12). Sugars, amino acids, organic acids, vitamins, minerals etc. exuded from roots and seeds affect phytopathogenic fungi in many ways. Exudates can stimulate the germination of fungal propagules, direct the movement of phytopathogenic inoculum to root or seed surfaces and increase the efficiency of inoculum in infection courts (9). Contrary to beneficial effects on disease development, exudates in the rhizosphere or spermosphere may activate microflora that are antagonistic to phytopathogenic fungi.

Pythium spp. are generally noted for their ability to attack seeds and succulent plants. Nutrients from plant tissue readily stimulate most propagules of Pythium spp.,

although in a few, such as oospores of P. myriotylum, germination is not greatly affected by exudates (5). Sugars and amino acids in soil have been shown to stimulate the germination of P. aphanidermatum and P. ultimum propagules (65, 66). Norton and Harman (55) showed that volatile exudates from germinating pea seeds did not increase populations of Pythium spp. in natural (nonsterile) soil; however in sterile soil infested with P. ultimum, populations of P. ultimum were increased by volatiles from pea seeds. The authors suggested that antagonistic microbial activity in natural soil was also activated to suppress P. ultimum populations. Seed and root exudates may also serve as chemoattractants for Pythium spp. zoospores. Recently, sloughed root cap cells of cotton were shown to act as attractants to zoospores of P. dissotocum (31). Zoospores were attracted to and rapidly killed isolated root cap cells.

The influence of peanut roots on Pythium populations in soil has received little attention (61, 62). Shay and Hale (63) reported that low levels of calcium in the culture medium containing peanut roots increased the exudation of sugars from the roots; however, the effect on growth of root-infecting fungi was not reported. More is known about fungal colonization of pods, because of their commercial importance. Populations of fungi and other microbes are generally several fold higher in the soil surrounding pods

(geocarposphere) than in the bulk soil (32, 51). McDonald (51) observed that as peanut pods developed, numbers of propagules of fungi other than Pythium spp. fluctuated in dilution platings of soil adhering to the pods. By 9-12 weeks after planting, the fungal population in the geocarposphere soil was relatively low and stable, but thereafter the population increased. At week 15 the population peaked and then declined until week 17 when it peaked and declined again.

Populations of Pythium spp. in soil containing peanut pods were monitored by Lewis and Filonow (46) in three fields in Oklahoma. They observed a proliferation and decline in the population of Pythium spp. in soil after pegging had commenced. The increase and decline were not directly influenced by soil temperature or matric potential. As an alternate hypothesis, Lewis and Filonow (46) proposed that the proliferation and decline of Pythium spp. populations in soils may have responded to the leakage of nutrients from developing pods.

Subramanyam and Prabhakar (67) showed that the rate of ^{14}C translocation into newly formed (10 days old) pods was low, but the amount of ^{14}C lost via exudation from pods was comparatively higher. In more developed pods (50 days old), ^{14}C translocated into pods was comparatively higher than ^{14}C lost by pod exudation. Similarly, Hale (33) reported that the concentration of sugars released by pods growing in

axenic culture, was greatest during the early development of pods. Mechanical injury to pods (34) and low concentrations of calcium in the pegging zone (33) may increase nutrient exudation from pods.

Lewis and Filonow (46) observed that the timing of Pythium spp. population peak was similar to the R4-R6 reproductive growth stages for peanut (7), during which plants have added significant pod numbers and weight. Prior to these stages, young, developing pods may have released sufficient carbon energy for a proliferation of microbial activity in soil. These workers (46) further suggested that following subsidence of nutrient exudation as pods matured, nutrient-starved microorganisms may have fed on hyphae of Pythium spp. in soil causing a decline in the population. Lysis and disappearance of hyphae may occur by various means of microbial antagonism (1, 6, 11, 41, 42, 48, 50, 54). Alternatively, hyphae of Pythium spp. may have moved from the bulk soil to colonize geocarposphere soil and the surface of pods as they matured. In this regard, Pattee et al. (57) have reported that maximal concentrations of sugars (mainly sucrose) and starch were found in the hull of developing pods before maxima in the seed. Maximum starch content in hulls occurred at early and middle pod maturity, whereas sugar content in hulls was greatest at near middle maturity. Species of Pythium can utilize both sucrose and starch as energy sources (68), and hyphae could move from

energy-deprived areas in nearby soil to exploit sucrose and starch as they become available in the hulls.

Relationship Between Populations of
Pythium spp. in Soil
and Disease

In steam-pasteurized soil artificially infested with Pythium propagules, workers often observe direct correlations between inoculum densities of Pythium propagules and disease. For instance, root rot severity of snap beans caused by P. ultimum in steam pasteurized soil was significantly correlated with inoculum density of sporangia (1-500/g soil) (58). Mitchell (52) reported that 15-43 oospores of P. myriotylum in pasteurized soil was needed for a 50% disease incidence of peanut, rye or soybean. The relationship between the inoculum density of Pythium spp. in natural soil and pod rot is more difficult to obtain. Csinos and Gaines (13) and Lewis and Filonow (46) found no clear cut relationship between populations of Pythium spp., as determined by plating soil dilution, and pod rot in peanut soil. Frank (25), however, found a significant correlation between recovery of Pythium spp. from sorghum baits incubated in peanut soil and pod rot incidence.

CHAPTER III

MATERIALS AND METHODS

Field Experiments

Ft. Cobb

Field studies were conducted in 1989 and 1990 at Ft. Cobb, Oklahoma. Soils in these plots was a fine sandy loam (62% sand, 24% silt and 14% clay). Other characteristics of this soil as determined by the Soil Fertility Laboratory, Oklahoma State University were: pH 7.0, 12.3 kg/ha surface nitrate, 0.216 ppm ammonia, 92 kg/ha phosphorus, 186 kg/ha potassium and 1093 kg/ha calcium. A plot consisted of four rows, 10.9 m long with 0.91 m row spacings, arranged in a randomized complete block design with five replicates per treatment. Treatments were peanut, (cv. Florigiant), soybean, (cv. Forrest) or fallowed soil. Peanut and soybean seeds were treated with Granox PMF (Gustafson) at 3.9 cc/kg of seed and planted at 10 seeds per meter on May 24 in 1989 and 17 seeds per meter on May 15 in 1990. Except for one application of Orthene at 265 cc/ha for thrips control in 1989, no pesticides were applied to the plots. Weeds were

hand hoed . All plots were irrigated with ca. 5 cm of water/irrigation every 7-10 days in the absence of rain.

Soil from each row in the plots was sampled on the day of planting and periodically thereafter up to harvest. A total of three random samples from a row were taken with a garden trowel to a depth of 7-10 cm and composited in a plastic bag to give one sample per row. In rows with peanut or soybean plants, samples were obtained from the pegging zone or the root zone of the rows, respectively. Soil sampling of fallowed plots was done along the middle of rows marked by stakes. Bags were kept in a styrofoam cooler in the field, transferred to 5 C within 8 h, and assayed for populations of Pythium spp. within 24-48 h after collection.

The soil in each bag was hand mixed and 10 g of a subsample was suspended in 90 ml of sterile 0.1% agar in water (w/v) in 250 ml flasks. One 10 g sample from each bag was also air dried at 80 C for 72 h and reweighed for dry soil weight calculation. Flasks were shaken for 30 min. on a reciprocating shaker. Populations of Pythium spp. in soil were estimated by plating 0.2 ml of this dilution (1/10) or 1/50 (if needed) on each of 5 dishes (9 cm dia.) of a Pythium selective medium (PSM) (46). Dishes were incubated at 23-25 C for 36-48 h, after which they were washed under running water and colonies were counted. Population data were expressed as propagules (p)/g oven dried soil.

During each soil sampling, soil temperature readings at one location in each row were obtained using thermistors which were buried at 7.5 cm deep in the soil (46). Resistance readings were taken with an ohmmeter and converted into temperature using a conversion table supplied by the thermistor manufacturer (Radio Shack).

Matric potential of soil obtained during sampling of plots was determined using a soil moisture release curve established from readings using a soil moisture pressure plate apparatus (Soil Moisture Equipment Corporation, Santa Barbara, CA) (46).

Soon after pegging, three peanut plants were periodically removed from each row to monitor Pythium spp. colonization of pods. Pods from the plants were combined into one sample per row. Pods were washed with water, cut into ca. 1 cm pieces, and five randomly selected pieces were plated on each of ten dishes of PSM. Five dishes were incubated at 23-25 C and five were incubated at 37 C. After 24-48 h, dishes were examined for colonies of Pythium spp. Selected colonies were subcultured and stored on corn meal agar for future identification.

At harvest, peanut plots were dug with a digger-invertor. Plants were threshed with a Kincaid stationary peanut thresher, and all pods from each row were collected in a large plastic bag. Pods were washed with water and air dried for 48 h at 23-25 C on absorbent towels.

The pods were returned to plastic bags and stored at 5 C until were rated for pod rot severity. The pods were rated on a pod rot pod rot severity index of: 1=no pod rot; 2=1-25% pod rot; 3=26-50% pod rot; 4=51-75% pod rot and 5=>75% pod rot. A mean pod rot index for each row was calculated by summing the number of pods in disease indices 3, 4 and 5 (which are the classes that cause the greatest economic losses), and dividing by the total number of pods. Isolations for Pythium spp. from pods were made as described above.

Other fields in 1990

Fluctuations of Pythium spp. in peanut soils from seven fields other than Ft. Cobb were also monitored in 1990. Three of these were in Caddo county, two were in Garvin county and two were in Marshall county. The fields in Caddo county were known to support pod rot caused by Pythium spp. (21). The other fields were chosen after preliminary population assays in late May, 1990 showed measurable populations (>10-20 p/g) of Pythium spp. in their soils.

Fields C4, C6 and C10 in Caddo County were planted on May 21, May 18 and May 20. Fields G1 and G2 in Garvin County were planted on May 15. Fields M1 and M2 in Marshall County were planted on May 17. Peanut plants in field C4 of Caddo County were planted in sourghum stubble, and one field (G2) in Garvin County was double-row-planted (0.65 m

sapcings between rows). Sampling commenced in these fields ca. 2-3 weeks after planting, when seedlings were 5-10 cm tall. All fields were sampled on the same day as the Ft. Cobb sampling.

At each field, a permanent reference (e.g. a telephone pole) on an outside corner of the field was used to align the direction of the traverse into the field for locating the sampling area. Including the first row of peanut at the edge of the field, the sampling area was 20 rows into the field on a perpendicular line from the reference. From the 20th row, 10 successive rows were sampled. Three random soil samples of the root and/or pegging zones (7-10 cm deep) of plants were taken along each row, composited into a plastic bag. Pythium spp. populations were assayed as described above. On the day of sampling, soil temperature in the root/pegging zones of the rows was measured with a bi-metal thermometer after 20-40 min. equilibration in soil. Soil moisture content of soils was determined as above.

Growth Chamber Experiments

Box Experiments

Styrofoam ice chests (30 X 40 X 60 cm) were filled to capacity with soil from field plots at Ft. Cobb. The interior length of a box was divided into three soil sampling zones, each 20 cm long (Figure 1). Peanut seed

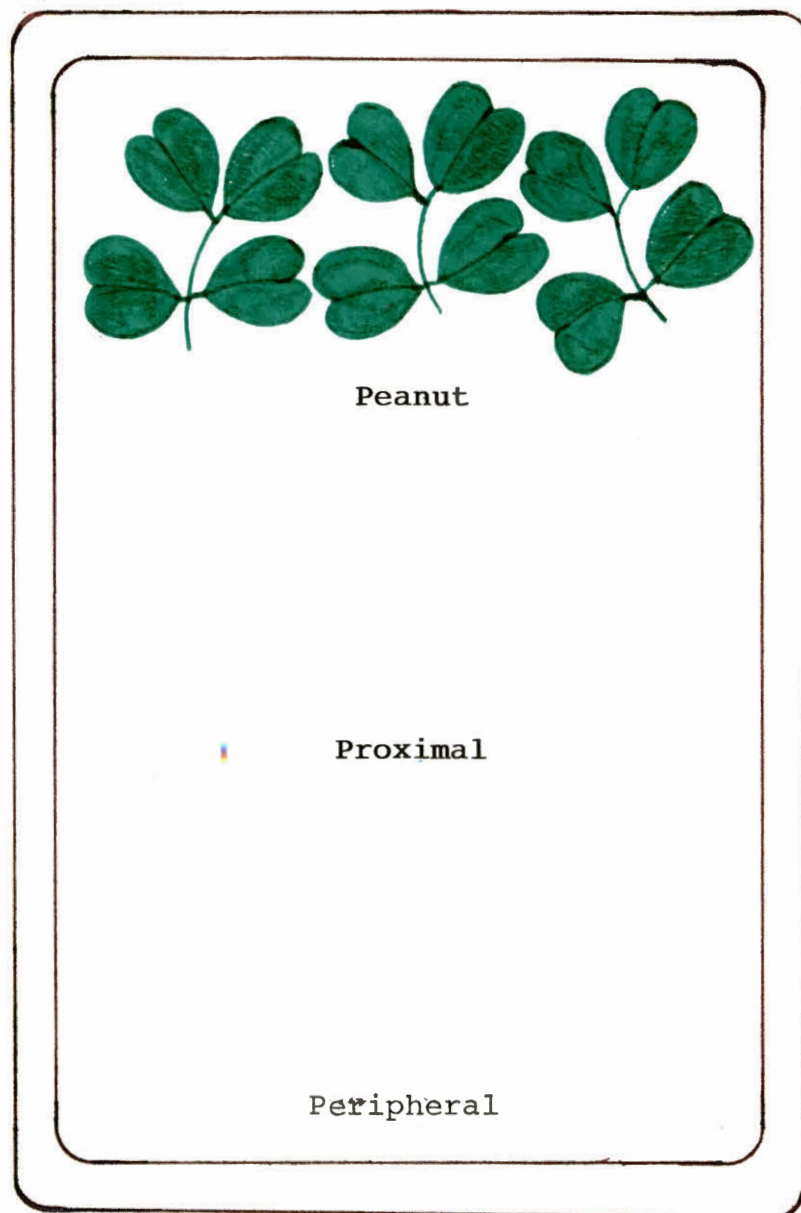


Figure 1. Peanut, proximal and peripheral zones for soil sampling in the box experiments.

(cv. Pronto) that had been treated with Granox PMF at 3.9 cc/kg were planted in one row 10 cm from the edge of the narrow (40 cm) side of each box. Eight seed per row (one/5 cm) were planted. The next zone out from the peanut zone (20-40 cm out from the edge) constituted the proximal zone of soil in the box. The zone of soil farthest away from the peanut plants (40-60 cm from the edge) was the peripheral zone of soil. There were 7 boxes in the first experiment which was conducted at 25-28 C on a laboratory bench under a tungsten, high intensity lamp ($550 \mu\text{E}/\text{m}^2/\text{sec}$; 12 h day/12 h night). In the second experiment 9 boxes of soil were used and these were incubated in a growth chamber under 10 h of light ($500 \mu\text{E}/\text{m}^2/\text{sec}$) at 27 C and 14 h of darkness at 24 C. The boxes were completely randomized in both experiments. Soil in the boxes were watered with 500 ml of deionized water in each zone of soil every 2-3 days. Every 10-14 days, 50 ml of a fertilizer solution (15-30-15) of (Miracle Gro Sterns Co., Port Washington, N.Y.) were added to each zone.

Three random samples of soil from each sampling zone of soil in a box were removed with a spatula (1.5 cm x 10 cm) and composited (ca. 40 g in a plastic bag). Populations of Pythium spp. and soil moisture content were determined on a monthly basis, as described previously. Plants in the first experiment were harvested at 161 DAP, and those in the second were harvested at 165 DAP. Pods were washed with

water and examined for pod rot symptoms. The presence of Pythium spp. was determined by plating pod pieces on PSM.

Pod Training Experiments

Pod training experiments were conducted in soil enclosed in a system of nested plastic pots (Figure 2). The inner pot was 17 cm dia. x 18 cm and it was nested inside a 24 cm x 28 cm pot. A piece of PVC pipe (2 cm i.d. x 12 cm) was cemented with silicone caulk (Dow Chemical) on the inside and at the bottom of each pot. The silicone rubber was allowed to cure for 2 days prior to filling the pots with soil. This pipe allowed for drainage of soil water from the inner pot without contaminating the soil in the outer pot. The inner pot had small holes on the sides at the bottom for drainage. The pots were filled with soil from the plots in Ft. Cobb.

Florigiant seed were surface disinfested in 1.05% (v/v) sodium hypochlorite for 4 min., rinsed several times in sterile water and incubated under sterile, moist paper towels for 3-4 days at 25 C. One germinated seed was planted in each inner pot. Nested pots were incubated in walk-in growth chambers at 26 C under $550 \mu\text{E}/\text{m}^2/\text{sec}$ of light for 12 h and under 12 h of darkness at 24 C. At pegging (ca. 45 DAP), pegs were trained or not trained into the outer pot to result in soil with no roots or pods, 50% of the available pods, and 100% of the available pods.

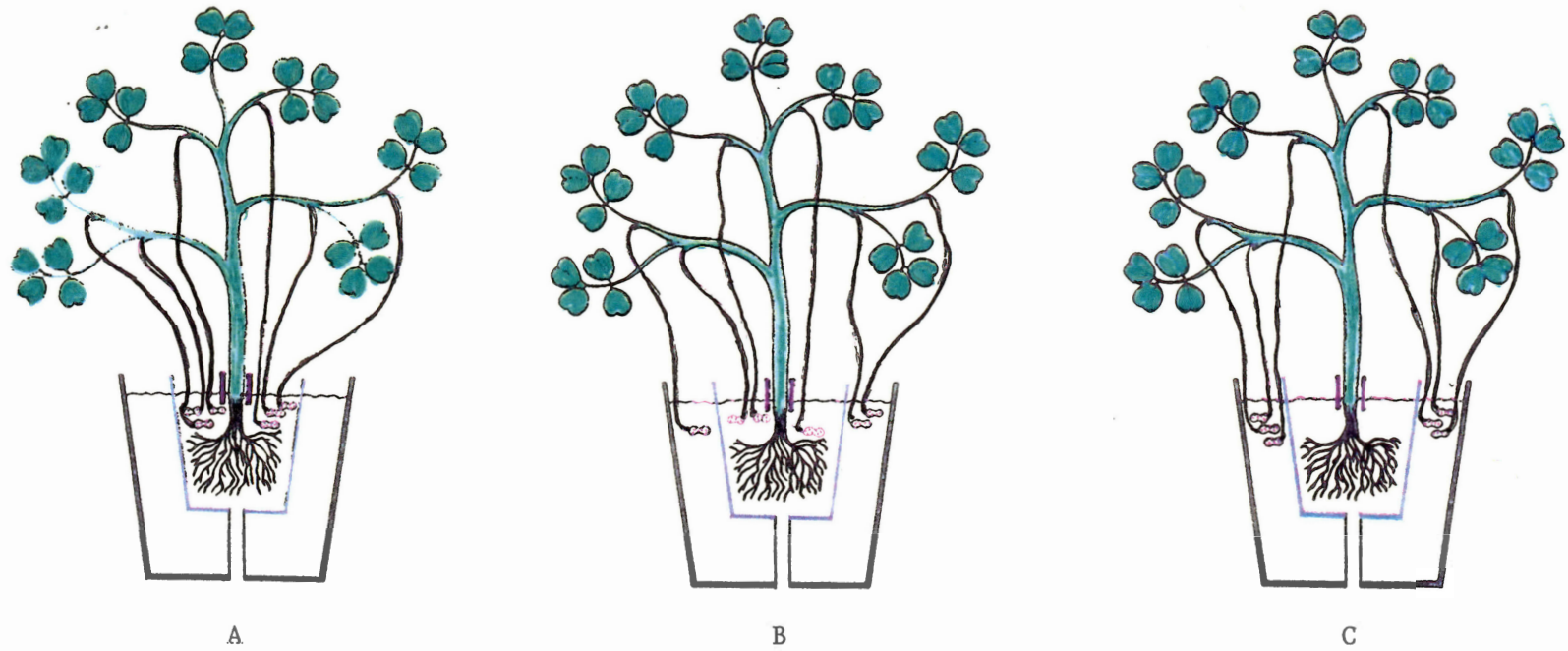


Figure 2. Nested pot arrangements for the pod training experiments. (A) no pods or roots in the outer pot and roots and 100% of the available pods in the inner pot; (B) 50% of the pods in the outer pot and roots plus 50% of the pods in the inner pot; (C) 100% of the pods in the outer pot and roots only in the inner pot.

Treatments in the inner pot were all roots, roots plus 50% of pods and roots plus 100% of the pods. There were six replicates per treatment. Plants were watered daily, and every two weeks 25 ml of Hoagland's solution was added to the inner and outer pots. A second experiment was conducted without the 50% pods and roots and the 50% pods treatments. In this experiment there were 6 replicates per treatments. Treatments in both experiments were completely randomized. Pythium spp. populations in soils were monitored on a monthly basis, as described previously. From each replicate there was one sample from the inner and one from the outer pot. Each sample was the composite of three 5-10 g subsamples. At harvest, pods were examined for pod rot symptoms and pod pieces were plated on PSM to confirm the presence of Pythium spp.

Pathogenicity of Pythium species to peanut pods

The following species were evaluated for their pathogenicity to pods of Pronto peanut: P. aphanidermatum, P. arrhenomanes, P. debaryanum, P. irregulare, P. myriotylum, and P. ultimum. Colonies of these species were maintained on CMA. Inoculum of each species was grown aseptically for 4 weeks in sterile corn meal/sand (5 g/95 g) cultures (22) in 250 ml flasks. Cultures inoculated with CMA plugs without the fungi were the controls. Cultures

were blended with water for one minute in a Waring blender (22) and mixed with steam-pasteurized soil (2 part sand; 1 part loam soil: 1 part peat moss, v/v). The population densities of Pythium spp. in infested soils were estimated as described above. Steam-pasteurized soil was used to dilute these initial densities to 30 p/g for all Pythium spp.

Pronto seeds were surface disinfested in sodium hypochlorite and germinated, as previously described. One germinated seed was planted in a 17 cm dia x 18 cm plastic pot containing steam pasteurized soil (2 part sand: 1 part loam soil: 1 part peat moss, v/v). Pots were incubated in a growth chamber at 27 C and $450 \mu\text{E}/\text{m}^2/\text{sec}$ for 10 h and at 24 C in darkness for 14 h. Plants were watered daily and fertilized with 50 ml of Hoagland's solution every two weeks. When pegs had begun to enter the soil in some pots, ca. 250 cc of soil from the pegging zone of a plant was removed and replaced with a 250 cc of infested soil. Plants receiving noninfested soil were the controls. Treatments were completely randomized with 6 replicates in the first experiment, and 10 replicates in the second experiment.

At harvest, pods were washed and rated for pod rot severity, as previously described. Plant height from the tip of the root to the top leaves was measured and the total number of pegs and pods per plant were recorded. Isolations for Pythium spp. from pods were made on PSM.

Statistical Analysis

Data were statistically analyzed using a Costat computer program (Version 3.0; CoHort Software; Berkeley, CA). In the field studies, populations, soil temperature, and soil moisture data were entered into the costat data base on a treatment by replicate by row basis. There was one datum per row per sampling date (20 data points per treatment per sampling date at Ft. Cobb in 1989 and 1990 and 10 data points per sampling dates at each of the other field sites in 1990). In growth chamber experiments there was one datum per treatment per sampling date. Data were subjected to one way or two way analysis of variance and significant differences between means determined from the Student-Newman-Keuls test at $P \leq 0.05$. The correlation between sampling date and fluctuations of Pythium spp. populations in soil was assessed. Influence of soil temperature and/or soil moisture on population fluctuations were also determined by polynomial or multiple regression analyses.

CHAPTER IV

RESULTS AND DISCUSSION

Field Experiments

Ft. Cobb 1989

Populations of Pythium spp. in fallowed soil (Figure 3) ranged from 12.2 to 44.9 p/g soil over the growing season. No significant ($P=0.05$) fluctuation in population over time was observed. Populations of Pythium spp. were not correlated ($P=0.05$) with soil temperature (Figure 4; $r=0.39$; $n=17$) or matric potential (Figure 5; $r=-0.13$; $n=17$). Multiple regression analysis showed no effect ($P=0.169$; $r^2=0.21$; $n=17$) of soil temperature and matric potential on populations of Pythium spp. in fallowed soil.

In soil planted with soybean (Figure 3), populations fluctuated from 15.6 to 127.0 p/g soil. At 100 DAP, the populations of Pythium spp. peaked to 127 p/g, which was greater ($P=0.01$) than all other population values for soil planted with soybean. This population peak in soybean soil was also greater ($P=0.05$) than populations in fallowed or peanut soils at 100 DAP. Fluctuation in populations were not correlated with soil temperature (Figure 6; $r=0.22$;

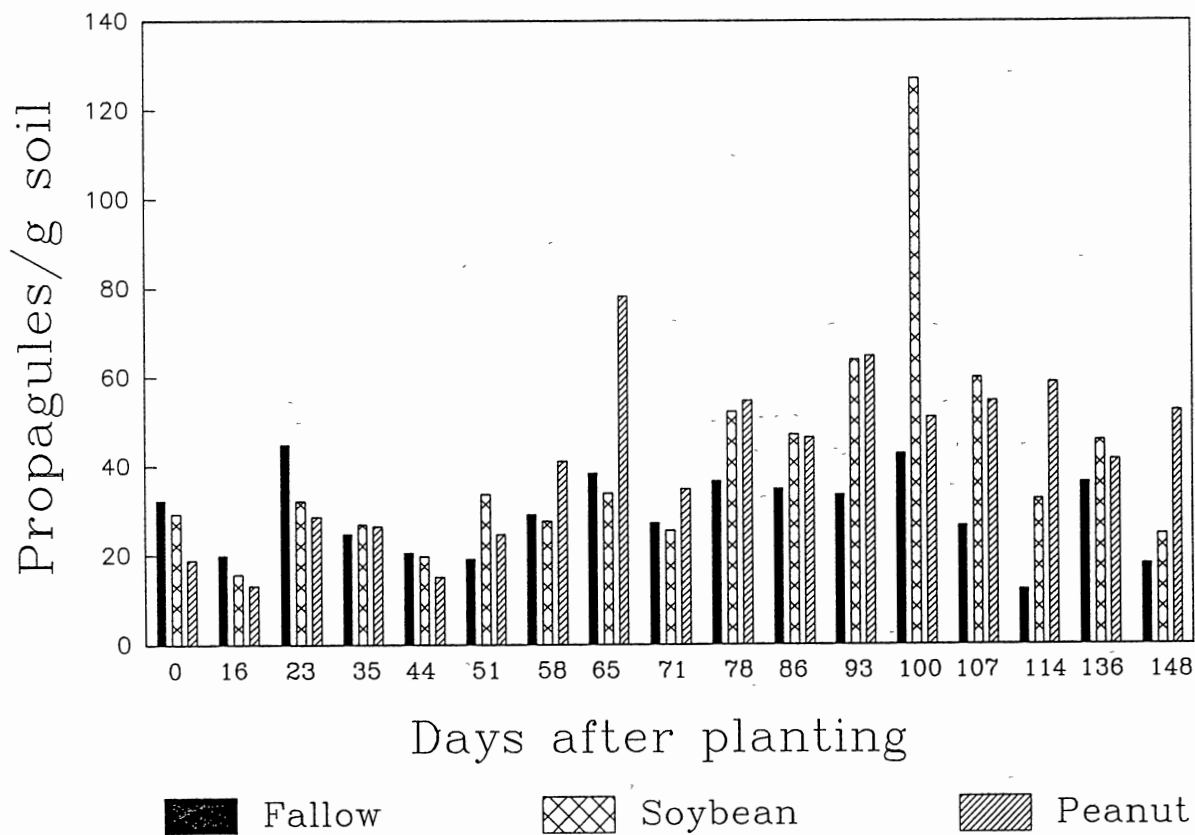


Figure 3. Populations of *Pythium* spp. in fallowed soil or in soils planted to peanut or soybean at Ft. Cobb, Oklahoma in 1989.

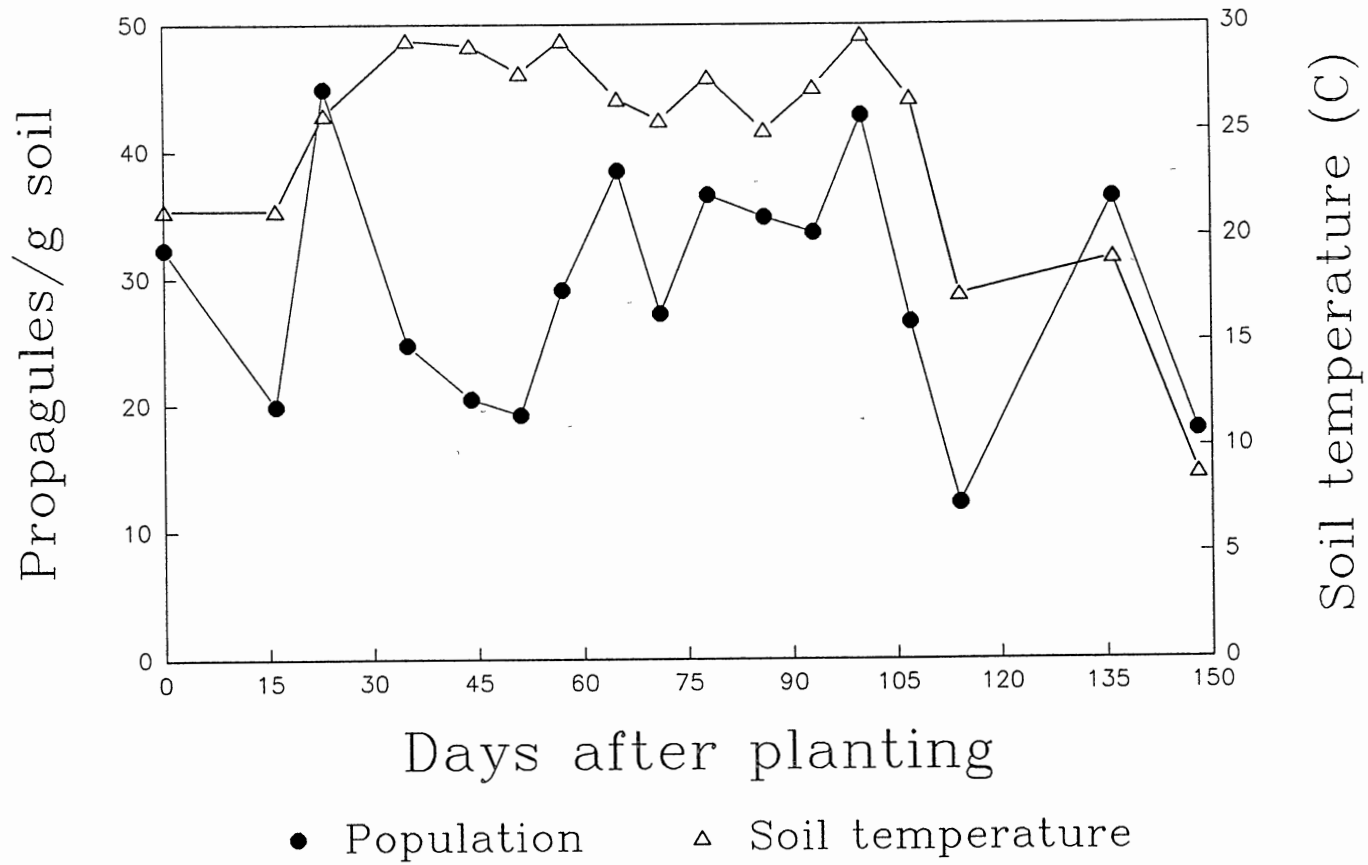


Figure 4. Populations of *Pythium* spp. in fallowed soil as related to soil temperatures at Ft. Cobb, Oklahoma in 1989.

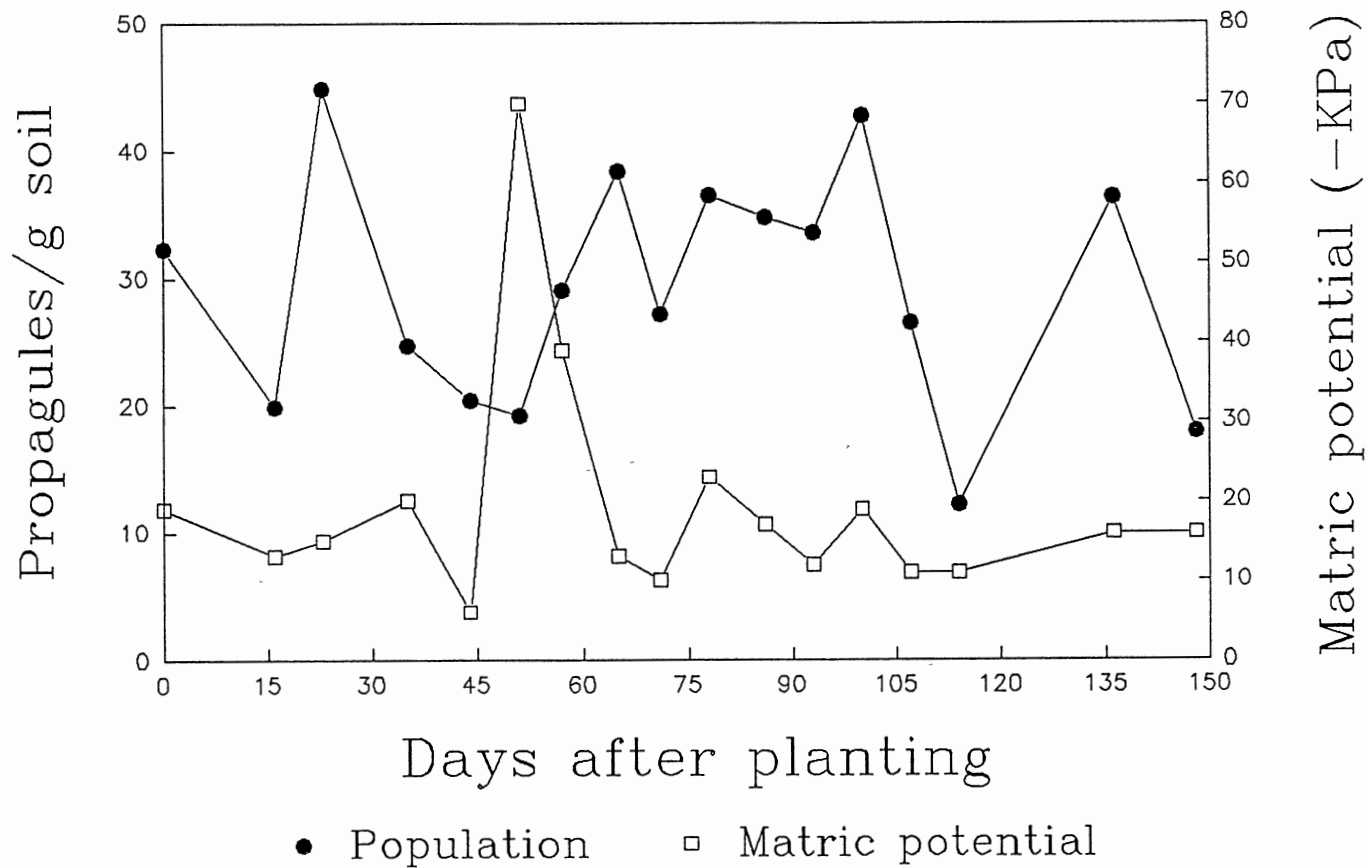


Figure 5. Populations of *Pythium* spp. in fallowed soil as related to soil matric potentials at Ft. Cobb, Oklahoma in 1989.

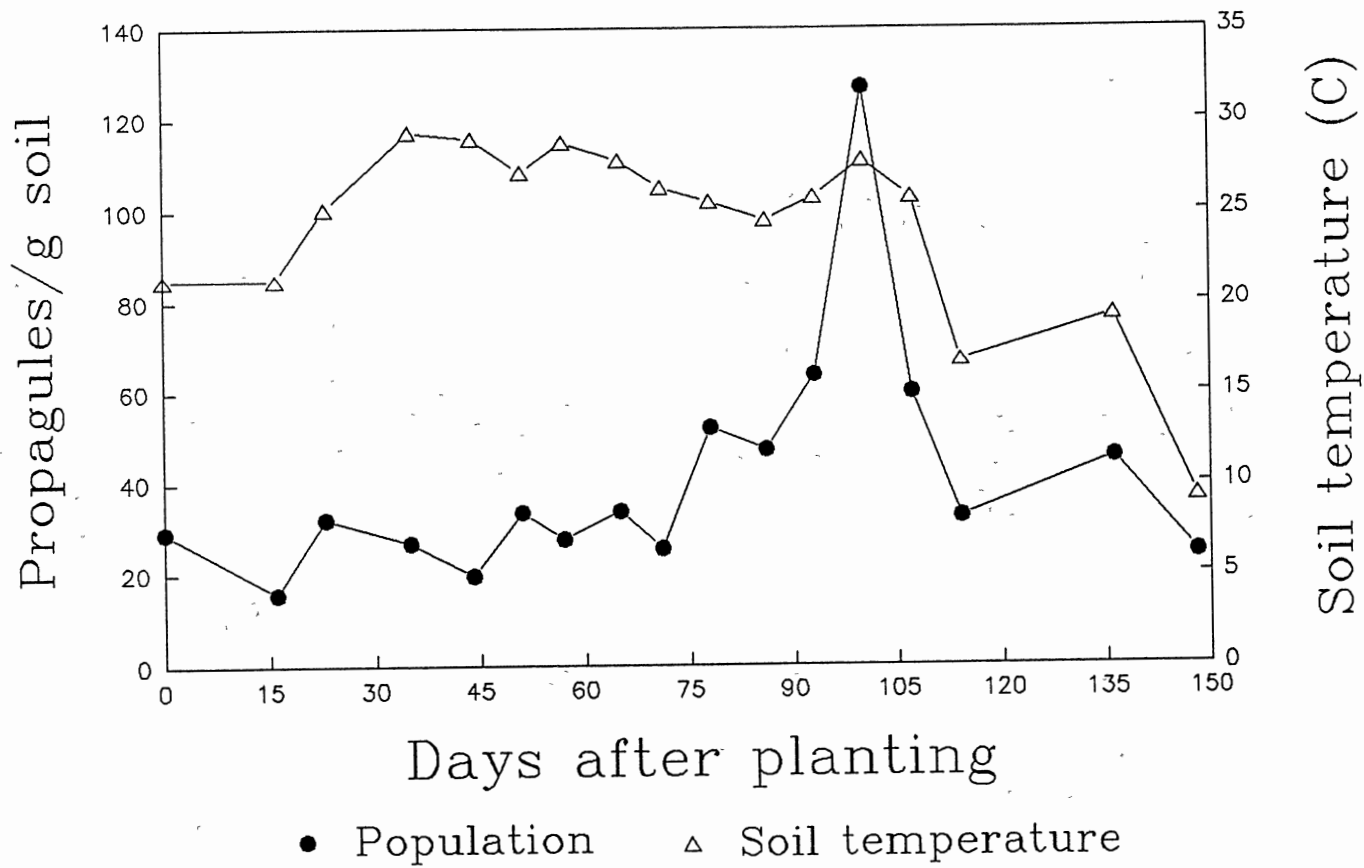


Figure 6. Populations of Pythium spp. in soil planted with soybean as related to soil temperatures at Ft. Cobb, Oklahoma in 1989.

n=17) or matric potential (Figure 7; $r=0.26$; $n=17$), nor were fluctuations due to the interactive effects of temperature and matric potential ($P=0.52$; $r^2=0.09$; $n=17$).

Populations of Pythium spp. varied from 13.1 p/g to 78.1 p/g in soil planted with peanut (Figure 3). At 65 DAP the population of Pythium spp. increased to 78.1 p/g. This increase was greater ($P=0.01$) than populations in peanut soil at 58 and 71 DAP. No other significant ($P=0.05$) peak in Pythium spp. populations was observed over time during the season. Populations of Pythium spp. were not correlated with soil temperature (Figure 8; $r=-0.14$; $n=17$) or matric potential (Figure 9; $r=0.01$; $n=17$). No interaction of soil temperature and matric potential with populations of Pythium spp. in soil was found ($P=0.86$; $r^2=0.02$; $n=17$).

Pod rot was severe in the peanut plots at harvest. Mean pod rot severity for all plots was 0.93. There was no significant ($P=0.05$; $n=5$) correlation between the mean pod rot severity in each plot and mean Pythium spp. populations per plot in peanut soil at harvest. Isolation of Pythium spp. from pods increased as the growing season progressed. Mean isolation frequency was 1.6%, 16.8%, 49.8% and 50.2% at 65, 78, 114 and 148 DAP, respectively. Forty six percent of the Pythium spp. isolated from pods at harvest (148 DAP) and subcultured on CMA at 37 C were P. myriotylum as indicated by rapid growth and abundant clusters of appressoria (68).

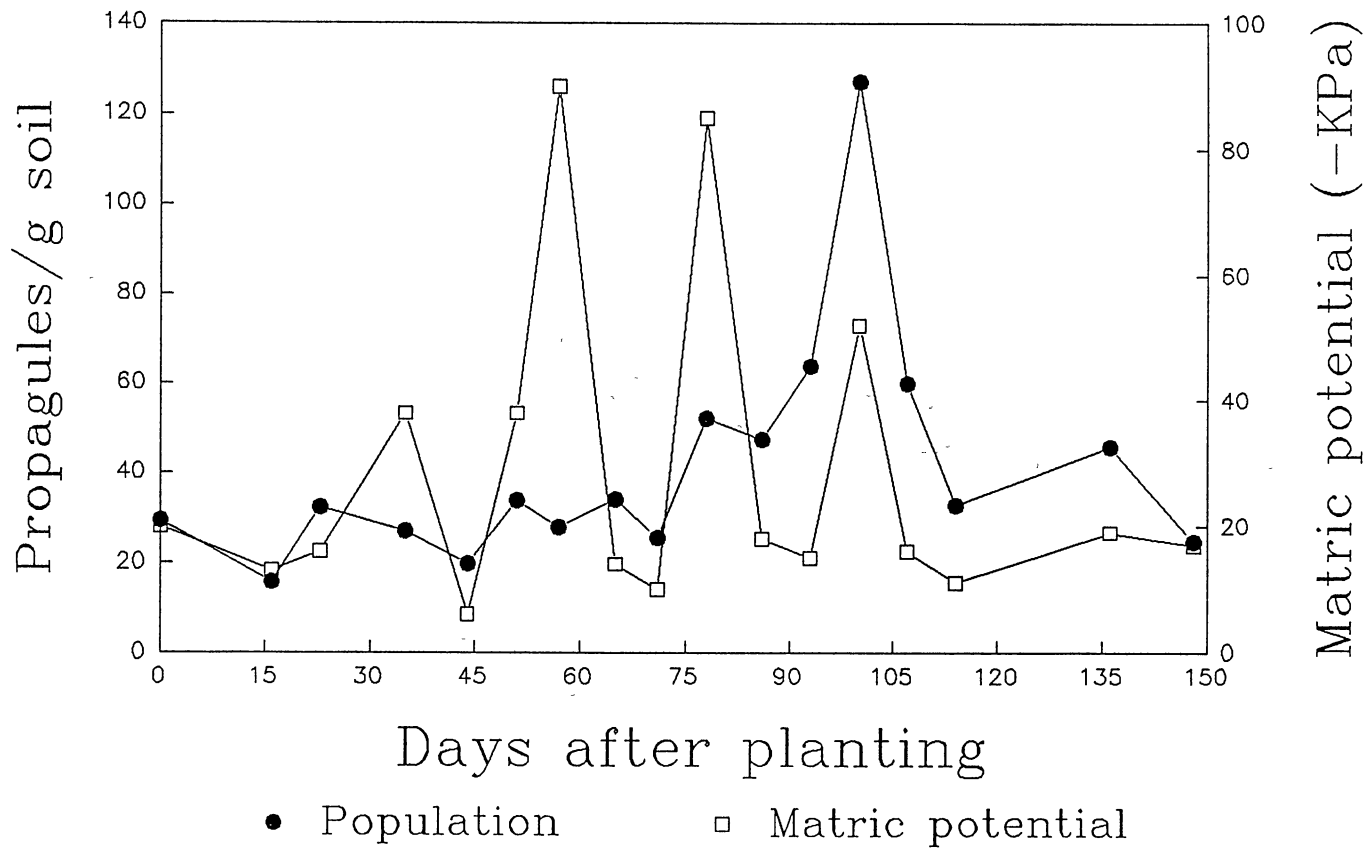


Figure 7. Populations of *Pythium* spp. in soil planted with soybean as related to soil matric potentials at Ft. Cobb, Oklahoma in 1989.

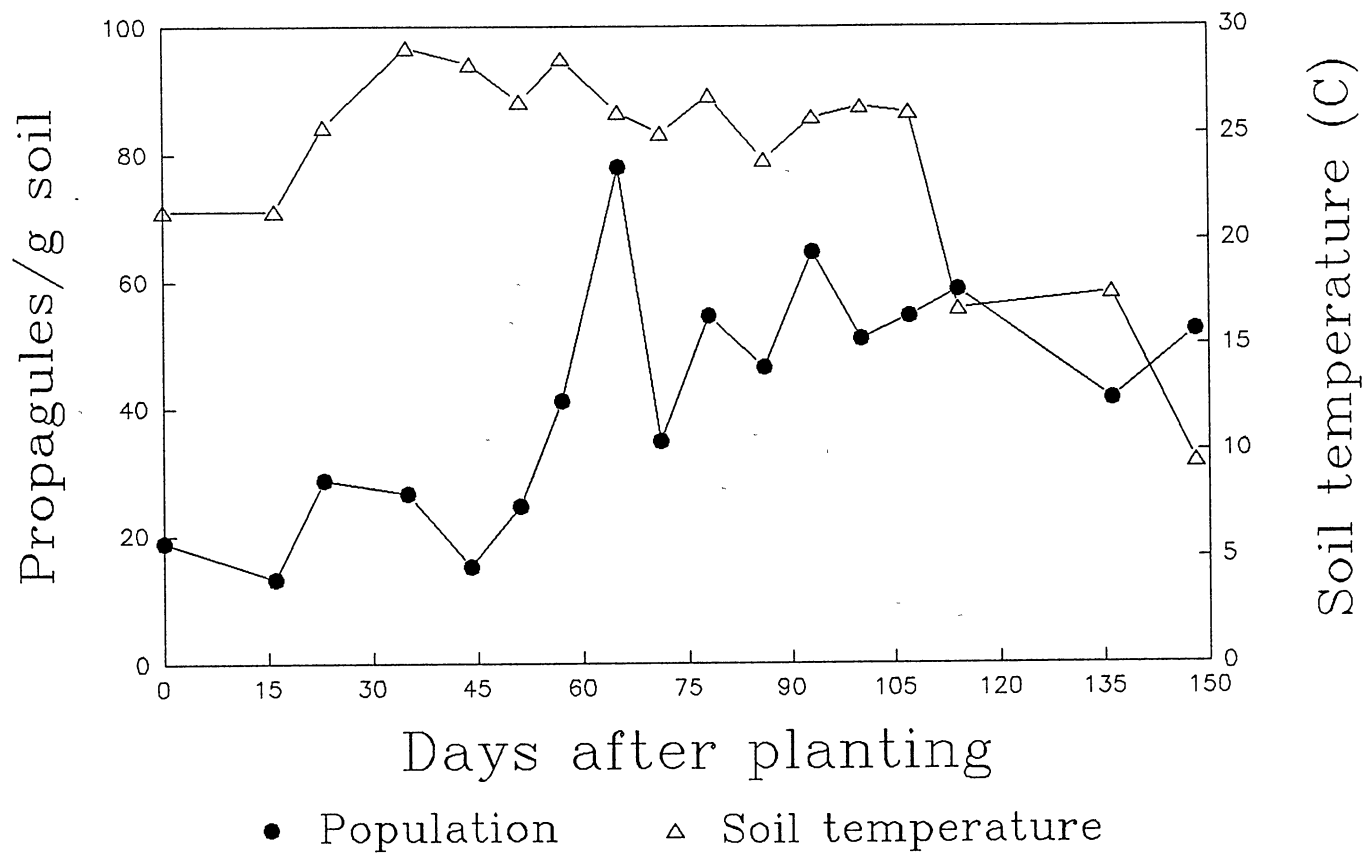


Figure 8. Populations of *Pythium* spp. in soil planted with peanut as related to soil temperatures at Ft. Cobb, Oklahoma in 1989.

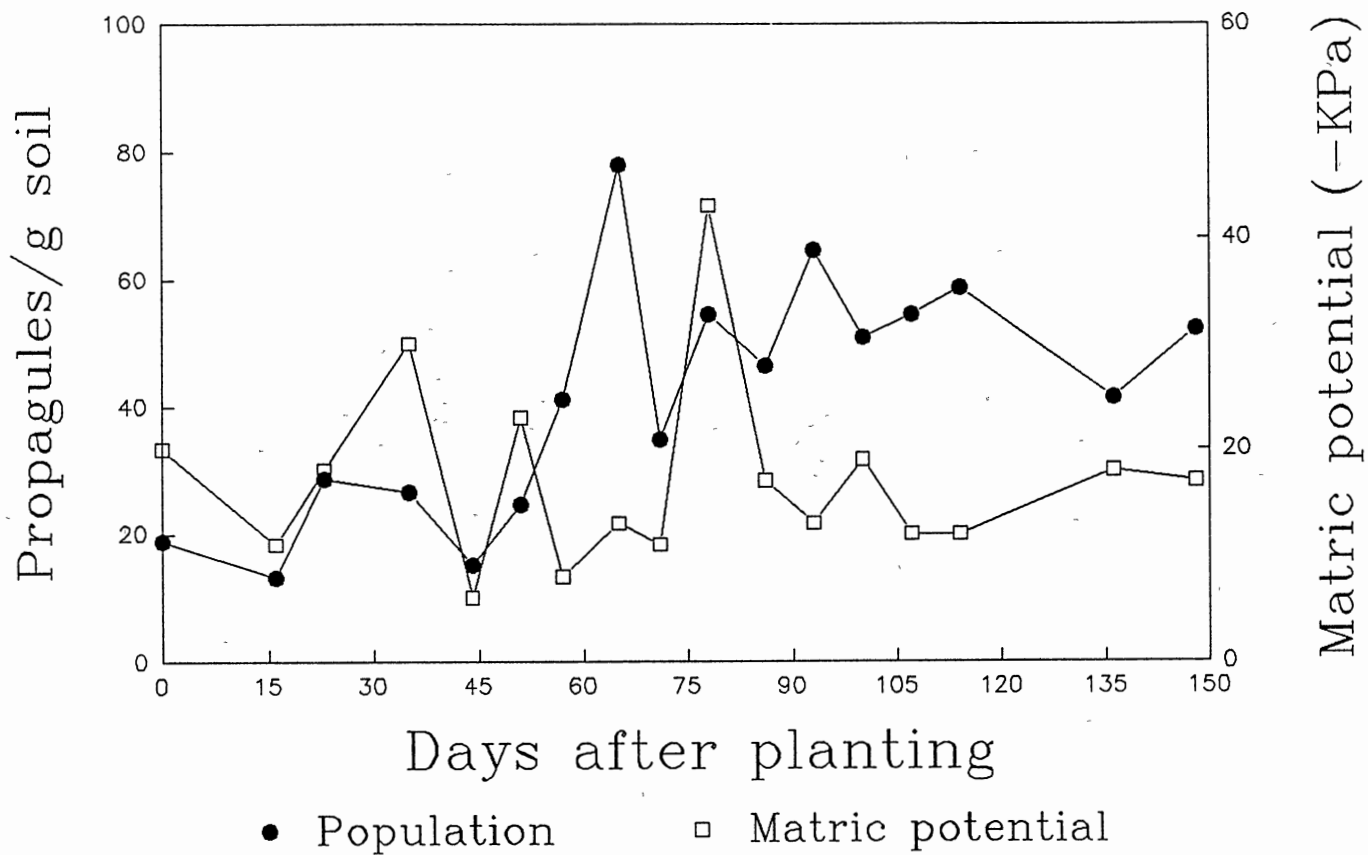


Figure 9. Populations of *Pythium* spp. in soil planted with peanut as related to soil matric potentials at Ft. Cobb, Oklahoma in 1989.

Averaged over the entire growing season, the mean population of Pythium spp. in fallowed soil (29.3 p/g) was lower ($P=0.05$) than those in soils planted to peanut or soybean. The mean seasonal population in peanut soil (41.5 p/g) was not greater ($P=0.05$) than in soybean soil (41 p/g).

At specific sampling dates, there were few differences ($P=0.05$) between treatment populations in soils until 65 DAP, when the peanut population was significantly greater ($P=0.05$) than populations in fallow or soybean soil (Figure 3). Thereafter, populations in fallowed soil were generally lower ($P=0.05$) than those in peanut or soybean soil. At 100 DAP, the population in soybean soil (127 p/g) was greater ($P=0.01$) than that in peanut or fallow soil.

Ft. Cobb 1990

Populations of Pythium spp. in fallowed soil (Figure 10) ranged from 4.7 p/g to 57.6 p/g. No differences ($P=0.05$) in populations over time were found, except at 101 DAP when the population (57.6 p/g) was greater ($P=0.01$) than at 93 DAP, but not greater ($P=0.05$) than at 109 DAP. No correlations between populations and soil temperature (Figure 11; $r=-0.31$; $n=13$) or soil matric potential (Figure 12; $r=-0.09$; $n=13$) were found. Multiple regression analysis showed no effect ($P=0.51$; $r^2=0.13$; $n=13$) of soil temperature and matric potential on populations of Pythium spp. in fallowed soil.

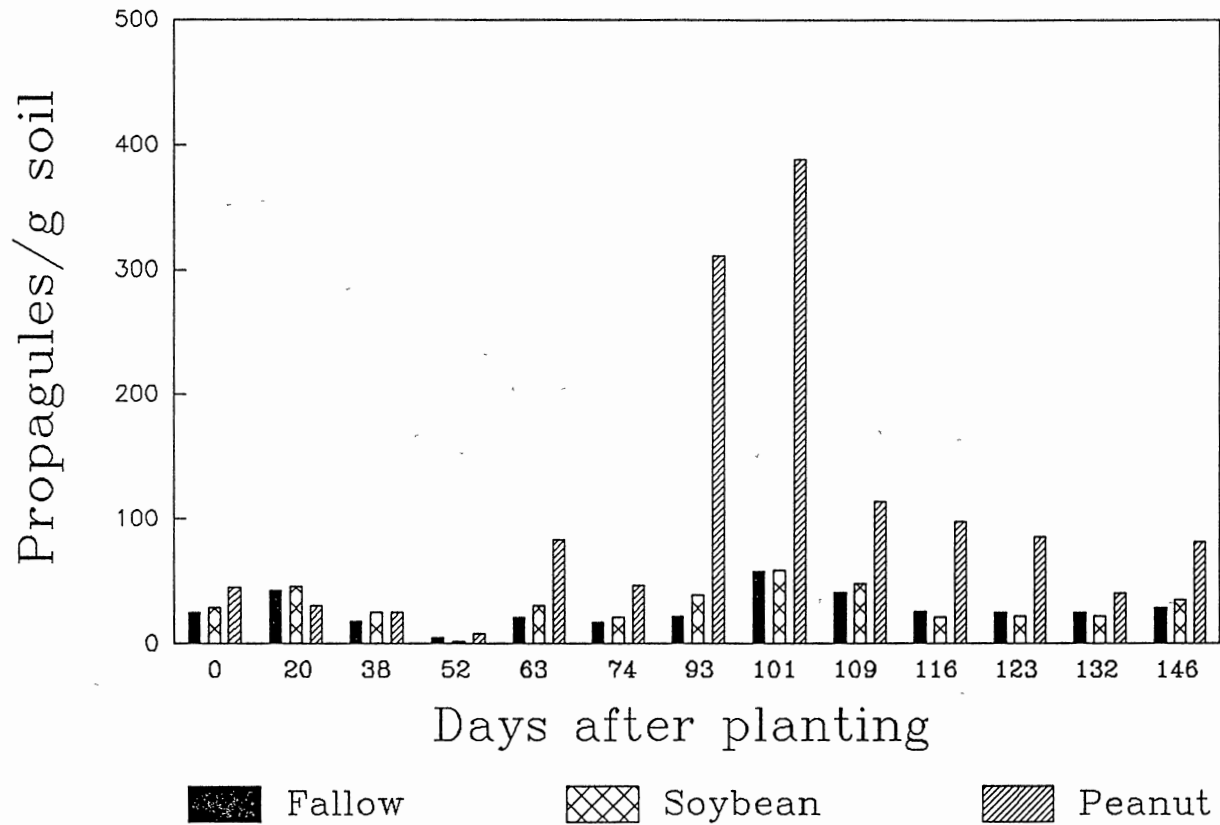


Figure 10. Populations of *Pythium* spp. in fallowed soil or in soils planted to peanut or soybean at Ft. Cobb, Oklahoma in 1990.

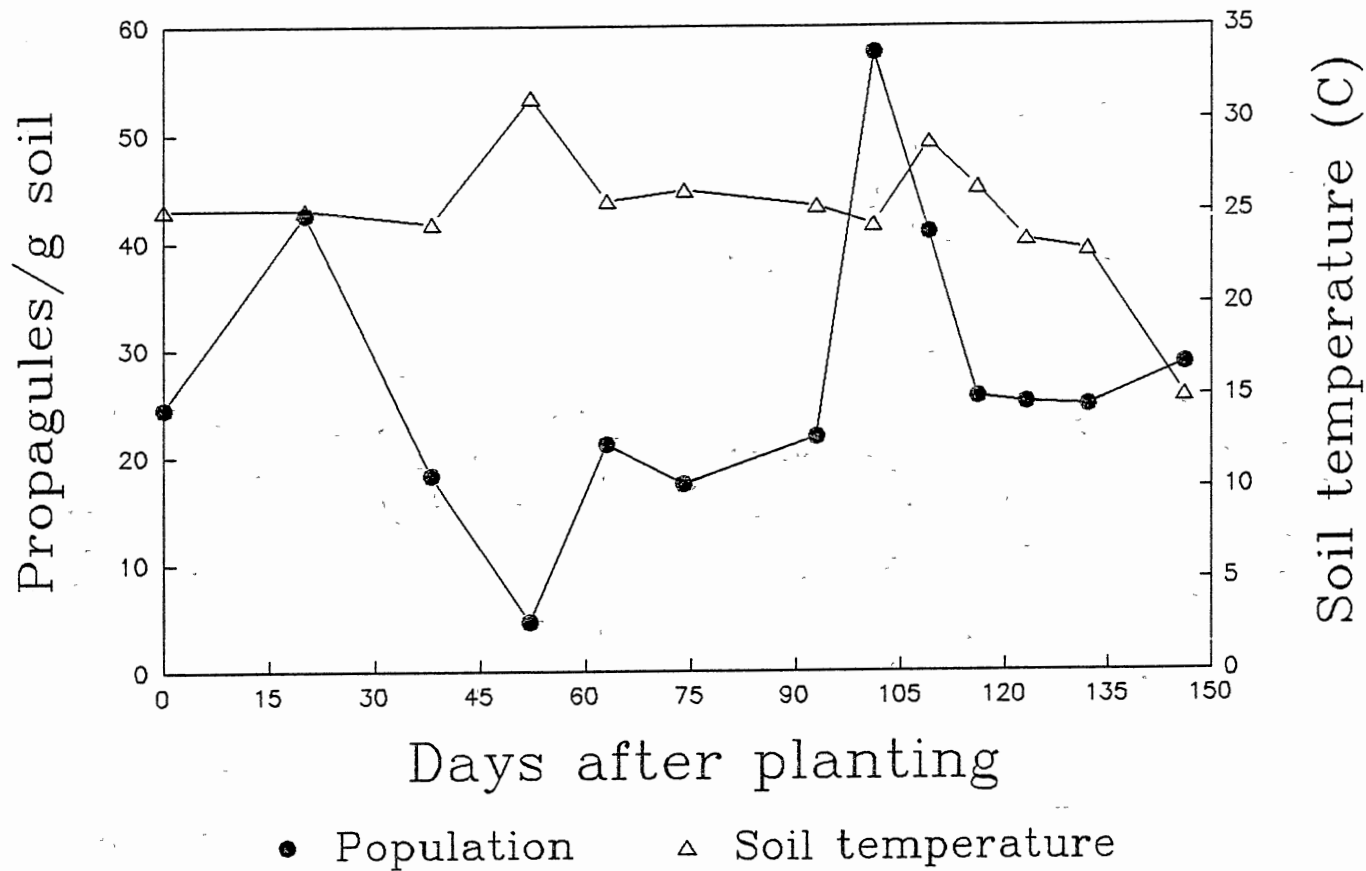


Figure 11. Populations of *Pythium* spp. in fallowed soil as related to soil temperatures at Ft. Cobb, Oklahoma in 1990.

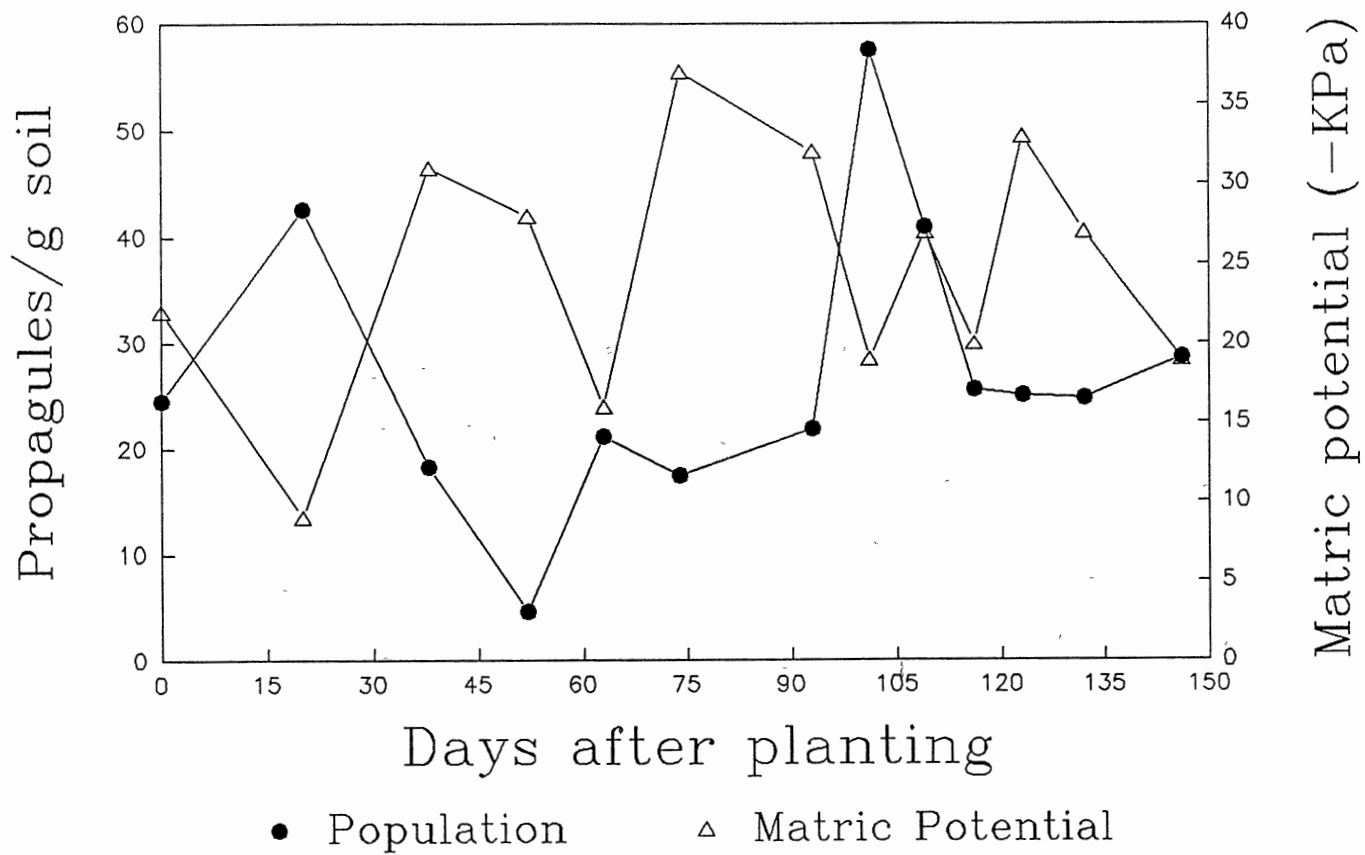


Figure 12. Populations of *Pythium* spp. in fallowed soil as related to soil matric potentials at Ft. Cobb, Oklahoma in 1990.

During the season, leaves and stems of soybean plants had been eaten repeatedly by deer and rabbits, leaving stunted plants with few pods at harvest. Populations of Pythium spp. in soil planted to soybean (Figure 10) varied from 2 p/g to 58.7 p/g during the growing season. No significant ($P=0.05$) fluctuations in populations over time were observed. Changes in populations were not correlated with soil temperature (Figure 13; $r=-0.03$; $n=13$) or matric potential (Figure 14; $r=-0.08$; $n=13$). No interactive effect of temperature and matric potential on population fluctuations ($P=0.94$; $r^2=0.01$; $n=13$) was found.

In peanut soil (Figure 10), populations of Pythium spp. fluctuated from 8.2 p/g to 388.5 p/g. After planting, populations waxed and waned over the season without significant ($P=0.05$) differences until 93 DAP, when the population increased to 311.2 p/g. This population increase was greater ($P=0.01$) than the population at 74 DAP (114 p/g). The population continued to increase, reaching a maximum (388.5 p/g) at 101 DAP, which was significantly ($P=0.05$) greater than all other seasonal populations in peanut soil except at 74 DAP. Thereafter, populations in peanut soil showed a precipitous reduction by 109 DAP followed further by a slow decline until harvest (146 DAP). Populations of Pythium spp. in peanut soil were not correlated with fluctuations in soil temperature (Figure 15; $r=-0.06$; $n=13$) or matric potential (Figure 16; $r=0.15$;

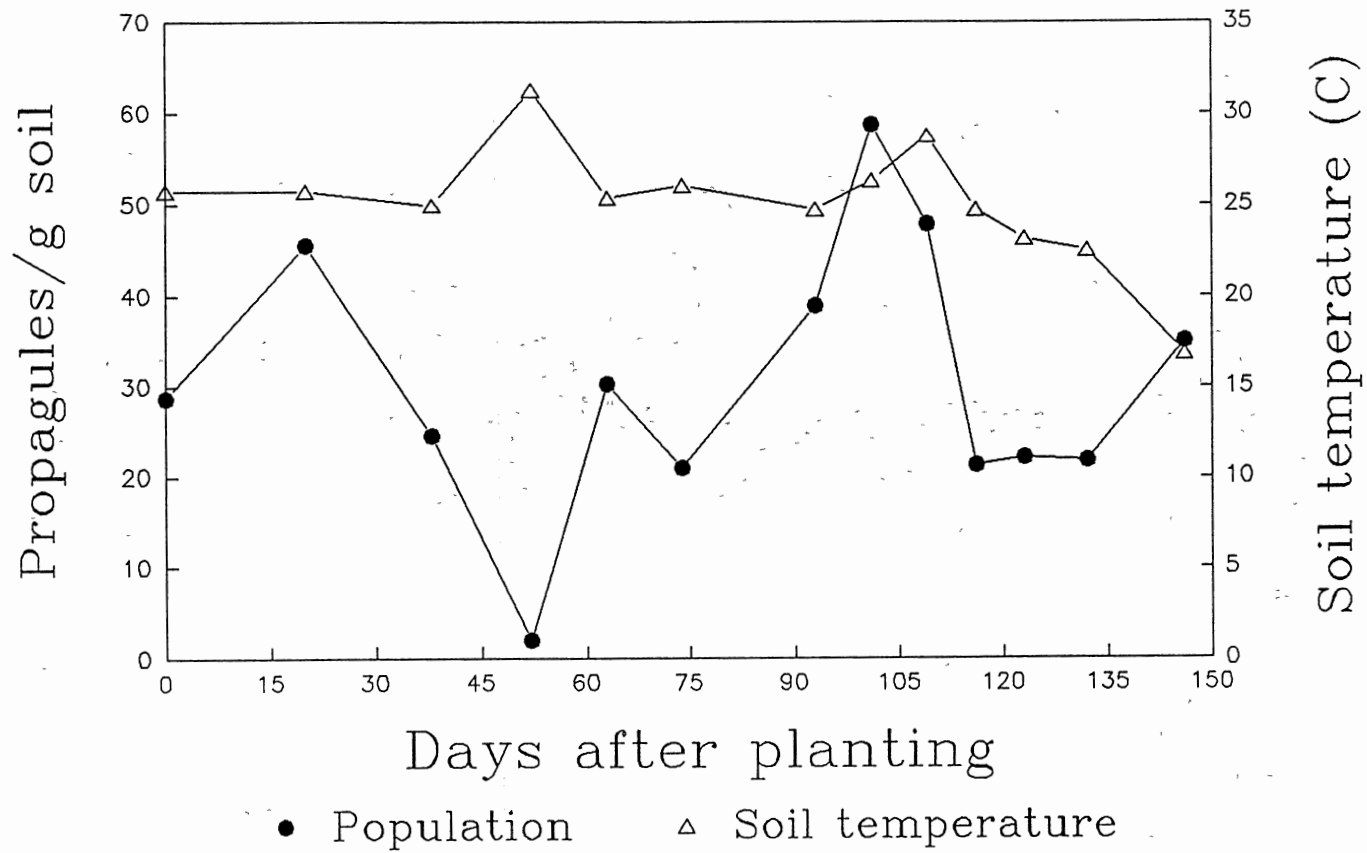


Figure 13. Populations of *Pythium* spp. in soil planted with soybean as related to soil temperatures at Ft.Cobb, Oklahoma in 1990.

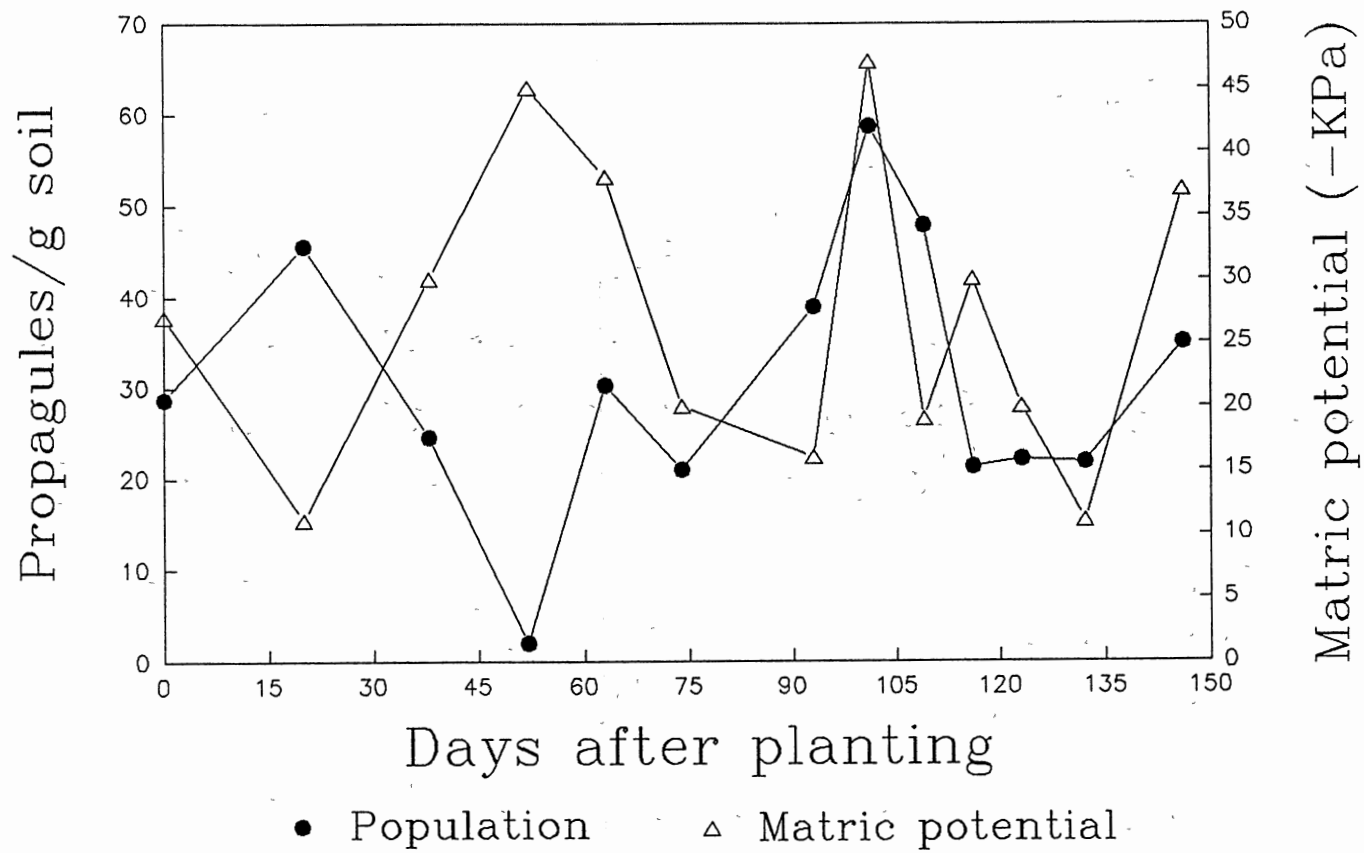


Figure 14. Populations of *Pythium* spp. in soil planted with soybean as related to soil matric potentials at Ft. Cobb, Oklahoma in 1990.

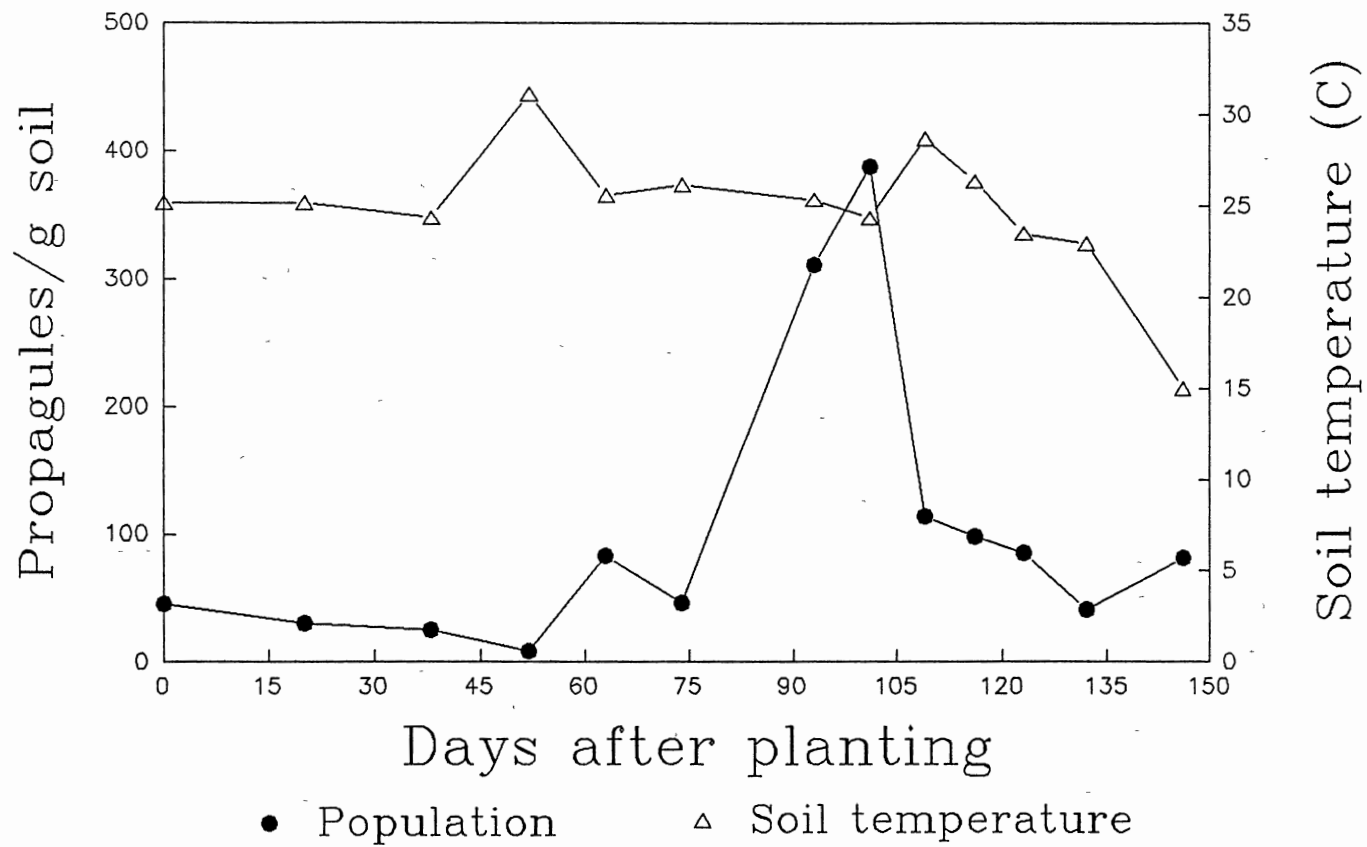


Figure 15. Populations of *Pythium* spp. in soil with peanut as related to soil temperatures at Ft. Cobb, Oklahoma in 1990.

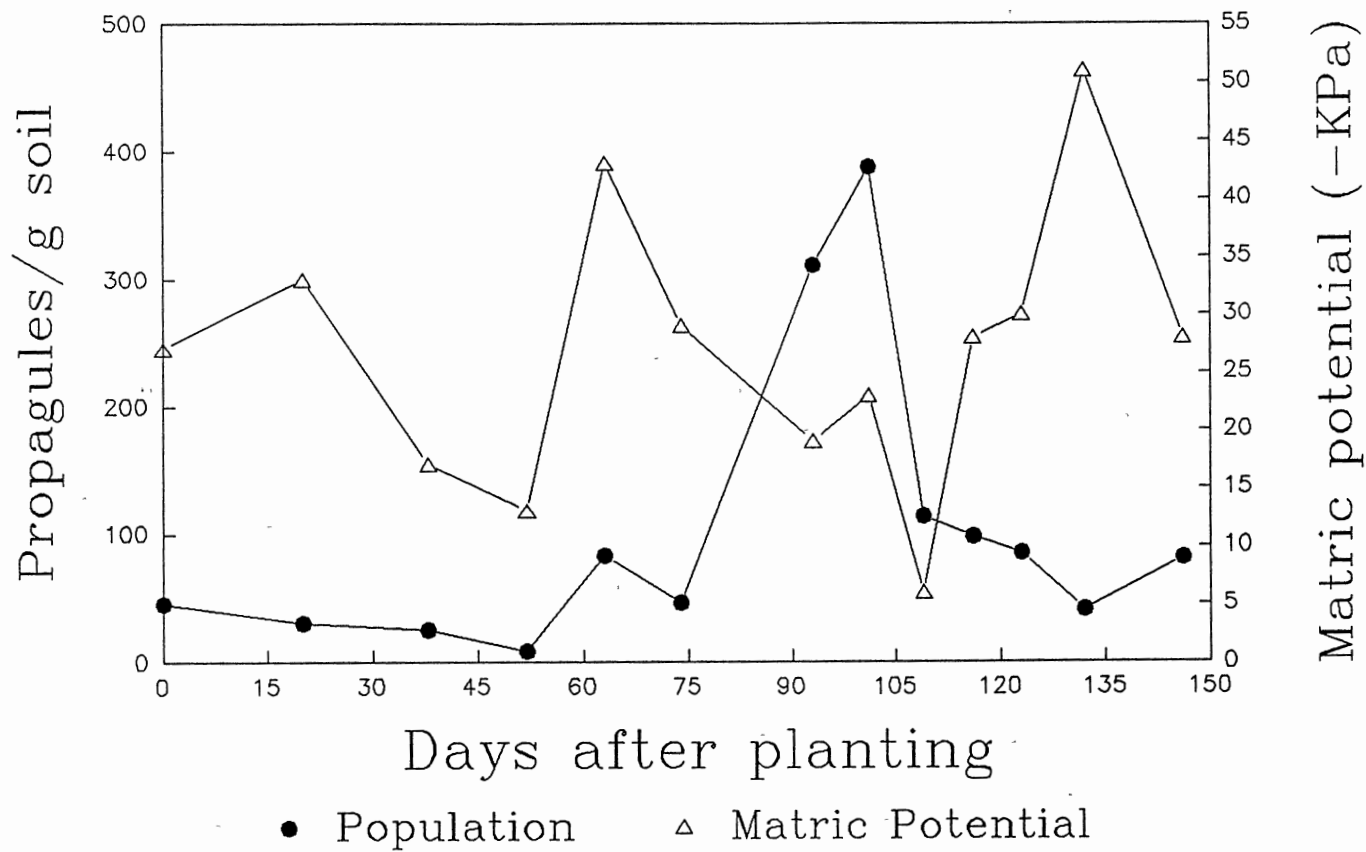


Figure 16. Populations of *Pythium* spp. in soil planted with peanut as related to soil matric potentials at Ft. Cobb, Oklahoma in 1990.

n=13), nor did these two variables have any significant interactive effect ($P=0.88$; $r^2=0.02$; $n=13$) on population changes during the season.

Mean pod rot severity for all plots at harvest was 0.94. There was no significant correlation ($P=0.05$) between the mean pod rot severity in a plot and Pythium spp. populations per plot in peanut soil at harvest. Isolation frequency of Pythium spp. from pods obtained at 74, 93, 116 and 146 DAP (harvest) was 16.3%, 21.8%, 37.2% and 42.6%, respectively. At harvest, 42% of the Pythium spp. that were isolated grew rapidly at 37 C and had morphological characteristics indicative of P. myriotylum.

The mean population of Pythium spp. in fallowed soil, when averaged over all sampling dates was 27.3 p/g, which was not different ($P=0.05$) when compared to the mean population in soybean soil (30.9 p/g). The mean population in peanut soil (103.6 p/g) over the season was greater ($P=0.01$) than those in fallowed or soybean soil. At planting (Figure 10), the population of Pythium spp. was greater ($P=0.05$) in peanut soil than in soils with other treatments. Thereafter, no differences ($P=0.05$) between populations at sampling dates were observed until 63 DAP, when populations in peanut soil peaked (83.3 p/g; $P=0.01$) compared to populations in the fallowed or soybean soil at 63 DAP. Significantly greater ($P=0.01$) populations of Pythium spp. in peanut soil compared to the other soil were

generally recorded at individual sampling dates from 93 DAP to harvest (146 DAP).

Other fields 1990

At site C4 in Caddo county (Figure 17) the population of Pythium spp. in the first soil sample was 118 p/g, but it markedly declined to 32 p/g one week later. There was a small, nonsignificant peak at 32, followed by significant ($P=0.05$) increases on 86 and 92. Thereafter, populations gradually declined. Fluctuations in populations of Pythium spp. were not correlated with soil temperature (Figure 17; $r=-0.41$; $n=11$) or soil moisture content (Figure 18; $r=0.16$; $n=11$), nor did temperature and moisture content have an interactive effect on population fluctuations ($P=0.58$; $r^2=0.17$; $n=11$).

Populations of Pythium spp. in soil at site C6 (Figure 19) peaked at 177.8 p/g at 94 DAP. This population was greater ($P=0.01$) than all other populations observed in the growing season. The population decreased at 102 DAP and then significantly ($P=0.05$) increased (90.2 p/g) at 108 DAP compared to the population at 102 DAP, but not the population observed at 116 DAP. No correlations between soil temperature (Figure 19; $r=-0.06$; $n=11$) or soil moisture content (Figure 20; $r=0.10$; $n=11$) and population fluctuations were observed. There was no interactive effect of the two variables on population ($P=0.94$; $r^2=0.01$; $n=11$).

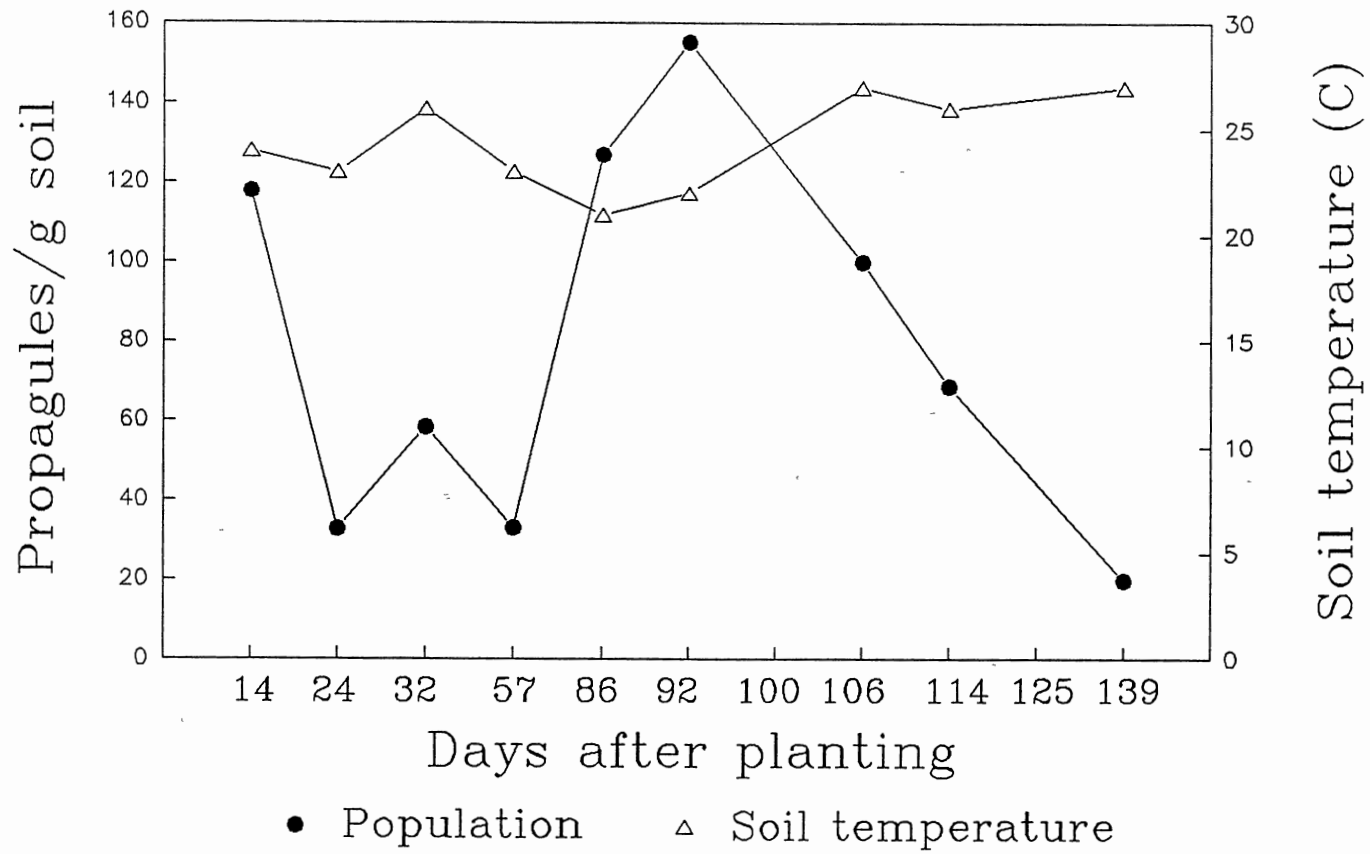


Figure 17. Populations of Pythium spp. in soil planted with peanut as related to soil temperatures at site C4 in Caddo County, Oklahoma in 1990.

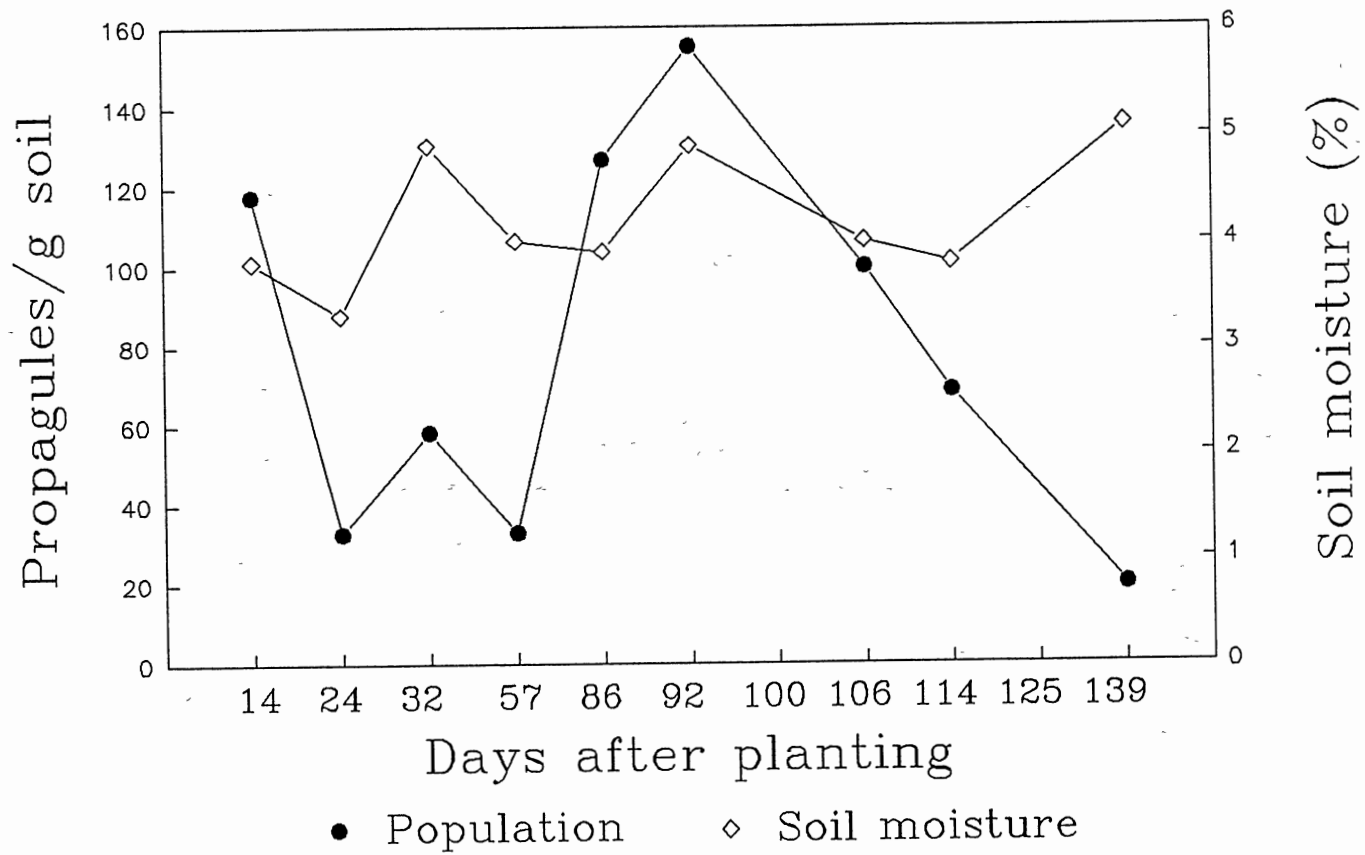


Figure 18. Populations of *Pythium* spp. in soil planted with peanut as related to soil moisture contents at site C4 in Caddo County, Oklahoma in 1990.

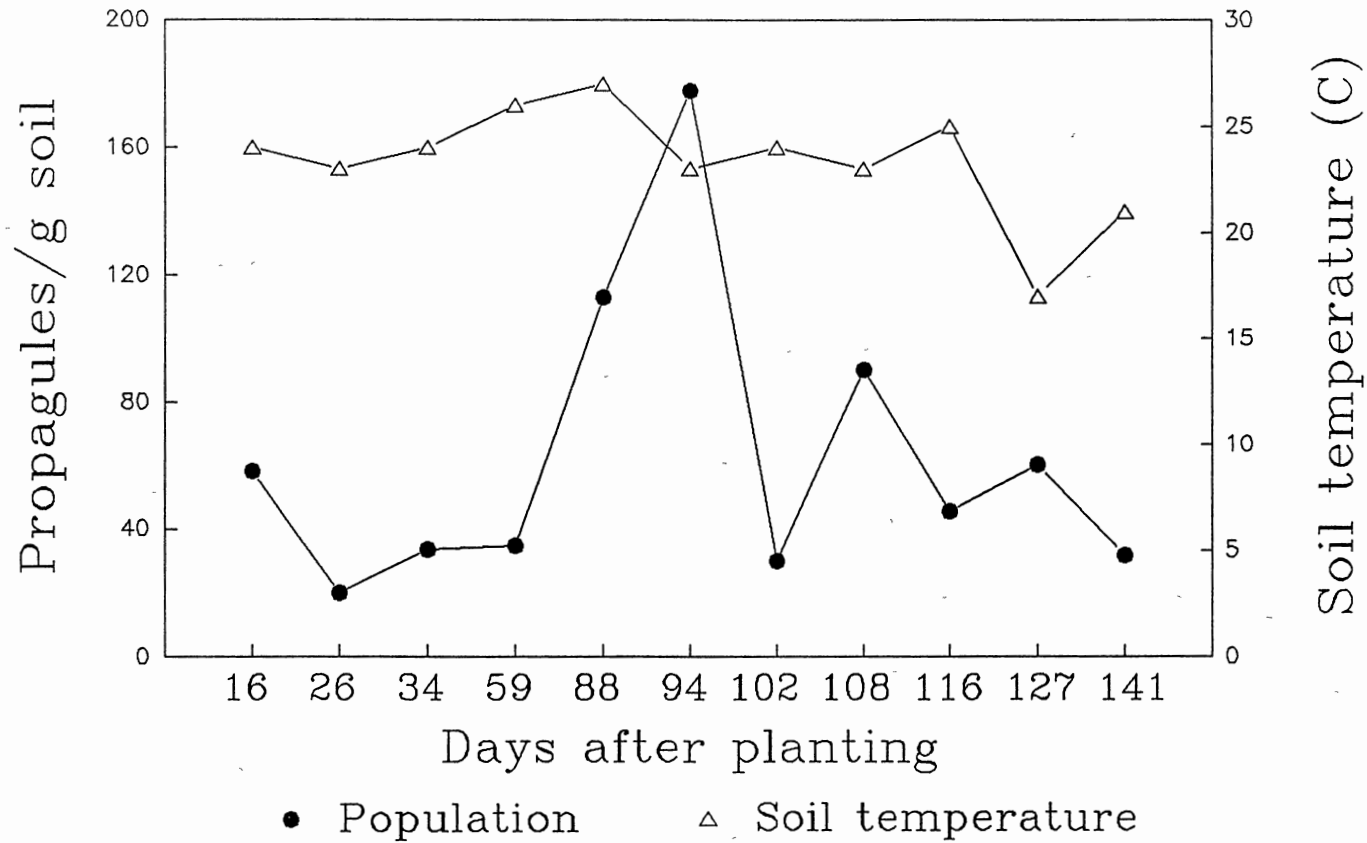


Figure 19. Populations of Pythium in soil planted with peanut as related to soil temperatures at site C6 in Caddo County, Oklahoma in 1990.

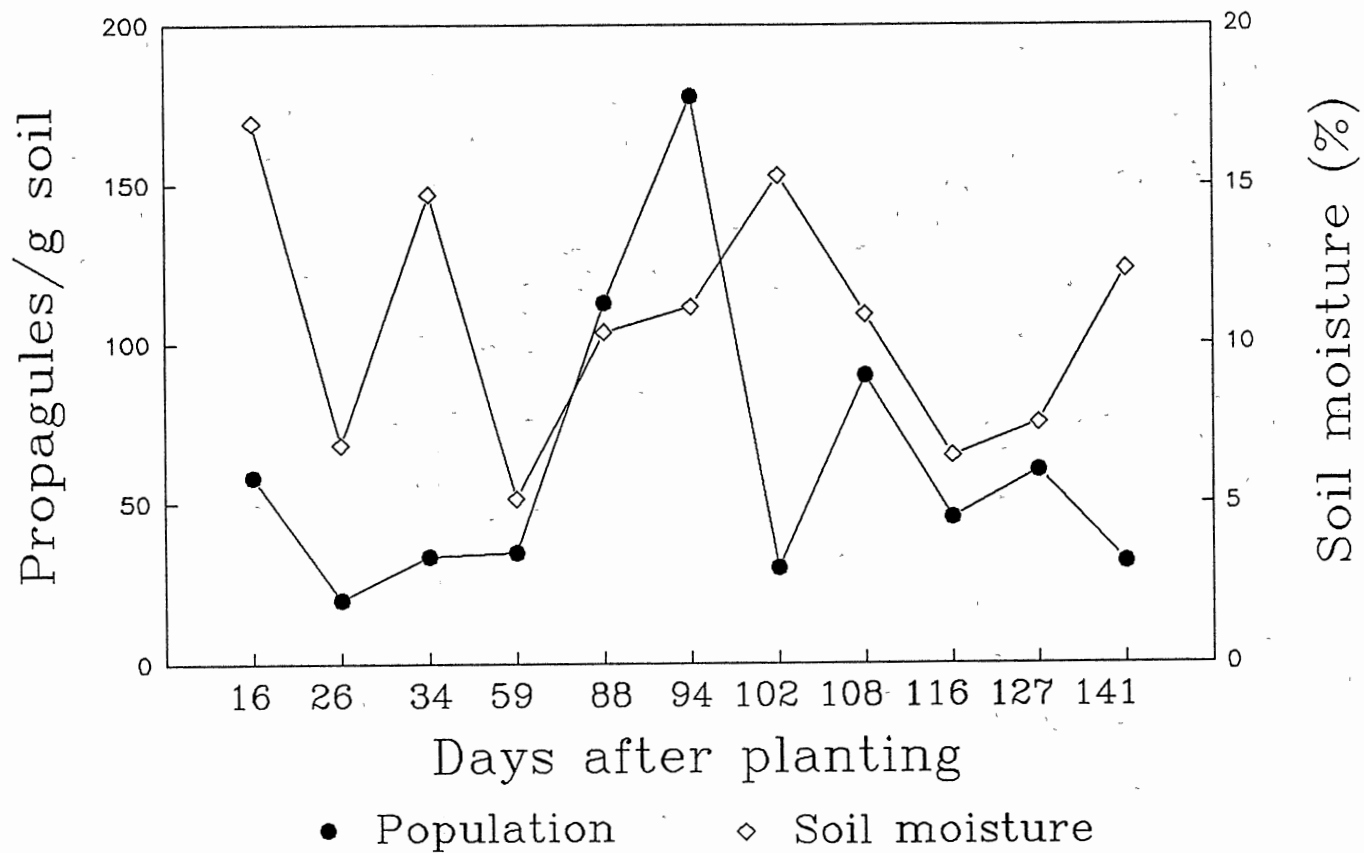


Figure 20. Populations of *Pythium* spp. in soil planted with peanut as related to soil moisture contents at site C6 in Caddo County, Oklahoma in 1990.

A peak in Pythium spp. populations at site C10 was also observed (Figure 21). At 56 DAP the population was 21.7 p/g, but by 91 DAP the population reached a maximum ($P=0.05$) of 140.3 p/g and then declined to 58.7 p/g (99 DAP). Thereafter, populations waxed and waned without significant increases up to harvest. Population fluctuations were not correlated with soil temperature (Figure 21; $r=0.21$; $n=11$) or soil moisture content (Figure 22; $r=-0.30$; $n=11$), nor did these variables have an interactive effect ($P=0.60$; $r^2=0.12$; $n=11$) on population fluctuations.

In Garvin county, at site G1 (Figure 23) and G2 (Figure 24) populations of Pythium spp. peaked at 80 DAP (122.4 p/g) and at 86 DAP (110.9 p/g) respectively. These increases were significantly ($P=0.05$) greater than the populations immediately preceding the increases. Another increase and decline in Pythium spp. population occurred at the end of the growing season at G1, but they were not significant ($P=0.05$). No correlation ($P=0.05$) between population fluctuations and soil temperature (Figures 23 and 24) and soil moisture content (Figures 25 and 26) were found. No interactive effect of soil temperature or moisture content on population was observed at G1 ($P=0.48$; $r^2=0.19$; $n=10$) or G2 ($P=0.98$; $r^2=0.01$; $n=9$).

Populations of Pythium spp. at site M1 (Figure 27) and M2 (Figure 28) in Marshall county generally increased toward

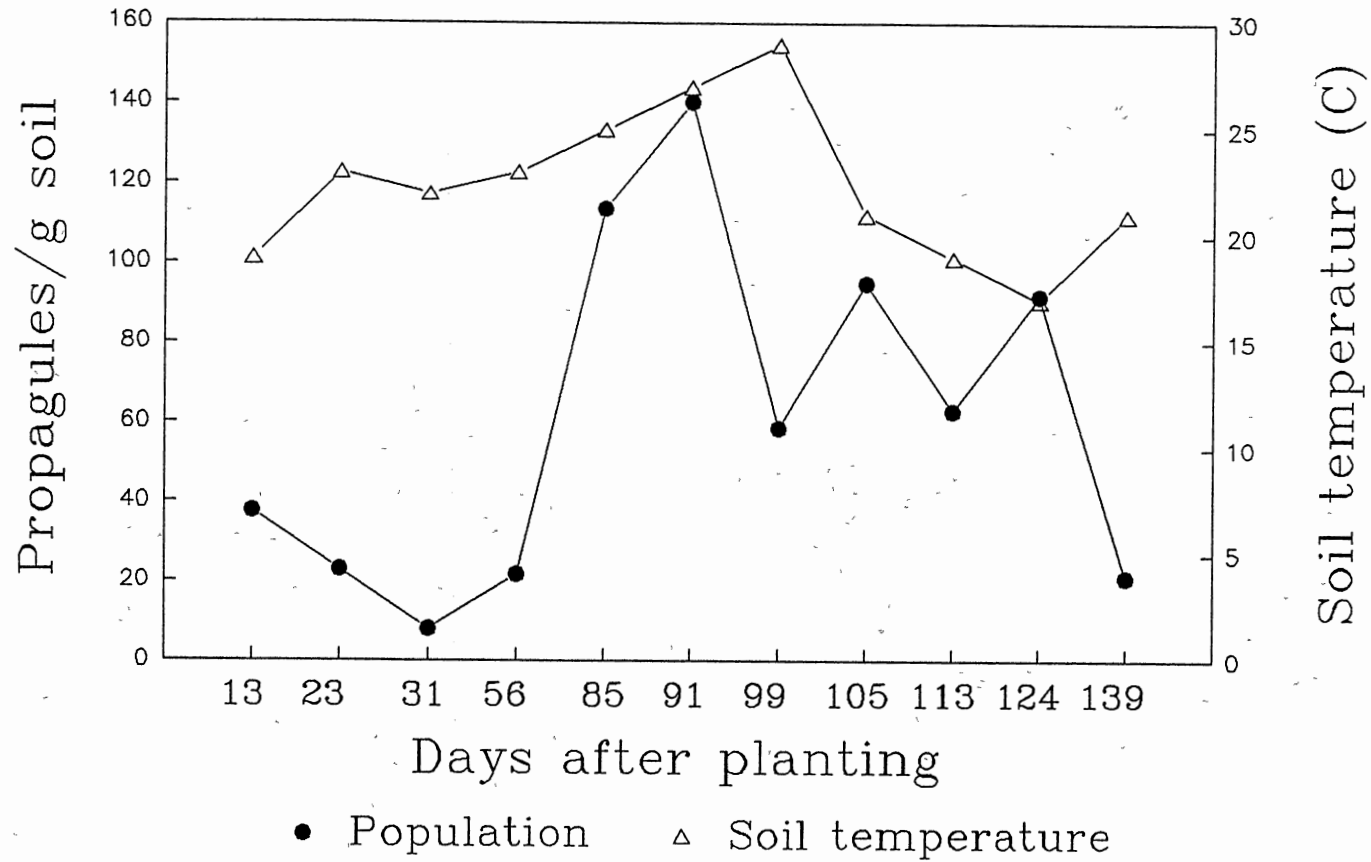


Figure 21. Populations of *Pythium* spp. in soil planted with peanut as related to soil temperatures at site C10 in Caddo County, Oklahoma in 1990.

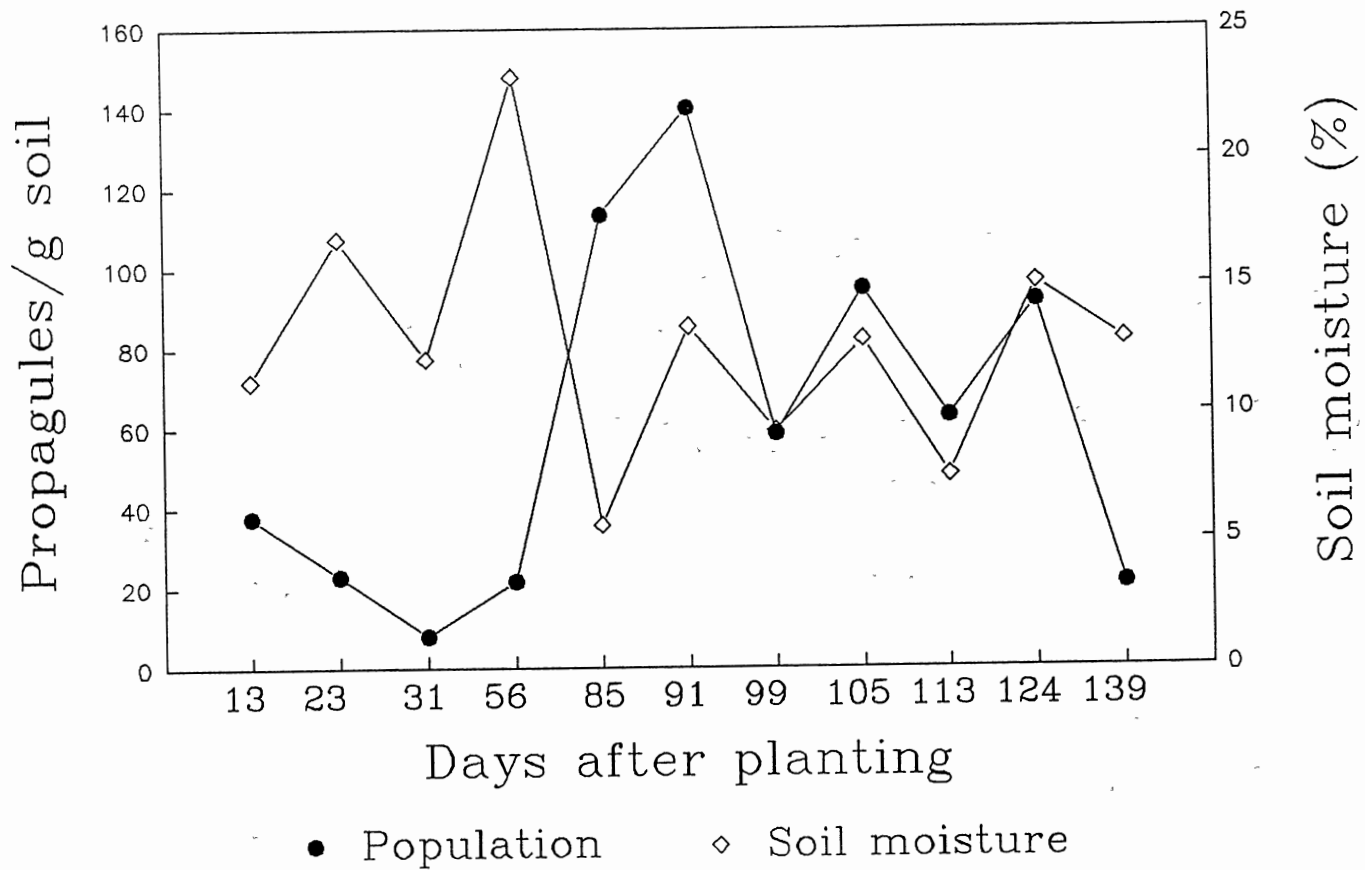


Figure 22. Populations of *Pythium* spp. in soil planted with peanut as related to soil moisture contents at site C10 in Caddo County, Oklahoma in 1990.

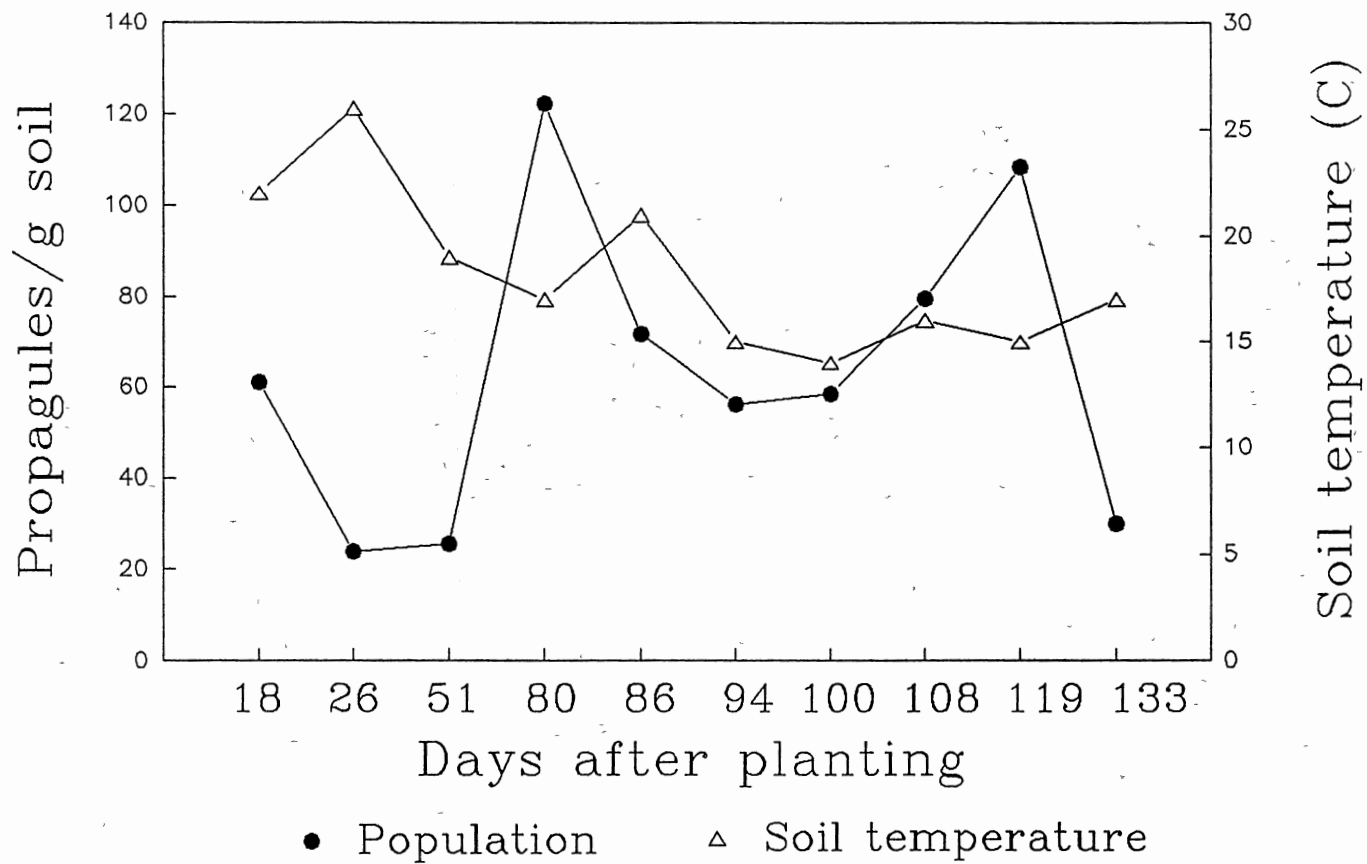


Figure 23. Populations of *Pythium* spp. in soil planted with peanut as related to soil temperatures at site G1 in Garvin County, Oklahoma in 1990.

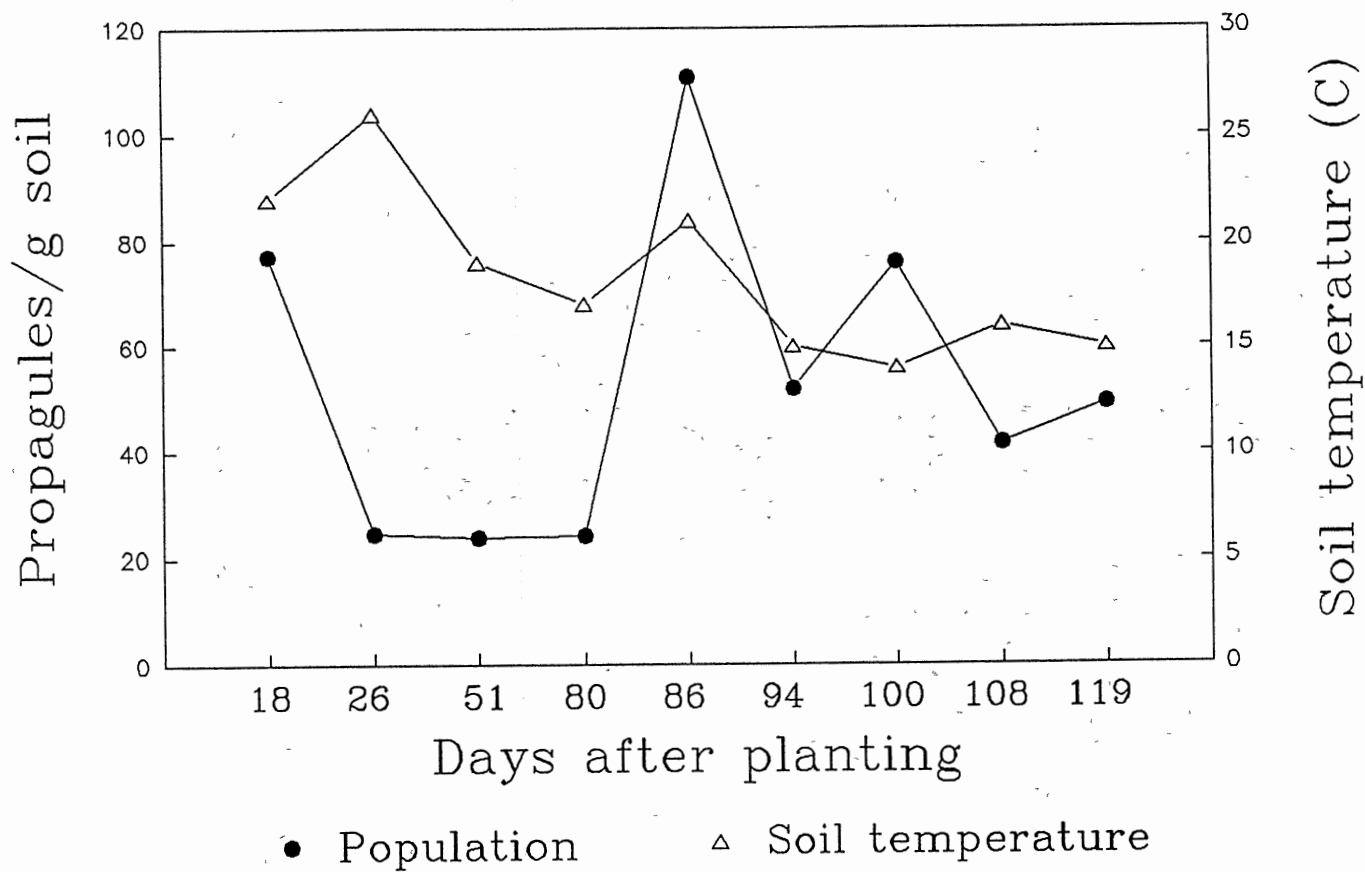


Figure 24. Populations of Pythium spp. in soil planted with peanut as related to soil temperatures at site G2 in Garvin County, Oklahoma in 1990.

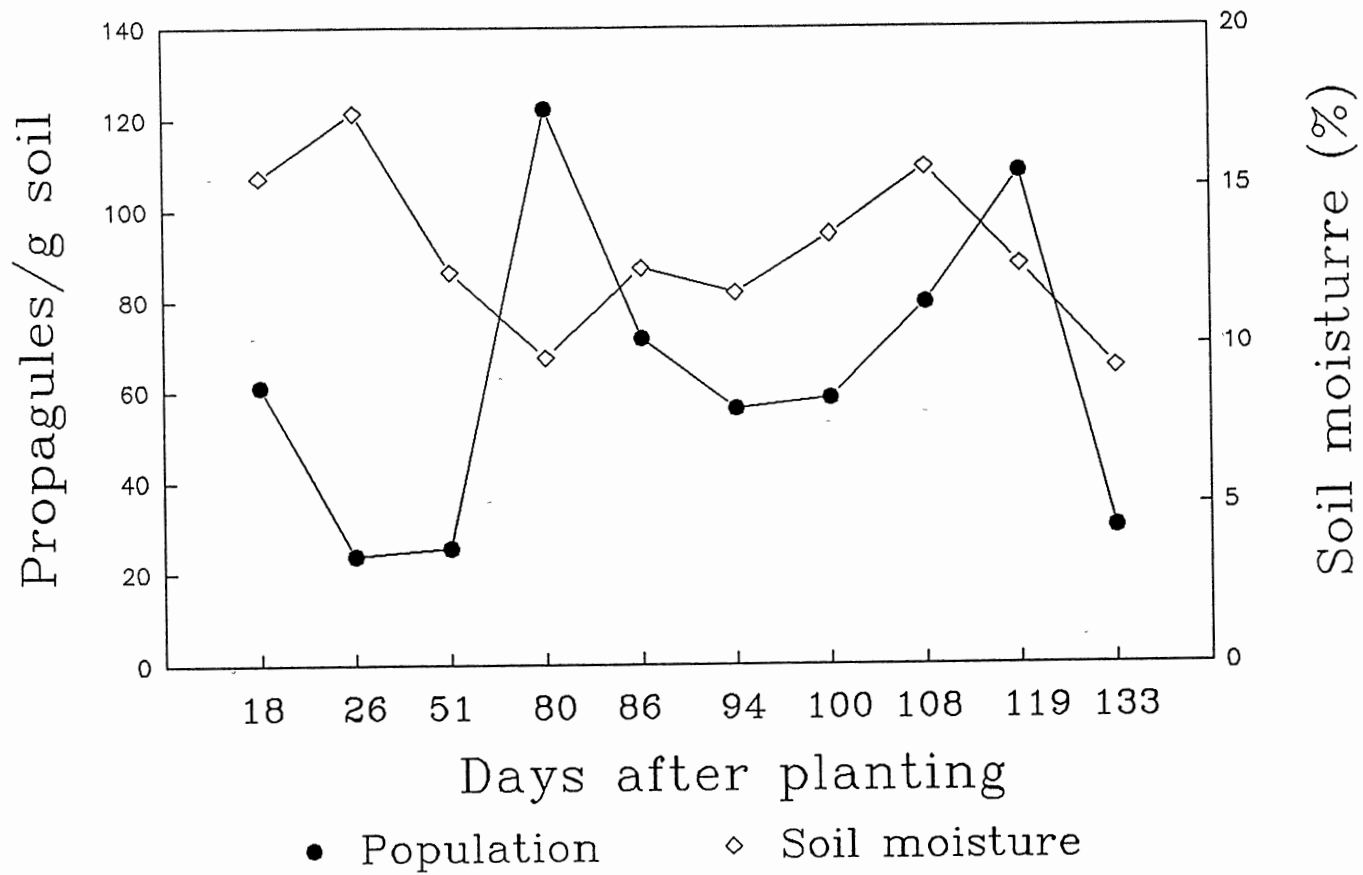


Figure 25. Populations of Pythium spp. in soil planted with peanut as related to soil moisture contents at site G1 in Garvin County, Oklahoma in 1990.

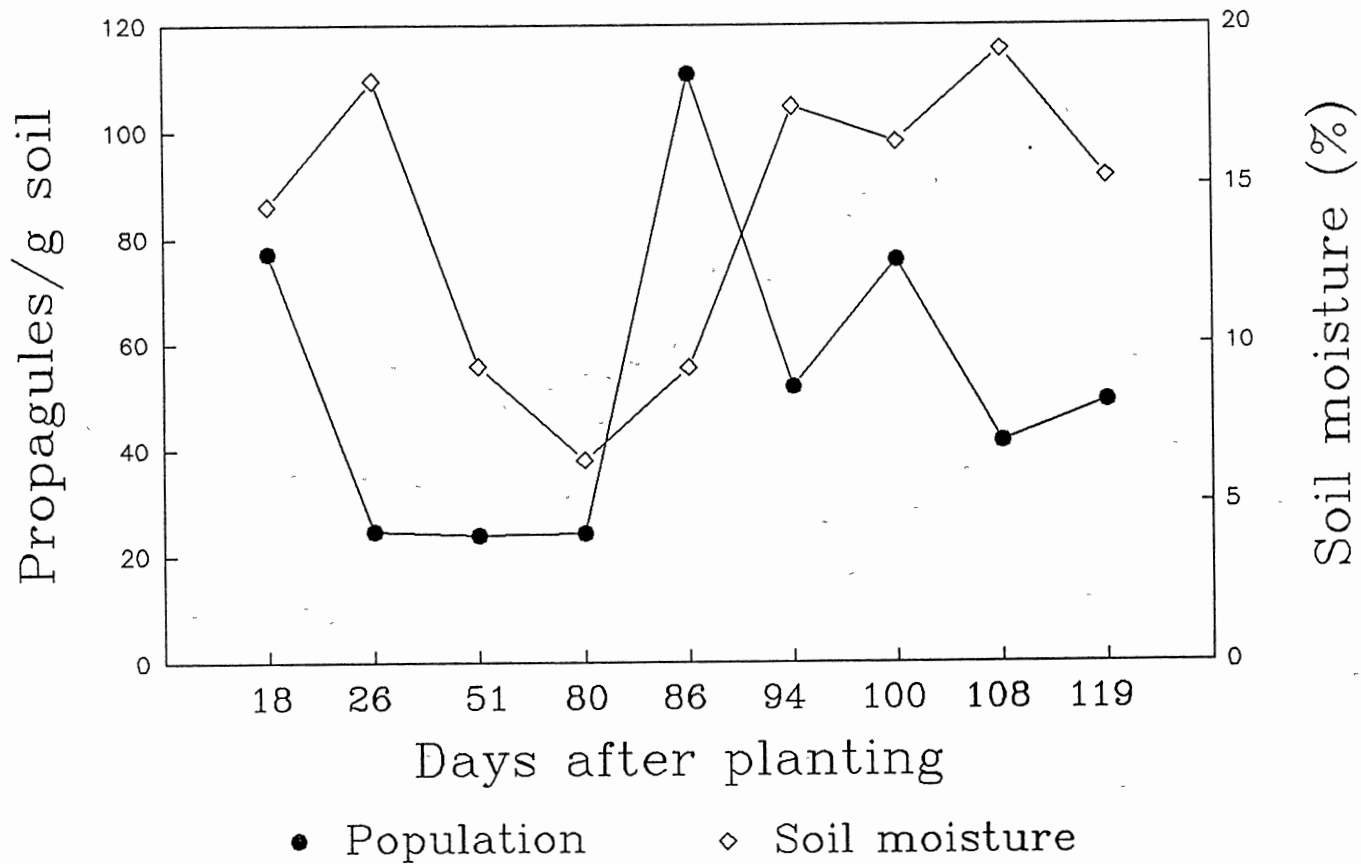


Figure 26. Populations of *Pythium* spp. in soil planted with peanut as related to soil moisture contents at site G2 in Garvin County, Oklahoma in 1990.

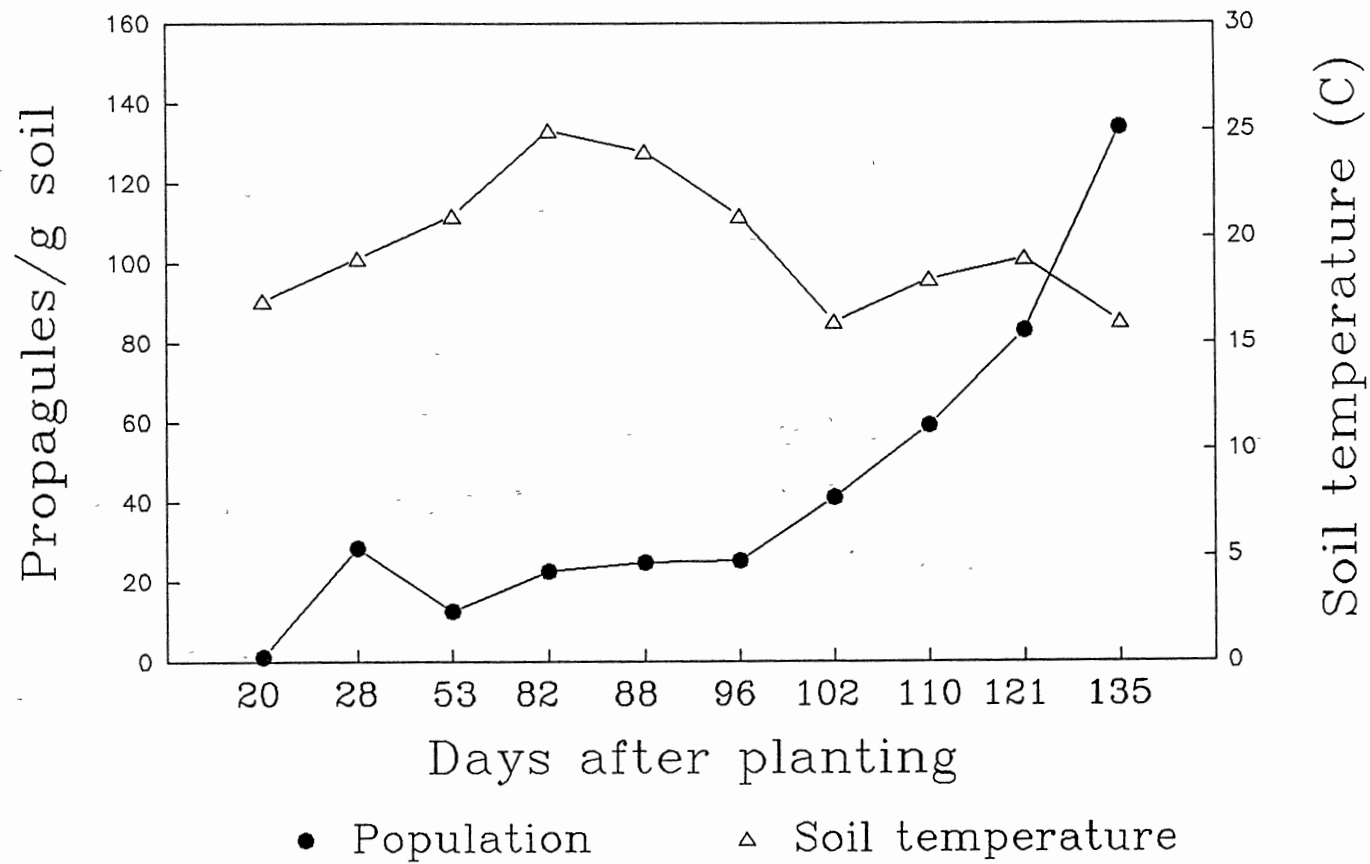


Figure 27. Populations of Pythium spp. in soil planted with peanut as related to soil temperatures at site M1 in Marshall County, Oklahoma in 1990.

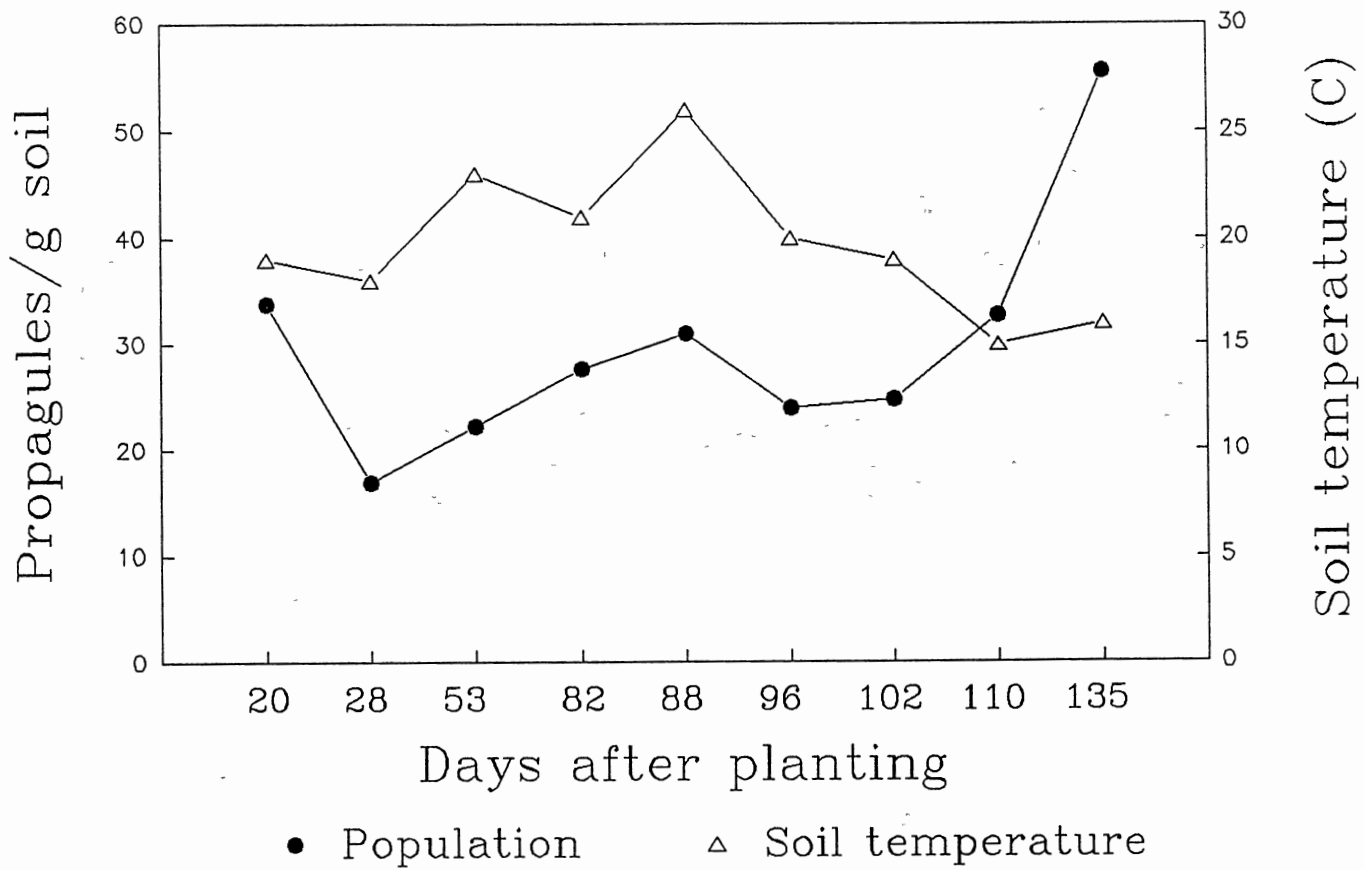


Figure 28. Populations of *Pythium* spp. in soil planted with peanut as related to soil temperatures at site M2 in Marshall County, Oklahoma in 1990.

the end of the growing season. Only at M1, however, was the population at harvest (134.1 p/g) greater ($P=0.01$) than populations at previous dates. The population increase at harvest at M2 was not significant ($P=0.05$). Population fluctuations in soil showed low, inverse correlations with soil temperatures at M1 (Figure 27; $r=-0.47$; $n=10$) and M2 (Figure 28; $r=-0.41$; $n=9$). No correlations between soil moisture contents and populations at either site was observed for M1 (Figure 29; $r=0.06$; $n=10$) and for M2 (Figure 30; $r=0.35$; $n=9$). Soil temperature and moisture content had no interactive effect on population fluctuations at M1 ($r^2=0.23$; $n=10$) or M2 ($r^2=0.24$; $n=9$).

Results from my study corroborate those of Filonow and Jackson (19) and Lewis and Filonow (46) who had previously reported a significant peak followed by a rapid decline in populations of Pythium spp. after pegging in soil planted to peanut at the Caddo Research Station, Ft. Cobb, Oklahoma. The temporal occurrence of the peaks found at Ft. Cobb in my study (65 DAP in 1989 and 101 DAP in 1990) were similar to those (75 DAP in 1986 and 60 in 1987) observed by Filonow and Jackson (19) and Lewis and Filonow (67 DAP in 1987 and 89 DAP 1988) (46). The magnitude of the populations in peaks reported by these workers was about 100-1000 p/g soil, whereas I observed peaks of 78 p/g in 1989 and 388 p/g in 1990. Thus there are many similarities in the population

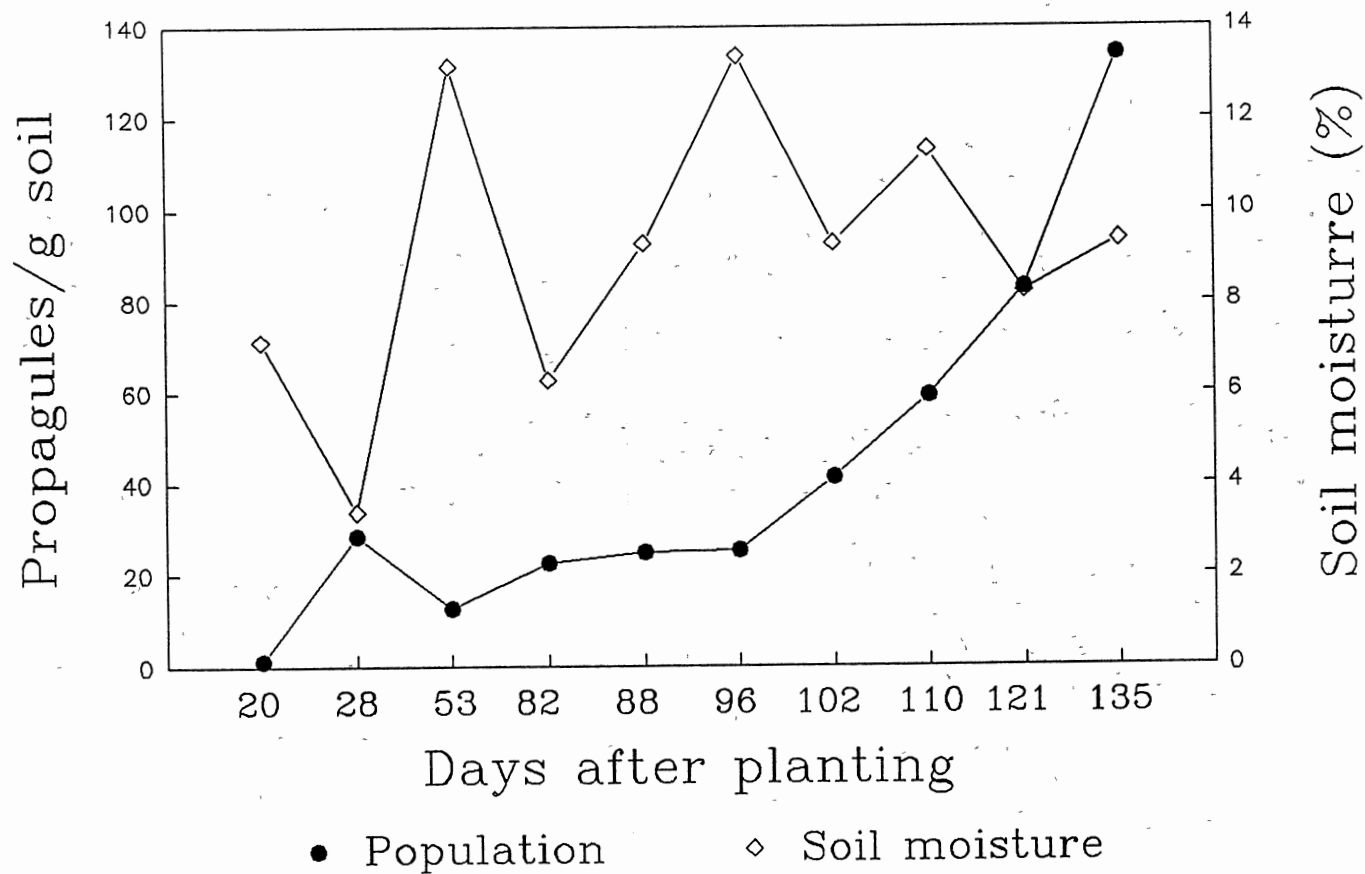


Figure 29. Populations of *Pythium* spp. in soil planted with peanut as related to soil moisture contents at site M1 in Marshall County, Oklahoma in 1990.

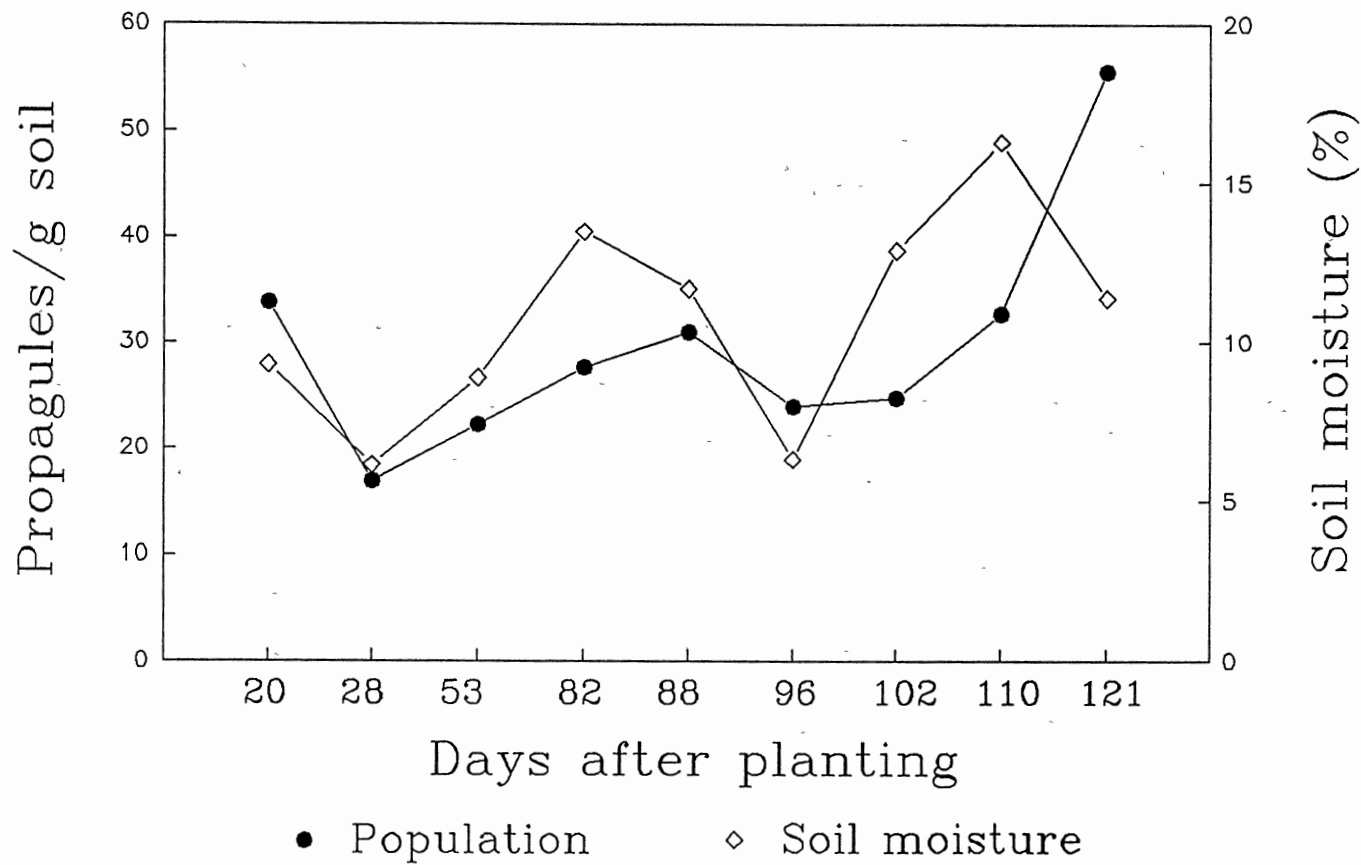


Figure 30. Populations of *Pythium* spp. in soil planted with peanut as related to soil moisture contents at site M2 in Marshall County, Oklahoma in 1990.

phenomena observed by different researchers over several years at Ft. Cobb.

The population phenomenon was not restricted to Ft. Cobb. In my study, a peak and decline pattern occurred in fields at Albert (C10), Hinton (C6), and in Ft. Cobb at a site other than the research station (C4) and in two fields at Stratford, OK (G1 and G2). Lewis and Filonow (46) reported a similar peak in population of Pythium spp. at Madill, Oklahoma in 1987. At Marshall County field sites, however, a different pattern in Pythium spp. populations was observed. After pegging, populations in these fields gradually increased to reach a maximum near or at harvest. These results confirm a similar population pattern found earlier at a field in Marshall County (46).

Soils from the fields sampled in 1990 and Ft. Cobb differed greatly in their characteristics (Appendix). Yet the peak and decline population effect was observed in 6 dissimilar soils (Ft. Cobb, C4, C6, C10, G1 and G2) located in geographically different areas of Oklahoma. The occurrence of this temporal population pattern may be common to peanut fields in Oklahoma and perhaps to other peanut producing areas of the U.S.

Fluctuations in Pythium spp. populations in soils other than those planted with peanut have been reported by others. Populations of Pythium spp. in soil cropped to a rough grass meadow in England (38) exhibited over time a population

periodicity in 1983. In 1984, no peaks were noticed in the same plots. Soil temperature was considered to be more important than rainfall in accounting for population changes in this study (38). In a longer study in England, Ali-Shtayeh et al. (3) observed a winter peak and a summer trough in populations of total Pythium spp. in soil. Population fluctuations were best explained by a sine curve model. Soil moisture was more important than soil temperature in improving the fit of the periodic curve to observed data (3). In another study, Ali-Shtayeh (2) observed that eighteen fields in the West Bank of Jordan and in the Gaza Strip had the highest populations of Pythium spp. during the winter and early spring, and the lowest during the summer. High soil moisture and cool temperatures during the winter and spring may have favored higher Pythium spp. populations, whereas in the summer, populations may have been less favored by higher soil temperatures and less soil moisture. Ali-Shtayeh (2), also observed that P. aphanidermatum which is typically a warm-temperature pathogen (68) had a different population pattern with a peak in late summer and a low population in winter. In addition, P. aphanidermatum was found only in irrigated fields. Lumsden et al. (49), however, reported greater populations of P. aphanidermatum during the winter in a vegetable field than in the spring and summer.

Soil populations of P. ultimum, which grows faster in cool temperatures (68) were highest in California cotton fields during the cooler months than in August or early September, when they were lowest (37). Seven of 10 fields exhibited this seasonal pattern. Temperatures during the summer months (30-37 C) were not favorable in the cooler (<28 C) months of the season.

The influences of soil temperature and/or soil moisture on Pythium spp. populations reported in some of the studies cited above have not been observed in soil planted with peanut. Fluctuations in populations of Pythium spp. in soils monitored in this study were not directly correlated with fluctuations in soil temperature and/or matric potential. These results confirm those of Lewis and Filonow (46), who had reported no direct effect of soil temperatures or matric potential on Pythium spp. populations in soil planted with peanut. However, soil temperature and moisture do affect the of the peanut plant (7).

Lewis and Filonow (46) have reported that the peanut host is the principal factor accounting for the temporal pattern of Pythium spp. populations observed in Oklahoma peanut soil. My results support this hypothesis. Populations of Pythium spp. in fallowed soil at Ft. Cobb in 1989 and 1990 waxed and waned and did not exhibit any significant peak, whereas populations of Pythium spp. in soil planted with peanut or soybean did. In plots planted

with soybean in 1989, a significant peak in population was found later in the season (100 DAP) compared to that observed in the peanut plots (65 DAP). In 1990, a significant peak was not found in the soybean plots; however, the leaves and shoots of these plants had been intermittently eaten by various animals during the season, so that the photosynthetic capacity of these stunted plants was most likely reduced compared to that of healthy soybeans in the 1989 experiment. Less exudate from the roots of these stunted plants would diminish the rhizosphere effect (9) imposed on populations of Pythium spp., whereas in soil containing healthy, well developed soybean plants, the rhizosphere effect would be more dramatic.

Growth Chamber Experiments

Box Experiments

In the first experiment (Figure 31) populations of Pythium spp. in soil of the peanut zone increased from 21.7 p/g at 19 DAP to a maximum of 255 p/g at 129 DAP, after which populations declined sharply to 67.4 p/g at harvest. The increase at 129 DAP was a significant ($P=0.01$) peak in population fluctuations. Populations in the proximal zone of soil also increased over time to a high of 135.1 p/g and 130.3 p/g at 129 DAP and 150 DAP, respectively; however, these populations were not greater ($P=0.05$) than other temporal populations. In the peripheral zone of the soil,

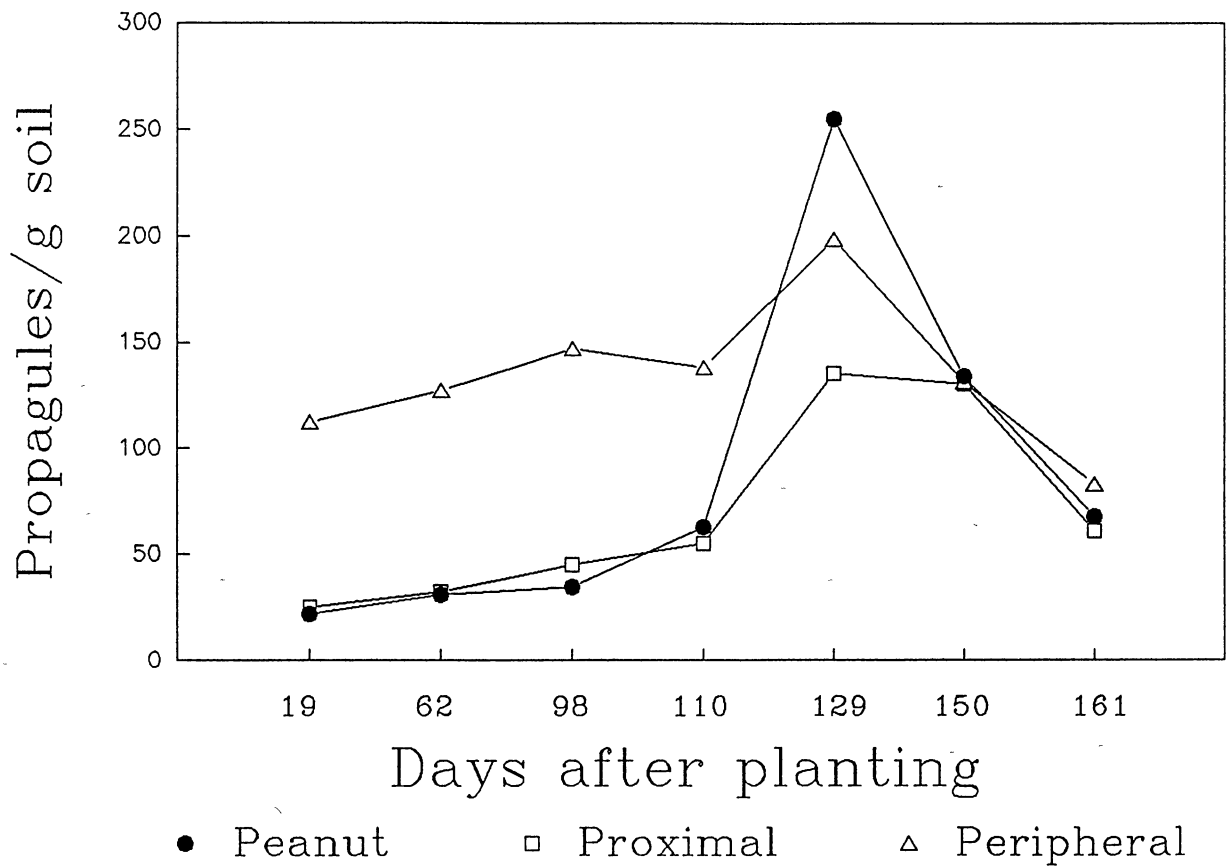


Figure 31. Populations of *Pythium* spp. in the peanut, proximal and peripheral zones of soil in boxes planted with peanut. First experiment.

populations of Pythium spp. were high (112.1 p/g) at the first sampling (19 DAP), and increased to 198.4 p/g at 129 DAP, which was not a significant ($P=0.05$) fluctuation. No difference ($P=0.05$) was observed between mean populations of Pythium spp. in soil sampled on 129 DAP from the three zones. Populations in the peanut, proximal, and peripheral zones of soil in the boxes were not correlated ($n=7$; $r=-0.29$, $r=-0.37$ and $r=0.11$, respectively) with the soil moisture contents of the soils. At harvest Pythium spp. were isolated from pods with pod rot symptoms.

In the second box experiment (Figure 32), populations of Pythium spp. in the peanut zone of soil gradually increased over time from 18 p/g at 13 DAP to 73.1 p/g at 165 DAP (harvest). The population at 165 DAP was greater ($P=0.05$) than all prior population estimates, except for that at 124 DAP (47.2 p/g). Pythium spp. were isolated from pods with symptoms of pod rot at harvest. Populations in soils of the proximal and peripheral zones of the boxes increased and decreased slightly over time with no significant ($P=0.05$) differences in their fluctuations. Only at 124 and 165 DAP were mean populations of Pythium spp. in soil from the peanut zone greater ($P=0.05$) than populations in soils from the other zones of the boxes. Populations in soils of the peanut and peripheral zones were not correlated ($n=6$; $r=-0.46$ and $r=0.15$, respectively) with their soil moisture contents. However, population fluctuations in soil

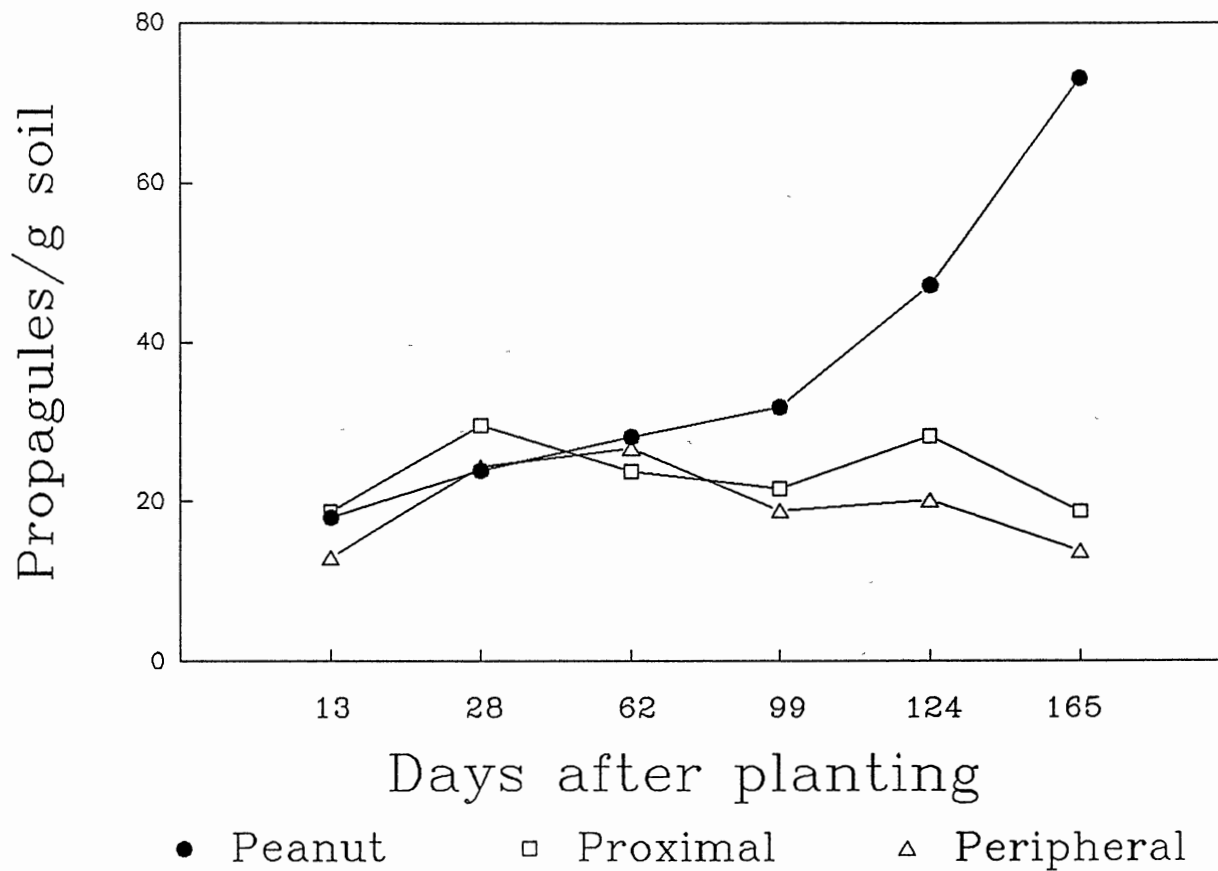


Figure 32. Populations of *Pythium* spp. in peanut, proximal and peripheral zones of soil in boxes planted with peanut. Second experiment.

from the proximal zone of the boxes were correlated ($r=0.83$; $n=6$) with soil moisture content.

Results from the box experiments also support the host influence hypothesis. In the first experiment, populations of Pythium spp. in soils from the peanut zones of the boxes significantly increased and declined over time, whereas those from the proximal and peripheral zones did not. In a second experiment, populations tended to increase over time with a significant increase in population at harvest (165 DAP) in the peanut zone. Populations in the proximal and peripheral zones of the boxes increased and declined over time with no significant fluctuations between sampling periods. Soil temperatures in zones of the boxes were not different during these experiments. There were small differences in soil moisture between zones; however, these differences were, for the most part, not correlated with populations.

Pod Training Experiments

In the first experiment (Figure 33), no significant ($P=0.05$) fluctuations in Pythium spp. populations were observed in soil which contained neither roots nor pods. The presence of pods and/or roots increased populations of Pythium spp. in soil. Populations in soils with pods or roots peaked at 79 DAP and these population peaks were greater ($P=0.05$) than populations at planting (0 DAP).

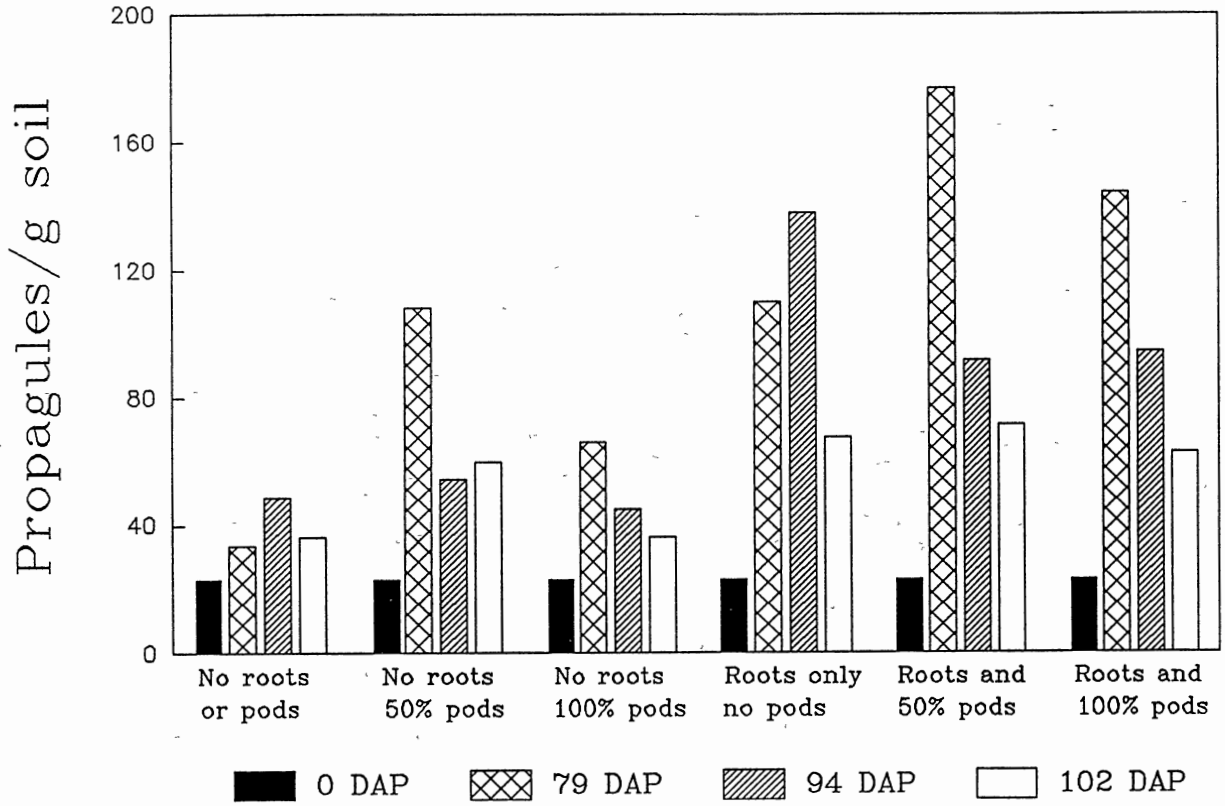


Figure 33. First pod training experiment. Populations of *Pythium* spp. in soil without roots or pods or in soil containing pods only, roots only or pods and roots.

Populations tended to decline after 79 DAP. In soil containing only roots, significant ($P=0.05$) population increases occurred at 79 and 94 DAP, with the maximum at 94 DAP.

In the second experiment (Figure 34), mean populations of Pythium spp. in soil were in decreasing order according to the following treatments: roots plus pods > pods only > roots only > no roots or pods. Population fluctuations over time in soil without roots or pods, and in soil containing roots were not significant ($P=0.05$). Population increases at 89 and 119 DAP in soil with pods were greater ($P=0.05$) than populations at other times. A significant ($P=0.05$) peak in Pythium spp. population was observed at 89 DAP in soil containing both roots and pods.

Results from the pod training experiments further supported the host-influence hypothesis. In soil without peanut roots and pods, populations of Pythium spp. were low and fluctuated little over the course of the experiments. Populations in soil containing roots and/or pods, however, exhibited significant peaks that were generally higher ($P=0.05$) than populations in soil without plant tissue at the same sampling dates. Moreover, the pod training experiments suggested that both peanut roots and pods exert an effect on populations of Pythium spp. This effect may occur at different times in the growing season, as indicated

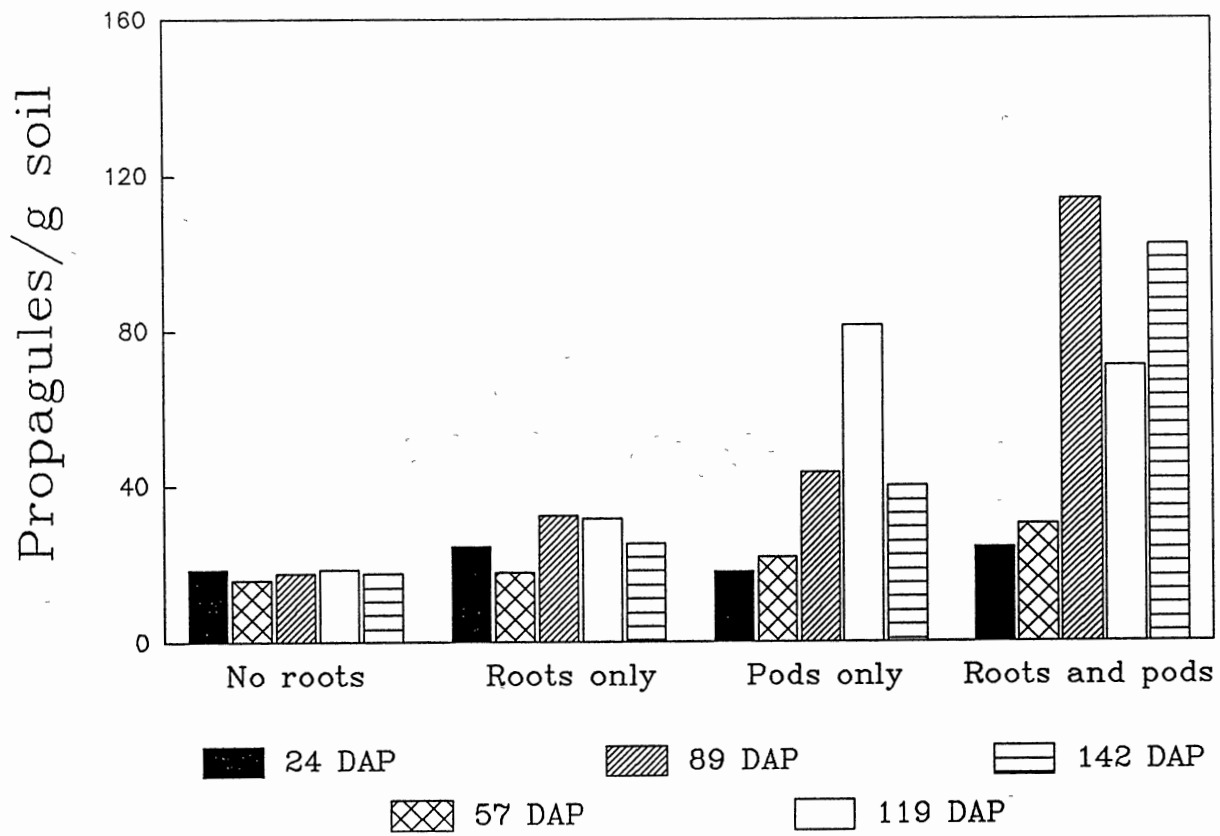


Figure 34. Second pod training experiment. Populations of *Pythium* spp. in soil without roots or pods or in soil containing pods only, roots only or pods and roots.

by the occurrence of population peaks at different times for soil containing roots compared to pods.

These results and those from Ft. Cobb and other fields clearly demonstrate the impact of the peanut host on populations of Pythium spp. in soil. The relative contribution of peanut roots compared to on this effect pods needs additional study. Little is known about the influence of peanut roots on populations of Pythium spp. in soil. Shay and Hale (63) reported that low levels of calcium in an axenic culture medium containing peanut roots increased the exudation of sugars from the roots; however, the study was not extended to the population dynamics of root-infecting Pythium spp. in natural soils. Further study in this area is needed.

Results from this study and those of others (46) indicate that pods influence the temporal dynamics of Pythium spp. populations in peanut soil. Lewis and Filonow (46) suggested that nutrient leakage from developing pods might supply energy for the proliferation of microbial growth, including Pythium spp. in soil. They based their hypothesis on their own observations (46) and those of MacDonald (51) and Griffin (32) who noted fungal increases in the geocarposphere, and Hale (33) who showed that developing pods exude sugars. Lewis and Filonow (46) further postulated that as pods matured, leakage of nutrients may decrease to a point where energy-deprived

microbes may feed on the hyphae of Pythium spp., resulting in a decline in soil population. The premise of a decrease in nutrient exudation from pods as they develop and mature is supported by the work of Hale (33) and by Subramanyan and Prabhakar (67). They showed that the rate of ^{14}C translocation into newly developed (10 day old) pods was low, but the amount of ^{14}C lost via exudation from pods was comparatively higher. In more developed pods (50 days old) ^{14}C translocation into pods was comparatively higher than ^{14}C lost via pod exudation.

An alternative explanation to account for the decline in Pythium spp. populations in soil after the peak was offered by Lewis and Filonow (46). They suggested that following the population peak of Pythium spp., hyphae may move from the soil to colonize pod surfaces (46). Soil populations would then be lowered. My results offer support for, and expand on this latter cause of the decline phase. At Ft. Cobb, recovery of Pythium spp. from pods as they developed over time was not synchronous with the increase in Pythium spp. soil populations leading to the peaks. Maximum recovery of Pythium spp. from pods was several weeks later than the maximum population of Pythium spp. in soil. This finding suggests that the growth of Pythium spp. in soil may need to reach a threshold population before colonization from soil to pods occurs. Alternatively, Pythium spp. colonization of pods may not occur until pods have reached a

developmental stage in which pod tissue can support sufficient hyphal growth. In this regard, Pattee et al. (57) have reported that maximal concentrations of starch in the hulls of pods occurred at early and middle pod maturity, whereas sugar (mainly sucrose) content in hulls was greatest at near middle maturity. Pythium myriotylum which composed >40% of the recovered Pythium spp. from pods at Ft. Cobb at harvest grows well on sucrose and starch (A.B. Filonow, unpublished observations). However, little is known about the effect of substrate preference (sucrose versus starch) on the colonization of pods by Pythium spp.

Pathogenicity of Pythium
species to peanut pods

P. myriotylum, P. aphanidermatum and, P. ultimum were pathogenic to pods of Pronto peanut (Table 1 and 2), whereas P. debaryanum, and P. irregulare, and P. arrhenomanes were not. Infected pods were black with various stages of hull decay, whereas noninfected pods were generally white with intact hulls (Figure 35).

Pythium myriotylum also significantly ($P=0.05$) reduced the mean number of pegs and attached pods formed per plant in both experiments. Peg number was also reduced ($P=0.05$) by P. aphanidermatum, but only in the second experiment (Table 2). In the second experiment, all species of Pythium reduced the number of intact pods.

TABLE 1

PLANT HEIGHT, NUMBER OF PEGS AND PODS AND POD ROT INDEX
OF PEANUT PLANTS GROWN IN SOIL INFESTED WITH
DIFFERENT SPECIES OF PYTHIUM:
FIRST EXPERIMENT

	Mean ^Z			
	plant height cm	peg number	pod number	pod rot index ^Y
<u>P. aphanidermatum</u>	16.9b	24.7a	14.6a	0.22b
<u>P. arrhenomanes</u>	19.7a	29.0a	19.7a	0.00c
<u>P. debaryanum</u>	19.2a	24.8a	14.3a	0.02c
<u>P. irregulare</u>	17.2b	26.7a	15.8a	0.00c
<u>P. myriotylum</u>	13.9c	11.8b	6.8b	0.95a
<u>P. ultimum</u>	16.9b	29.0a	18.7a	0.21b
Noninfested	19.9a	28.7a	17.3a	0.00c

^Z Mean of 6 replicates; one plant per replicate. Means within a column followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls test.

^Y Number of pods with pod rot in indicis 3, 4 and 5 in a replicate were summed and divided by the total number of pods.

TABLE 2

PLANT HEIGHT, NUMBER OF PEGS AND PODS AND POD ROT INDEX
OF PEANUT PLANTS GROWN IN SOIL INFESTED WITH
DIFFERENT SPECIES OF PYTHIUM:
SECOND EXPERIMENT

	Mean ^Z			
	plant height cm	peg number	pod number	pod rot index ^Y
<u>P. aphanidermatum</u>	9.2a	4.3b	2.5cd	0.44b
<u>P. arrhenomanes</u>	11.3b	10.0a	5.6bc	0.02c
<u>P. debaryanum</u>	12.2b	9.7a	6.5b	0.00c
<u>P. irregulare</u>	12.3b	9.7a	6.0bc	0.00c
<u>P. myriotylum</u>	8.6a	5.7a	1.8d	0.94a
<u>P. ultimum</u>	12.2b	9.6ab	5.9bc	0.47b
Noninfested	11.7b	10.2a	9.8a	0.00c

^Z Mean of 6 replicates; one plant per replicate. Means within a column followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keuls test.

^Y Number of pods with pod rot indices of 3, 4 and 5 in a replicate were summed and divided by the total number of pods.



***P. myriotylum* *P. irregulare* Noninoculated**

Figure 35. Pathogenicity of *P. myriotylum* and *P. irregulare* to Pronto peanut as compared to a noninoculated control.

Plant height was reduced ($P=0.05$) by P. myriotylum and P. aphanidermatum in both experiments. Pythium irregulare and P. ultimum reduced plant height only in the second experiment.

Root rot severity was more obvious in pots with P. myriotylum, P. aphanidermatum, P. ultimum and P. irregulare than in pots infested with P. arrhenomanes and P. debaryanum.

The above results demonstrate that other species of Pythium in addition to P. myriotylum can cause root and pod rots of peanut. How common these other Pythium spp. are in Oklahoma peanut fields is not known. Although P. myriotylum was routinely isolated from rotted pods from Ft. Cobb in this study and others (19, 46), other isolates of Pythium spp. have been obtained from pods with pod rot. These isolates have yet to be speciated; however, based on growth on CMA at temperatures from 5-45 C and morphological characteristics of the isolates, several distinct groups of Pythium spp. may inhabit peanut soil at Ft. Cobb and elsewhere in Oklahoma (Filonow, unpublished observations). Pythium aphanidermatum and P. ultimum are ubiquitous fungi in agricultural soils (68) and most likely reside in peanut soils in Oklahoma and elsewhere. Therefore, it is important for plant breeders and pathologists to consider these other species when evaluating new peanut genotypes and fungicides for pod rot control.

CHAPTER V

SUMMARY AND CONCLUSIONS

Results from Ft. Cobb in 1989 and 1990 and from seven other peanut fields sampled in 1990 corroborate the findings of previous Oklahoma researchers that populations of Pythium spp. in soils planted with peanut have temporal patterns to their fluctuations during the growing season. These patterns are a common occurrence in Oklahoma peanut fields and may be likewise in all soils where peanut is grown. The patterns in Oklahoma soils appear to be of two general types, both occurring after pegging and pod development have begun: (1) a proliferation in population followed by a usually rapid decline and (2) a gradual increase in population over the season, reaching a maximum near or at harvest. Temporal patterns in the fluctuations of Pythium spp. populations in soil were all type 1, except for those (type 2) observed in Marshall county, Oklahoma.

In regard to the second objective of the study, results indicate that the peanut host has a dominant influence on the fluctuations of Pythium spp. populations in soils planted with peanut. This finding is supported by the following lines of evidence: (1) the absence of population

peaks in fallowed soil compared to the presence of peaks in soil planted to peanut, (2) the lack of any correlation between population fluctuations and soil temperature and/or soil moisture at Ft. Cobb and seven other peanut cropping locations, (3) the greater populations of Pythium spp. found in soil containing roots and/or pods compared to soil without roots and pods in the pod training experiments and (4) the reproduction of the proliferation and decline pattern in Pythium spp. populations observed in the peanut zones of field soil in boxes incubated at controlled temperatures. Although both roots and pods influence Pythium spp. populations in soil, results from this study suggest that pods may have an important effect. Peaks in Pythium spp. populations in field and growth chamber experiments occurred only after pegging and not before.

Based on the above findings, the peak and decline effect in temporal populations of Pythium spp. in soil in fields in Caddo and Garvin Counties can be explained. It is suggested that populations of Pythium spp. in soil increase in response to nutrients exuded from developing pods. As pods grow toward maturity, exudation dramatically subsides, hyphae of Pythium spp. move from the geocarposphere to colonize the surface of pods in their later stages of development. This latter premise is supported by the finding that the frequency of Pythium spp. isolated from pods at Ft. Cobb increased linearly with the age of the pod

and that the maximum isolation percentage was not synchronous with the peak of Pythium spp. population in soil.

Therefore, the introduction of energy into the soil for growth of Pythium spp., the proliferation and decline of the Pythium spp. population and Pythium spp. colonization of pods are coupled components of an ecosystem predominantly driven by the peanut host. It should be possible using the findings obtained from this study to develop a model for predicting timely application of Pythium active fungicides.

Results of the last objective showed that Pythium aphanidermatum and P. ultimum, in addition to P. myriotylum, could cause root and pod rots of Pronto peanut. Pythium irregulare, P. arrhenomanes, and P. debaryanum did not cause pod rot; however, P. irregulare was generally pathogenic to roots, whereas P. arrhenomanes and P. debaryanum were not. In evaluations of peanut genotypes or fungicides for pod rot control, inclusion of P. aphanidermatum and P. ultimum in addition to P. myriotylum should be considered.

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APPENDIX

CHARACTERISTICS OF FIELD SOILS SAMPLED IN 1989 AND 1990

Site	pH	kg/ha				$\mu\text{g/g}$
		P	K	Ca	$\text{NO}_3\text{-N}$	$\text{NH}_4\text{-N}$
Ft. Cobb	7.0	92	186	1093	12	0.22
C4	6.9	141	245	1989	27	0.23
C6	6.6	197	521	1661	19	0.20
C10	7.4	74	138	1131	19	0.08
G1	7.4	65	389	4317	17	0.42
G2	5.8	112	231	2807	20	0.37
M1	4.9	66	69	283	25	0.30
M2	6.4	202	256	998	18	0.13

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