To the Graduate Council:

I am submitting herewith a thesis written by Danna Darlene Baird entitled "Seasonal Fluctuations in Cotton Seedling Pathogens in Three West Tennessee Soils." I recommend that it be accepted in partial fulfillment of the requirements for the degree Master of Science, with a major in Agricultural Biology.

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SEASONAL FLUCTUATIONS IN COTTON SEEDLING PATHOGENS IN THREE WEST TENNESSEE SOILS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee

Danna Darlene Baird

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ACKNOWLEDGEMENTS

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ABSTRACT

The total number of *Pythium* isolates found had a significant seasonal variation in incidence. *P. ultimum* also showed significant seasonal variation. The genus *Fusarium* was found to have a highly significant seasonal variation. Only one pathogen, a mycelial form of *Pythium* not producing sexual spores, had a significant variation with regard to soil type. Of all discolored hypocotyls there was a significantly greater incidence in soils not cropped to cotton than in soils cropped to cotton. *Thielaviopsis* showed a reversal of this trend and had a significantly greater incidence in soils cropped to cotton than in soils not cropped to cotton.

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INTRODUCTION

Seedling blight or damping off is one of the most destructive diseases of cotton. It is caused by a variety of pathogens including Pythium, Fusarium, Rhizoctonia, and Thielaviopsis. Reduction of stands commonly caused by seedling blight often necessitates replanting. Replanting results in losses by reduction in yield. Seedling diseases are estimated to cause 4 to 5 percent loss in cotton annually in Tennessee. This does not include replanting cost or losses due to lateness of replanted cotton (21). Tennessee, being in the northern most part of the cotton belt, frequently has environmental conditions favorable to infection by seedling disease pathogens while cotton is in the seedling stage of growth.

Control of seedling disease is at present unsatisfactory. Johnson (33) indicated that seed treatment with fungicides for damping-off very rarely resulted in increases in cotton yield. Application of fungicides to soil has resulted in better control than seed treatment, but this method is expensive and often fails to increase yields. Very little is known about the distribution of cotton seedling pathogens in soils, their seasonal incidence and survival or their occurrence in soils of different crop histories. Basic information about these pathogens is needed as a basis for the development of better methods of control.

The purposes of this study were: (1) to monitor seasonal fluctuations in the incidence of cotton seedling blight pathogens,

(2) to find which of these pathogens were present in each soil tested and their relative incidence, and (3) to determine differences in the incidence of these pathogens in soils cropped to cotton against the same soils not cropped to cotton.

CHAPTER I

PRELIMINARY CONSIDERATIONS

I. History

Damping-off organisms have been studied in various parts of the world and on a large variety of host plants. The history of damping-off studies goes all the way back to Antone DeBary (54). Various methods have been used in studying damping-off pathogens and a volumnous amount of work has been done since the time of DeBary including morphology, physiology, host specificity, and fungicide effects. In 1943 Middleton (38) published a large comprehensive work on the genus Pythium. Since that time a particularly interesting finding by Hendrix (22) is that there are heterothallic species of Pythium.

There are a great many pathogens which are known to cause damping-off of a spectrum of hosts including such diverse hosts as pine and strawberry. Fortunately not all damping-off organisms are found on Gossypium hirsutum.

II. Environmental Factors

Environmental conditions affect the incidence of the major pathogens of cotton seedlings. The findings of Johnson (33) correlated high moisture and low temperature with cotton seedling disease severity in field studies. These same studies indicate that in some years one pathogen, Rhizoctonia, was more prevalent while in other years another

pathogen, Pythium, was more prevalent. Later, Johnson (35) recovered damping-off pathogens from soils initially and subsequently from the same samples after storage.

Observations of this kind led to questions about the influence of other factors which are intrinsic to an understanding of the nature of inoculum potential and disease severity. Since temperature and moisture fluctuate annually perhaps there could be found seasonal variations in inoculum potential.

For a long time crop rotations have been recommended to help prevent the build-up of organisms pathogenic to cotton seedlings, i.e., Hadden (21) and Johnson (32). Perhaps there are some differences in which pathogens are found in greater numbers and which are found in lesser numbers in soils cropped to cotton for several years.

III. Pedological Factors

Vicksburg soils are Typic Udifluvents. They are coarse-silty, mixed, acid, thermic soils which were formed mostly from loess with some alluvium. They are well drained with slow runoff and moderate permeability (52).

The Memphis series are fine-silty, mixed, thermic Typic Hapludalfs. They are formed in loess and are well drained. Runoff in this stries is medium to rapid with moderate permeability (51).

Dexter soils are fine-silty, thermic Ultic Hapludalfs. They are formed in silty alluvium of mixed mineralology. They are well drained with moderate permeability and medium runoff (50).

The difference most pertinent here is the slow runoff of Vicksburg soils which could indicate a moisture level more favorable to cotton seedling disease. Since the Memphis soils have moderate to rapid runoff they could be expected to have fewer cotton seedling pathogens.

CHAPTER II

MATERIALS AND METHODS

Bulk samples of surface soil were collected from 12 sites, 4 of these from the Tennessee Agricultural Experiment Station at Milan, Tennessee, and 8 from the West Tennessee Experiment Station at Jackson, Tennessee. Each bulk sample consisted of 50 subsamples taken from an area 40 by 40 feet. Collections were made from three soil types, Dexter, Memphis, and Vicksburg. Cotton had been grown for three or more years previously on 2 of the sites of each type. On the 2 additional sites, crops other than cotton had been grown for four or more years previously. An outline of the soils with their location and history is given in Table I.

Soil samples were passed through screens with 6-mm openings and then placed in 15-cm diameter pots. Nine pots from each sample were prepared in this manner. Seven cotton seeds of a composite of 32 varieties were planted in each pot; they were then placed in a constant temperature growth chamber at 27°C. After nine days, data were taken on emergence, at which time 48 plants in each sample were transferred to a growth chamber with a constant temperature of 19°C for 12 days. Plants were removed, washed, and examined for discoloration of the hypocotyls. Portions of discolored or necrotic hypocotyls (0.5 - 1.0 cm each) were cut from the plants and washed in flowing tap water for 24 hours. They were then washed in three successive changes of sterile distilled water and blotted with sterile filter paper. Each

TABLE I

IDENTIFICATION, LOCATION, AND HISTORY
OF SOILS COLLECTED FOR ANALYSIS

Sample Number	Soil	Location	Identifying Designation	Crop History
1	Vicksburg Fine Sandy Loam	Milan	S-4 field	Cotton for over 5 years
2	Vicksburg Fine Sandy Loam	Milan	S-1 field	Cotton for over 5 years
3	Vicksburg Fine Sandy Loam	Jackson	Herbicide test field	No cotton for over 10 years (corn in 1974)
4	Vicksburg Fine Sandy Loam	Jackson	Nematode area	No cotton for over 10 years (soybeans for over 5 years)
5	Memphis Silt Loam	Milan	North tract	Cotton for over 3 years
6	Memphis Silt Loam	Milan	North tract	Cotton for over 3 years
7	Memphis Silt Loam	Jackson	RR field	No cotton for over 10 years (corn in 1974 soybeans in 1973 corn in previous years)
8	Memphis Silt Loam	Jackson	Hort. field	No cotton for over 10 years (tomatoes in 1974 and for several years)
9	Dexter Loam	Jackson	Breeder seed field	Cotton for over 3 years

TABLE I (continued)

Sample Number	Soil	Location	Identifying Designation	Crop History
10	Dexter Loam	Jackson	Strains field	Cotton for over 3 years
11	Dexter Loam	Jackson	Plant Path. range 4	No cotton for 4 years (wheat in 1974, barley 1971-73)
12	Dexter Loam	Jackson	Hort. field	No cotton for over 10 years (tomatoes in 1974 and for several years)

hypocotyl segment was placed on the surface of 2 percent agar containing 10 mg/liter Aureomycin in a petri dish. After three to five days of incubation at room temperature resulting fungal colonies were identified to genus. Hyphal tips of Pythium growing from the segments were transferred to tubes of corn meal agar (CMA). Isolates of Pythium were grown on hempseed agar (HSA) to induce the formation of sexual structures for identification to species. Keys used in identifications were Gilman (14), Middleton (38), and Waterhouse (53).

Crosses were made among those isolates which failed to produce sexual spores on HSA. The test isolate was placed in the center of a petri dish containing HSA. Three other isolates were placed in peripheral positions in the same dish. After four to five days crosses were examined for sexual spores.

Crosses were made with known cultures of P. sylvaticum using the same procedure for determination of that species.

Collections and isolations were made from each of the 12 sites four times during 1974-75: November, 1974, February, 1975, June, 1975, and August, 1975. After collection samples were stored in polyethylene bags for no more than two weeks until plantings were made.

CHAPTER III

RESULTS

I. Genera Found

A total of 2,304 cotton plants were employed as the means of obtaining soil borne pathogens. A little more than half of all the plants tested, 1,174, showed necrotic hypocotyls. The pathogen most frequently encountered was Pythium, which was recovered from hypocotyl sections of 582 plants. Fusarium was recovered from 401 test plants and was second in order of frequency. The pathogen found to have the third highest incidence was Rhizoctonia which was recovered from 140 discolored hypocotyls. Forty necrotic hypocotyls were found to have Thielaviopsis. One isolate of Alternaria was recovered and six isolates of an unidentified septate fungus with intercalary chlamydospores which failed to produce conidia on HSA or CMA.

The largest nubmer of discolored hypocotyls, 342, were found in the August samples. November samples had 336 necrotic hypocotyls and June samples produced discoloration in 330 plants. February samples yielded the fewest necrotic hypocotyls with only 166.

Data were transformed to $\sqrt{n} + 1$ and an analysis of variance was performed on the coded data.

The seasonal distribution for Pythium is given in Figure 1. The largest number of isolates were recovered in November. The seasonal differences in the incidence of Pythium was found to be statistically significant.

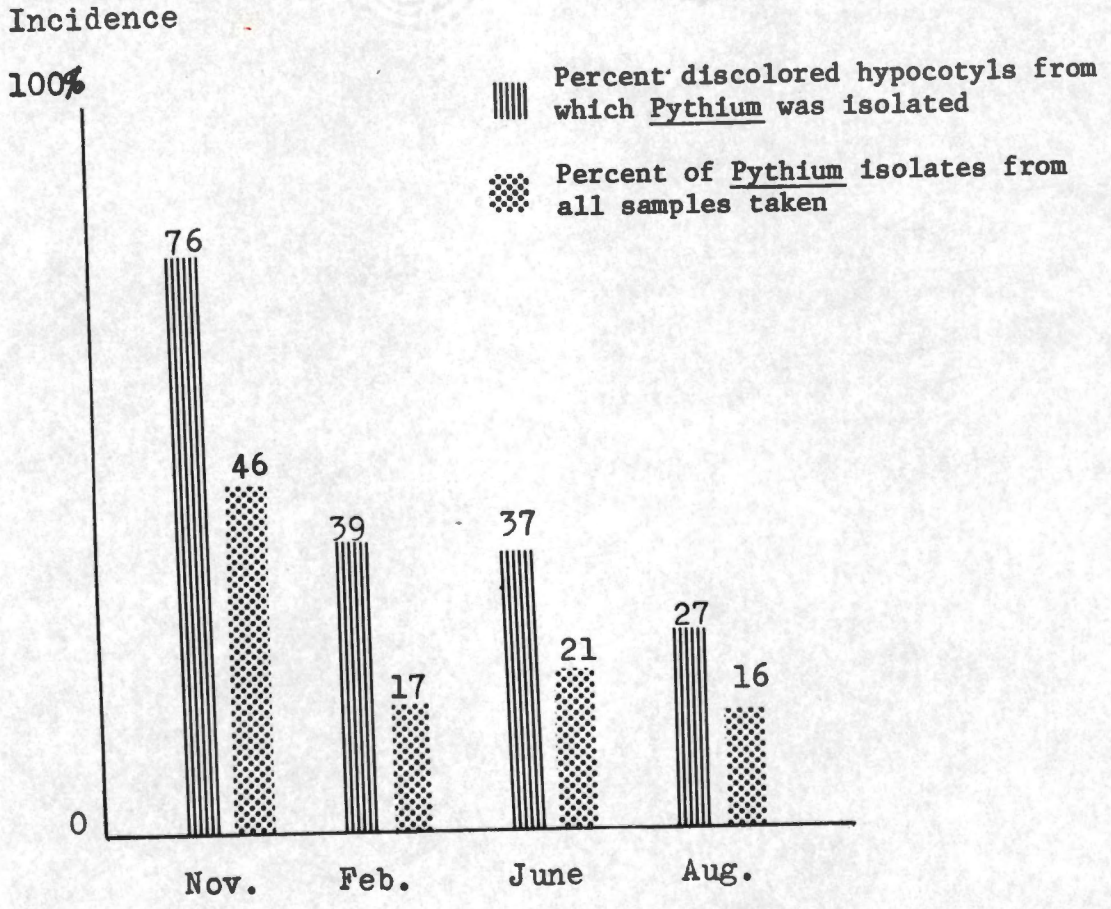

FIGURE 1. Incidence of Pythium in four collection months. The striped bars compare Pythium with other genera found in each collection month. The stippled bars show the seasonal distribution of Pythium for the duration of the experiment.

Fusarium, by contrast, in Figure 2 showed the fewest numbers in November with subsequent gains in other months. Statistically Fusarium was found to be highly significant in its seasonal fluctuations.

In Figure 3, Rhizoctonia is shown to have its greatest incidence in August as does Thielaviopsis in Figure 4; however, these figures are not statistically significant.

In the three soil types tested, the primary pathogen, Pythium, was found to have its greatest frequency in Vicksburg (Figure 5). By contrast, in Figure 6, Fusarium had its least incidence in Vicksburg. Rhizoctonia (Figure 7) and Thielaviopsis (Figure 8) have higher values in Vicksburg as did Pythium. The overall incidence of pathogens in each of the three soils tested is shown in Figure 9.

Two cropping histories were compared to determine whether soils cropped to cotton tend to build up a large inoculum reservoir on cotton plant residue. The soils not cropped to cotton were not cropped to cotton for at least three years previously. Those cropped to cotton were cropped to cotton for at least three years previously. The relative distribution of pathogenic genera under these two crop history categories is given in Figure 10. Rhizoctonia and Thielaviopsis were found more frequently in the soils cropped to cotton. However, the pathogens most frequently encountered were found more often in soils not cropped to cotton. The statistical analysis revealed that those differences were significant for the total number of discolored hypocotyls found. Thielaviopsis showed a significant difference between the two cropping histories.

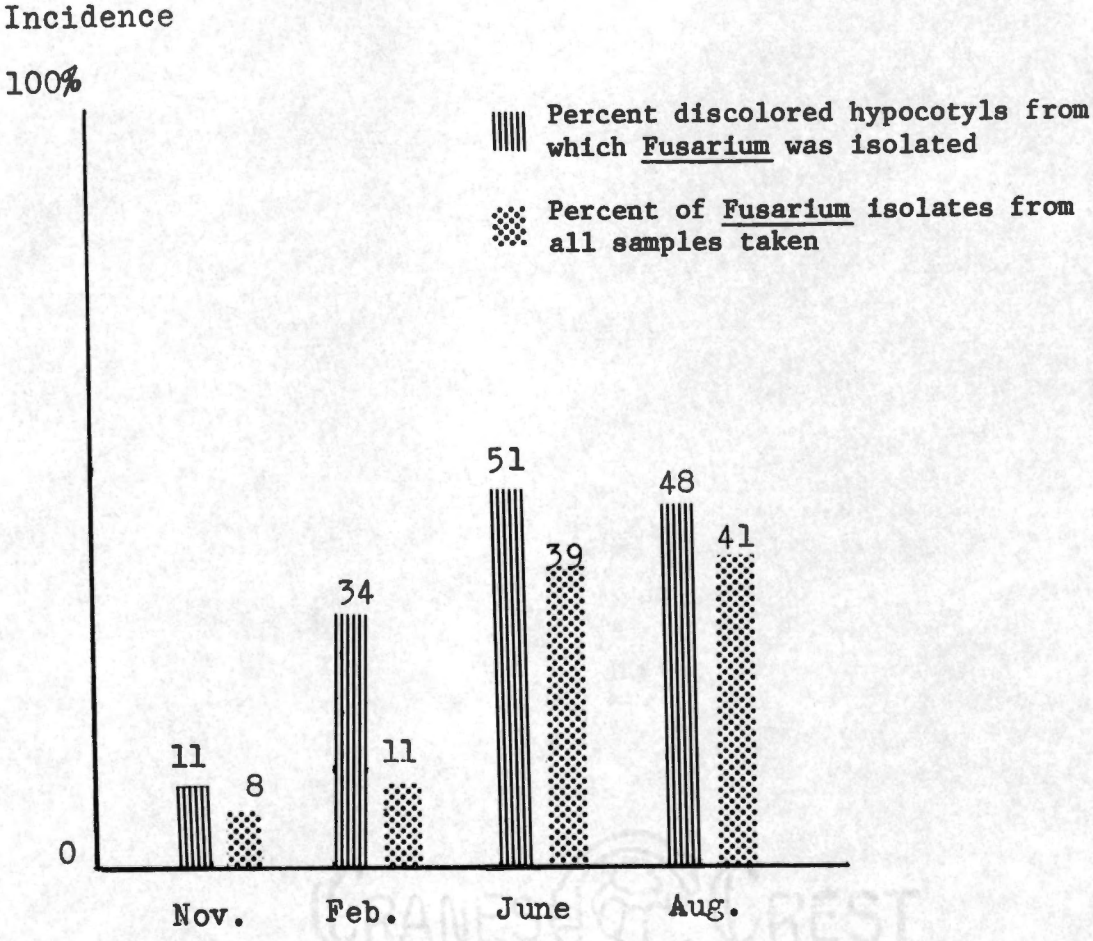

FIGURE 2. Incidence of Fusarium in four collection months. The striped bars compare Fusarium with other genera found in each collection month. The stippled bars show the seasonal distribution of Fusarium for the duration of the experiment.

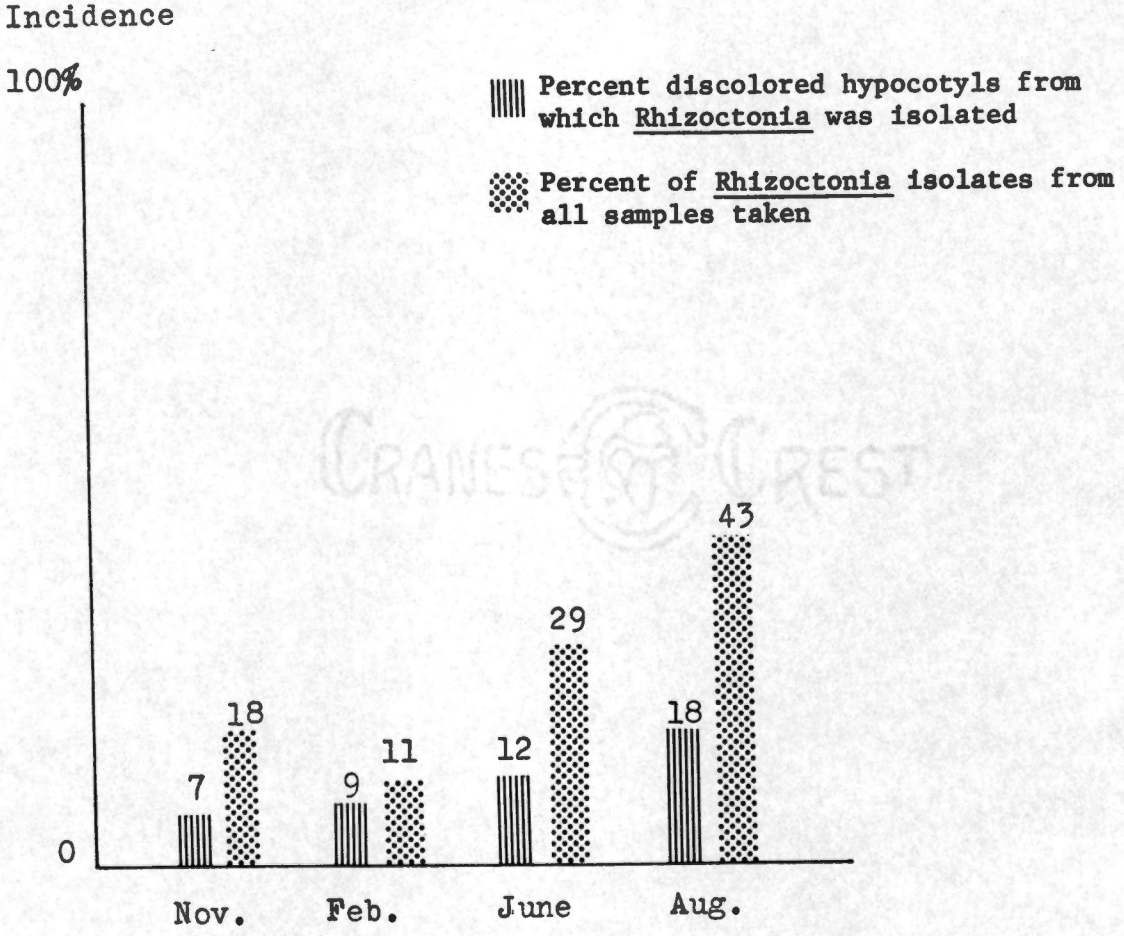

FIGURE 3. Incidence of Rhizoctonia in four collection months. The striped bars compare Rhizoctonia with other genera found in each collection month. The stippled bars show the seasonal distribution of Rhizoctonia for the duration of the experiment.

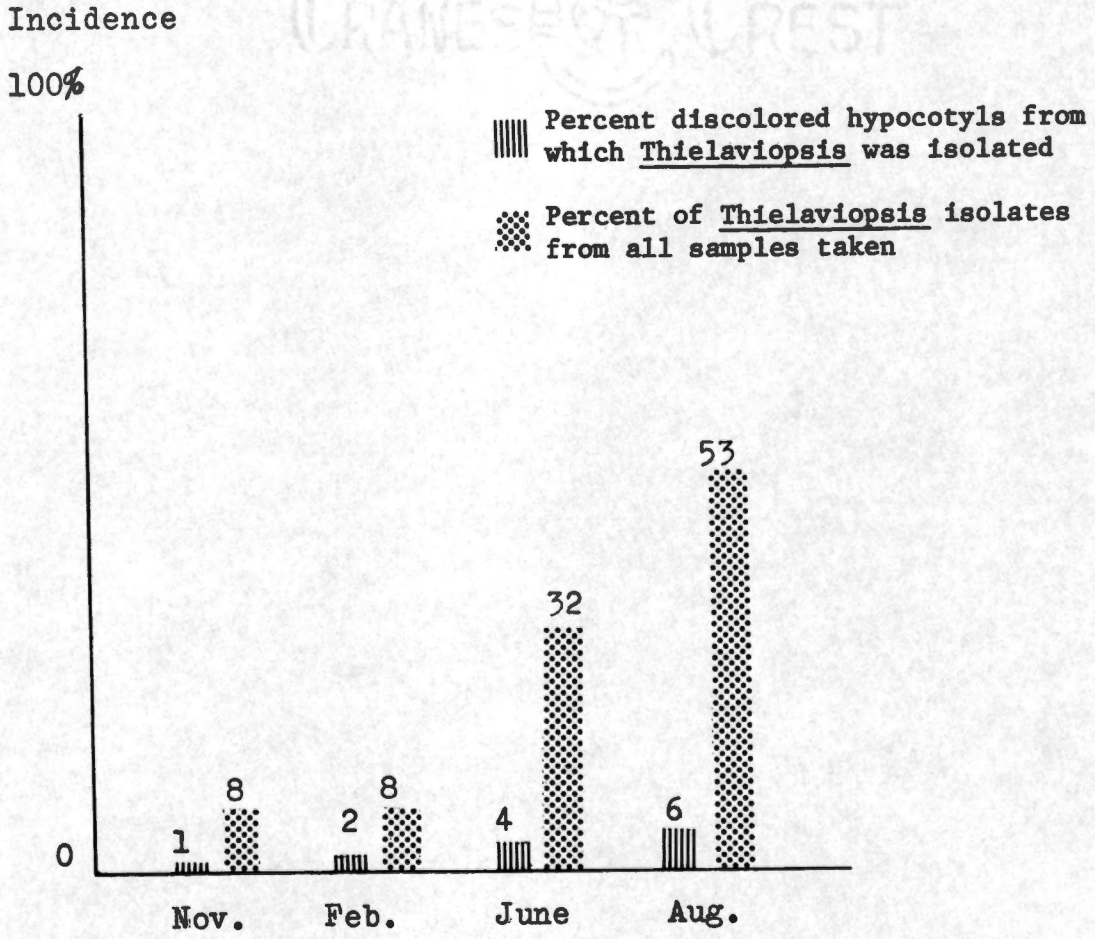

FIGURE 4. Incidence of Thielaviopsis in four collection months. The striped bars compare Thielaviopsis with other genera found in each collection month. The stippled bars show the seasonal distribution of Thielaviopsis for the duration of the experiment.

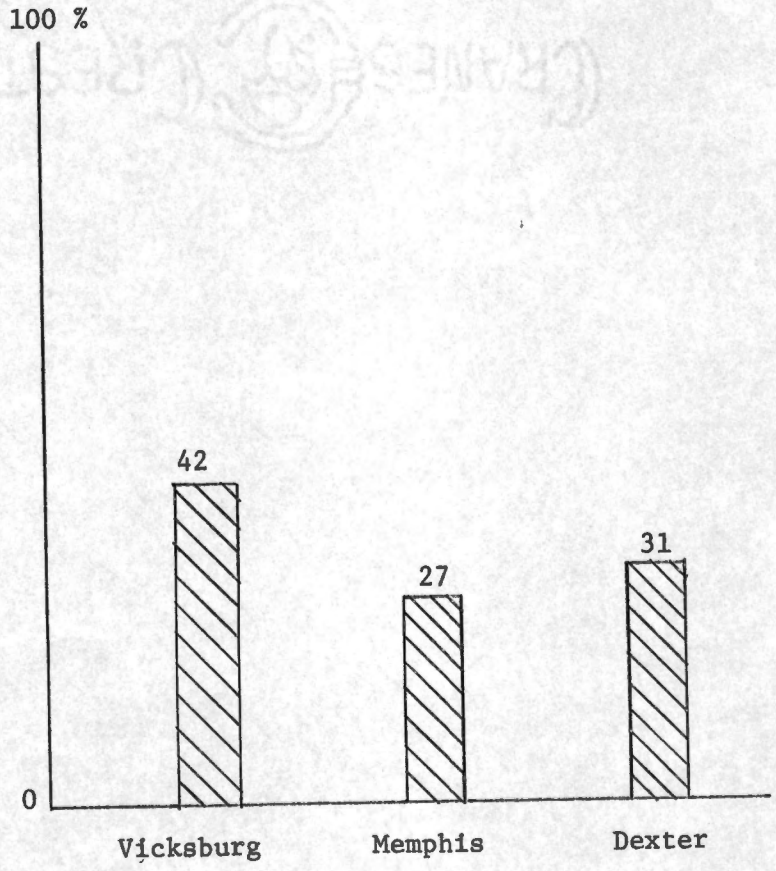

FIGURE 5. Incidence of Pythium in each of three soil types as a percentage of the total number of isolates of Pythium.

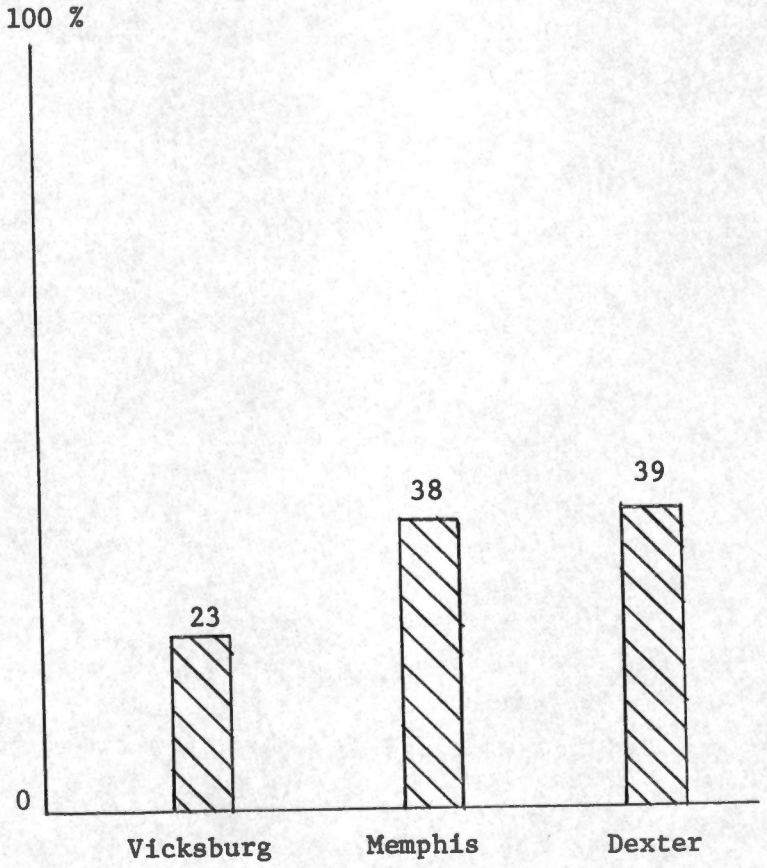

FIGURE 6. Incidence of Fusarium in each of three soil types as a percentage of the total number of isolates of Fusarium.

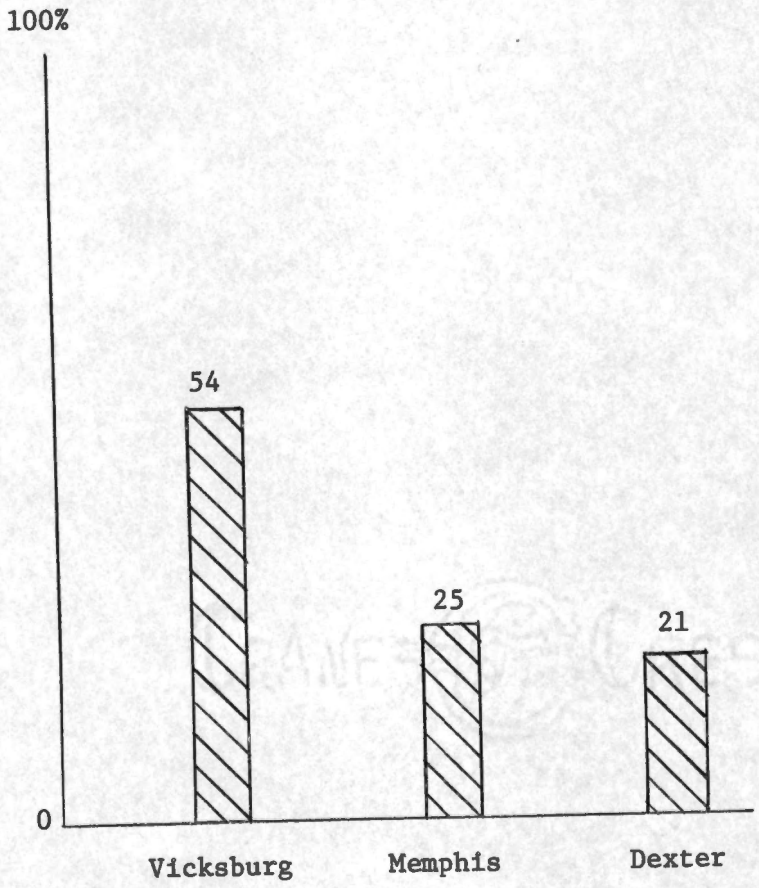

FIGURE 7. Incidence of Rhizoctonia in each of three soil types as a percentage of the total number of isolates of Rhizoctonia.

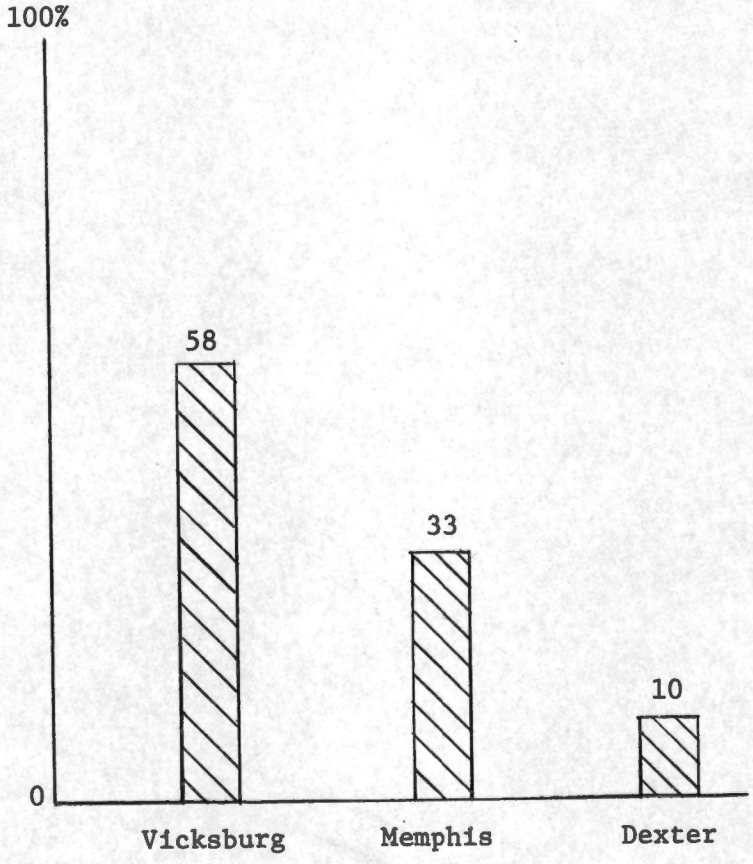

FIGURE 8. Incidence of Thielaviopsis in each of three soil types as a percentage of the total number of isolates of Thielaviopsis.

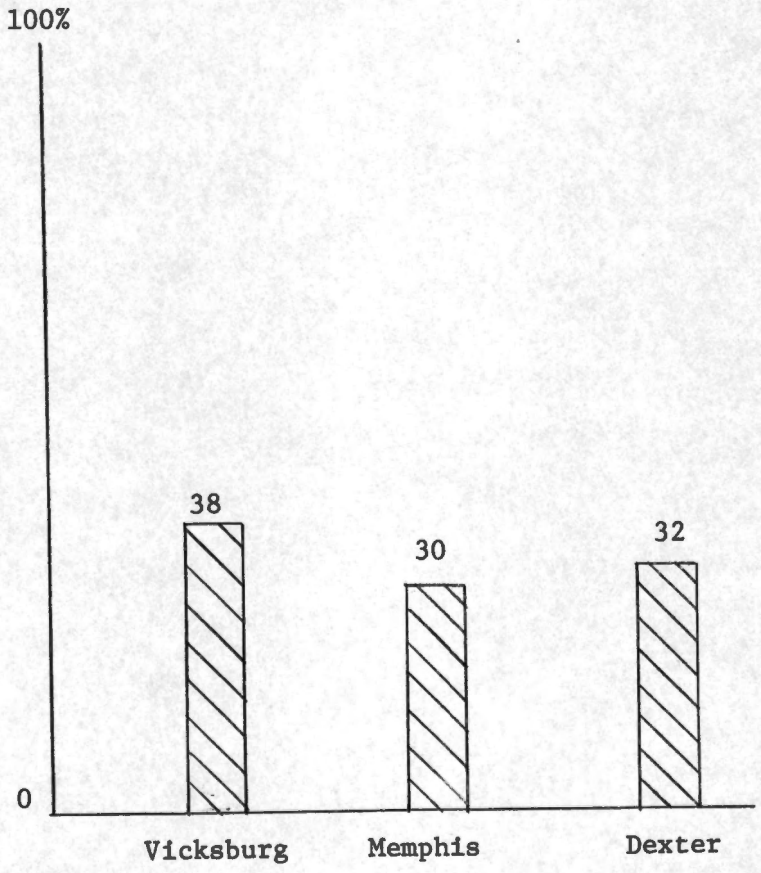

FIGURE 9. Incidence of pathogens isolated from each of three soil types as a percentage of the total number of necrotic hypocotyls.

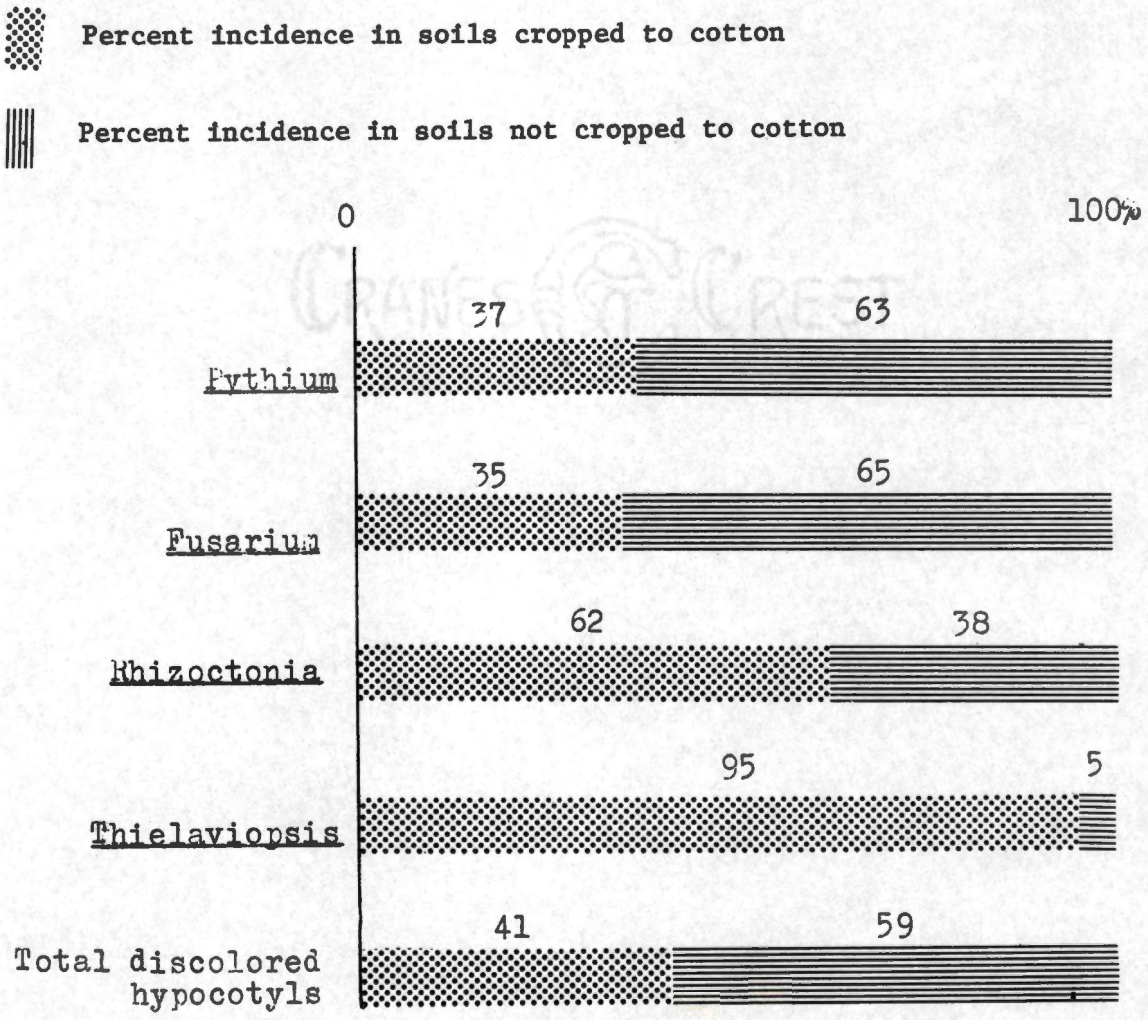

FIGURE 10. Incidence of pathogens in soils of different crop histories.

The amount of Rhizoctonia was found to be inconsistent from soil to soil in the soils cropped to cotton versus soils not cropped to cotton. Over the seasons sampled the incidence of Pythium in soils of different crop histories did not vary with consistence among the soils tested, nor did the incidence of Thielaviopsis.

II. Species of Pythium Found

P. ultimum was the species of Pythium found most often. A total of 367 isolates were recovered. In Figure 11 it is shown that most P. ultimum was found in November. The variation with seasonal change was found to be statistically significant. It did not, however, change in the two crop history categories with consistency among the soils tested over the seasons tested. Some seasons yielded more P. ultimum in soils cropped to cotton or in soils not cropped to cotton than did other seasons.

The mycelial form of Pythium not producing sexual spores also had its greatest incidence in November (Figure 12), as did P. sylvaticum (Figure 13), and P. irregulare (Figure 14). P. heterothallicum was found most frequently in February, however. Figure 15 gives the seasonal distribution of P. heterothallicum.

The numbers of P. irregulare and P. heterothallicum were too low for statistical differences of a significant nature to be indicated.

Of the three soil types tested P. ultimum had its greatest incidence in Vicksburg (Figure 16).

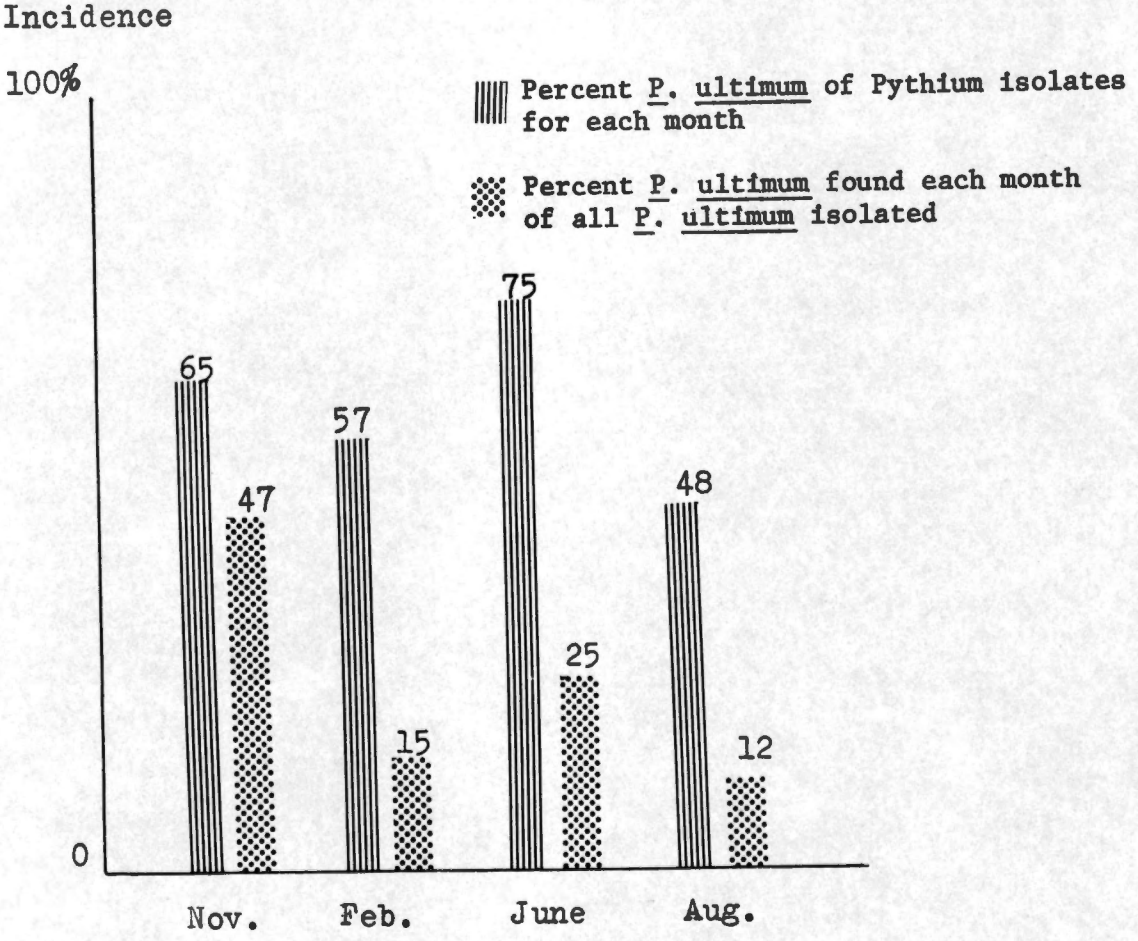

FIGURE 11. Incidence of P. ultimum in four collection months. The striped bars compare P. ultimum with other species of Pythium in each collection month. The stippled bars show the seasonal distribution of P. ultimum for the duration of the experiment.

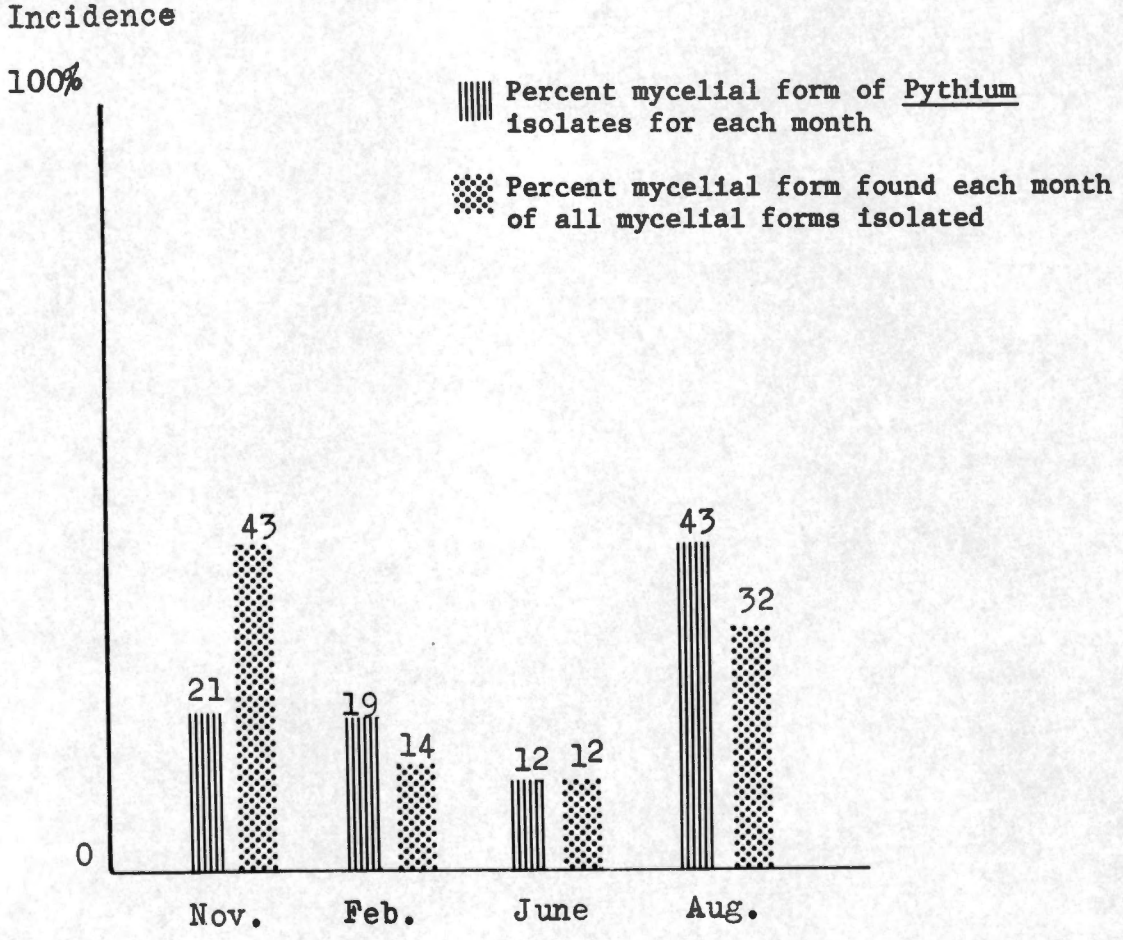

FIGURE 12. Incidence of an unidentified mycelial form of Pythium not producing sexual spores. The striped bars compare this form with other species of Pythium in each collection month. The stippled bars show the seasonal distribution of this form for the duration of the experiment.

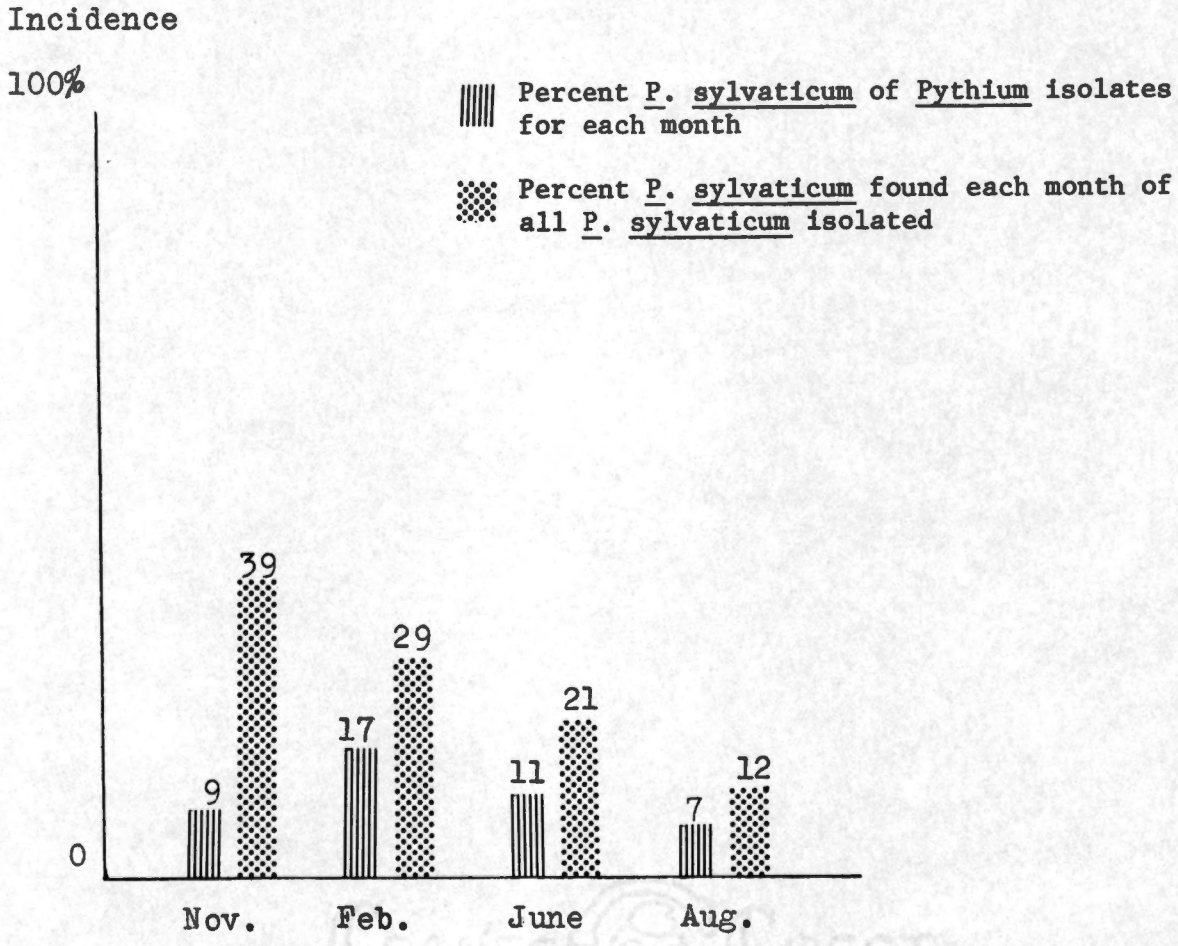

FIGURE 13. Incidence of P. sylvaticum in four collection months. The striped bars compare P. sylvaticum with other species of Pythium found in each collected month. The stippled bars show the seasonal distribution of P. sylvaticum for the duration of the experiment.

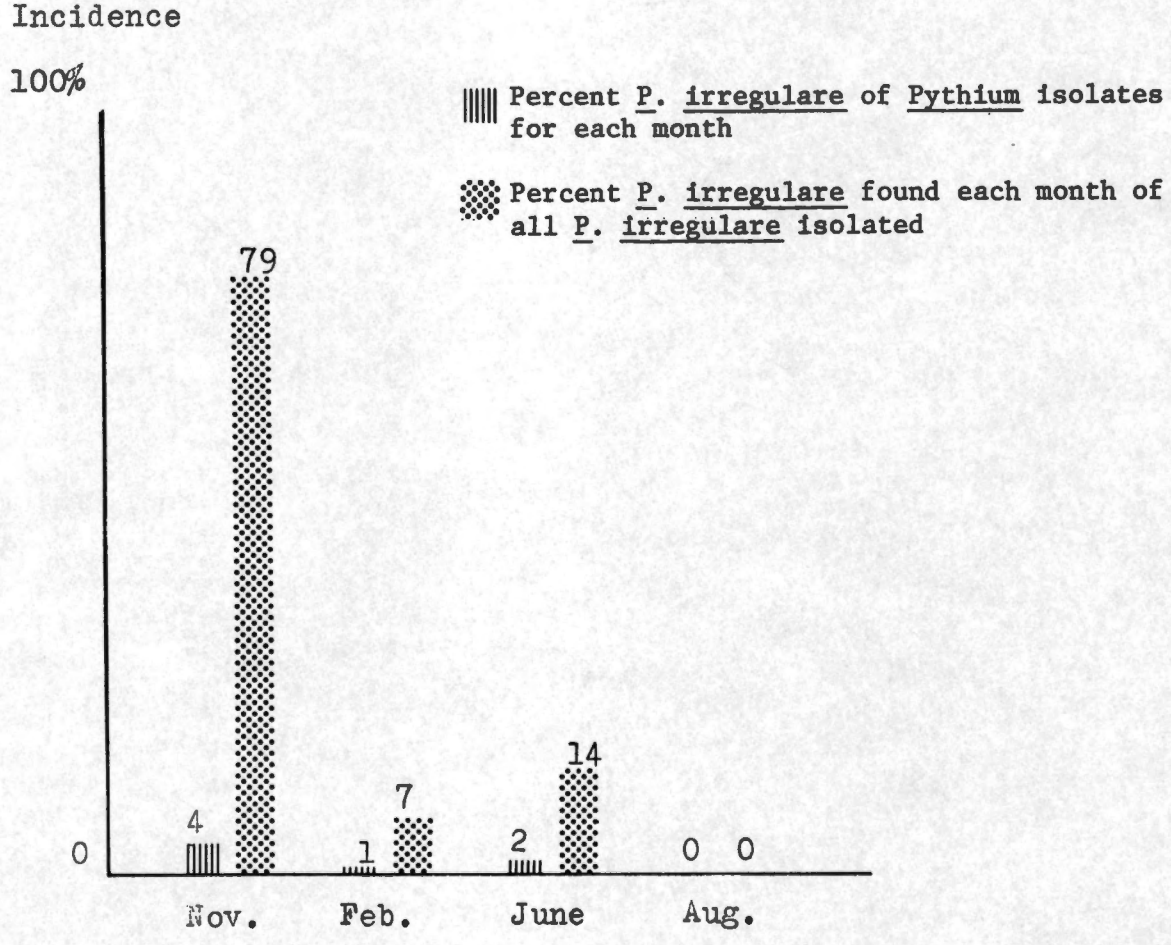

FIGURE 14. Incidence of P. irregulare in four collection months. The striped bars compare P. irregulare with other species of Pythium found in each collection month. The stippled bars show the seasonal distribution of P. irregulare for the duration of the experiment.

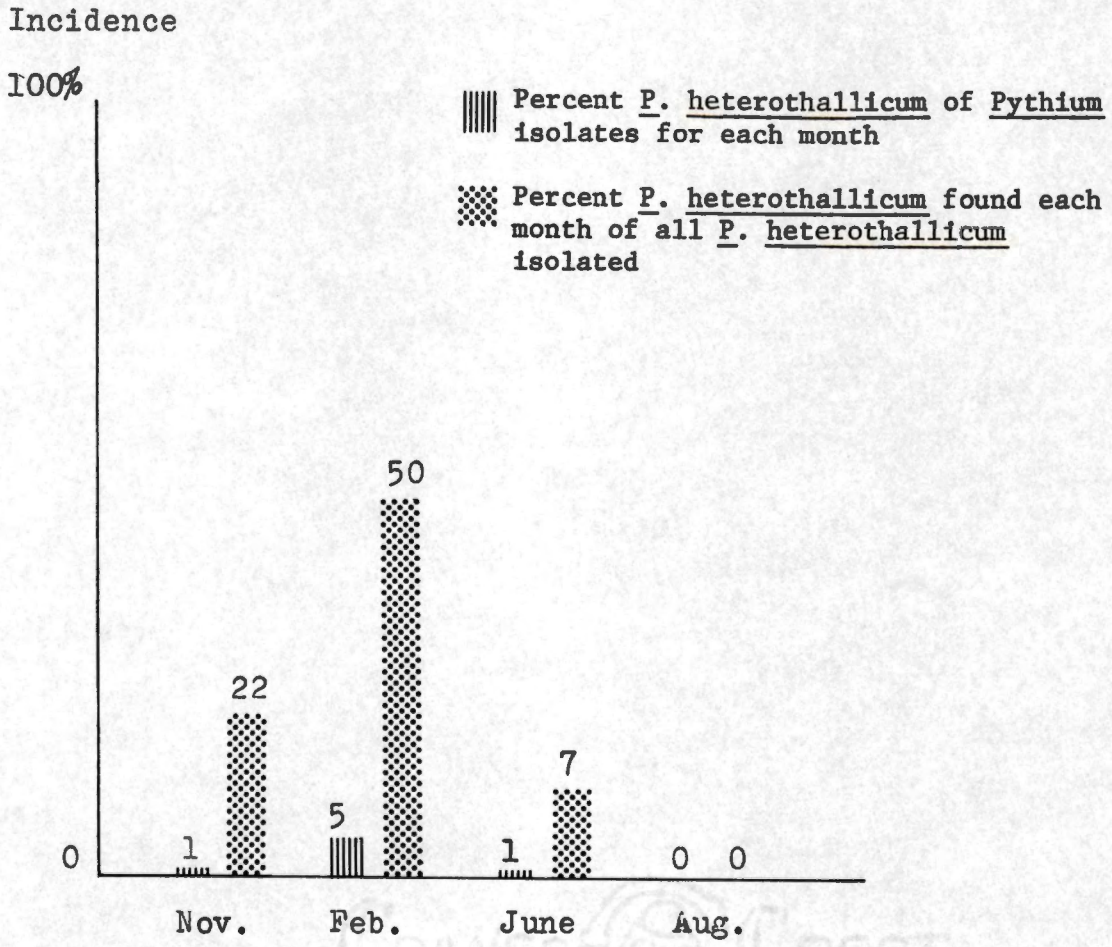

FIGURE 15. Incidence of P. heterothallicum in four collection months. The striped bars compare P. heterothallicum with other species of Pythium found in each collection month. The stippled bars show the seasonal distribution of P. heterothallicum for the duration of the experiment.

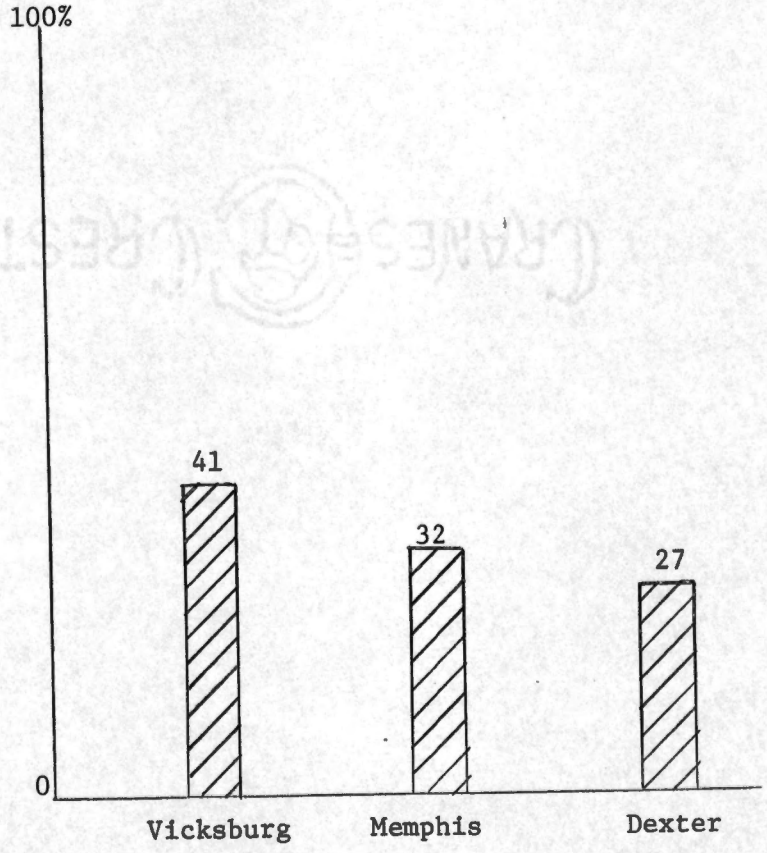

FIGURE 16. Incidence of P. ultimum in each of three soil types as a percentage of the total number of isolates of P. ultimum.

The mycelial form of Pythium not producing sexual spores was the only pathogen found to be statistically significant in its variation among the soils tested. Figure 17 shows the striking distribution among the soils tested. Vicksburg and Dexter appear to be equal in inoculum whereas Memphis has a much lower level of inoculum.

P. sylvaticum in Figure 18 is shown to be more prevalent in Memphis soils as is P. irregulare in Figure 19. P. heterothallicum (Figure 20) was recovered most frequently from Vicksburg.

The incidence of species of Pythium in soils of different crop histories is given in Figure 21. All species found were more prevalent in soils cropped to cotton except P. ultimum which was the major species of Pythium found.

One isolate of P. prolatum was recovered.

Table II gives pH values found for sites sampled and disease incidence for those sites. No correlation was found at these normally occurring pH values.

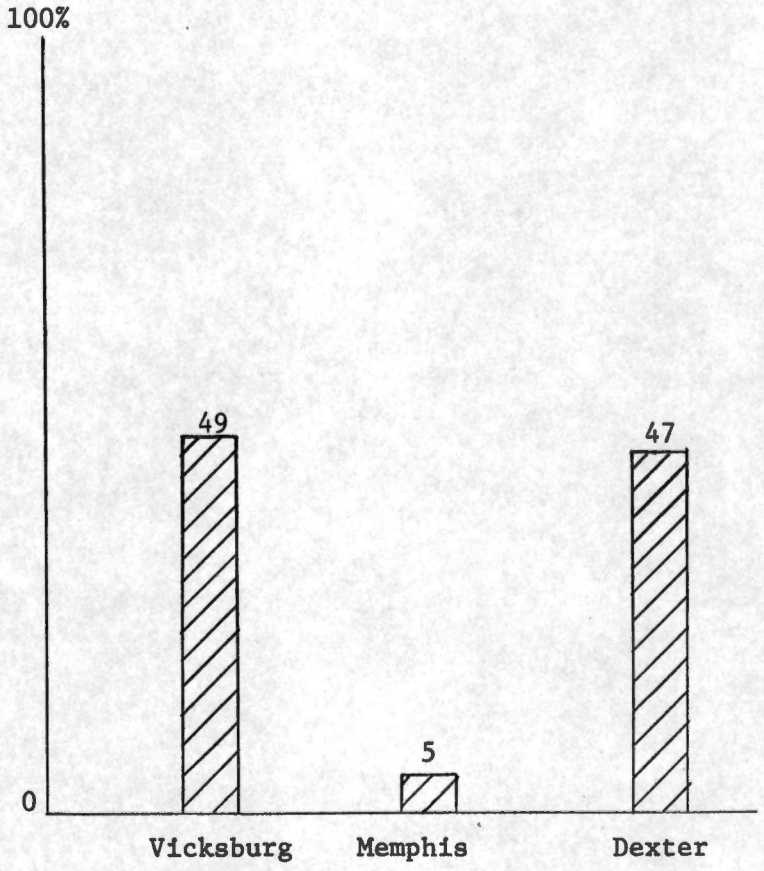

FIGURE 17. Incidence of a mycelial form of Pythium not forming sexual spores in each of three soil types as a percentage of the total number of isolates of this form.

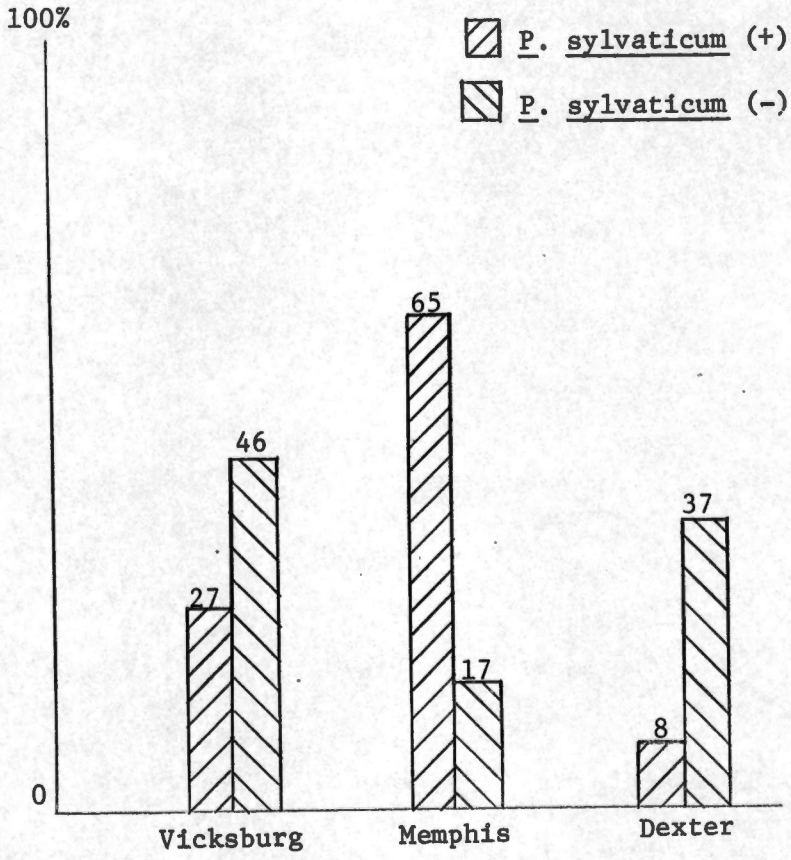

FIGURE 18. Incidence of P. sylvaticum in each of three soil types as a percentage of the total number of isolates of P. sylvaticum.

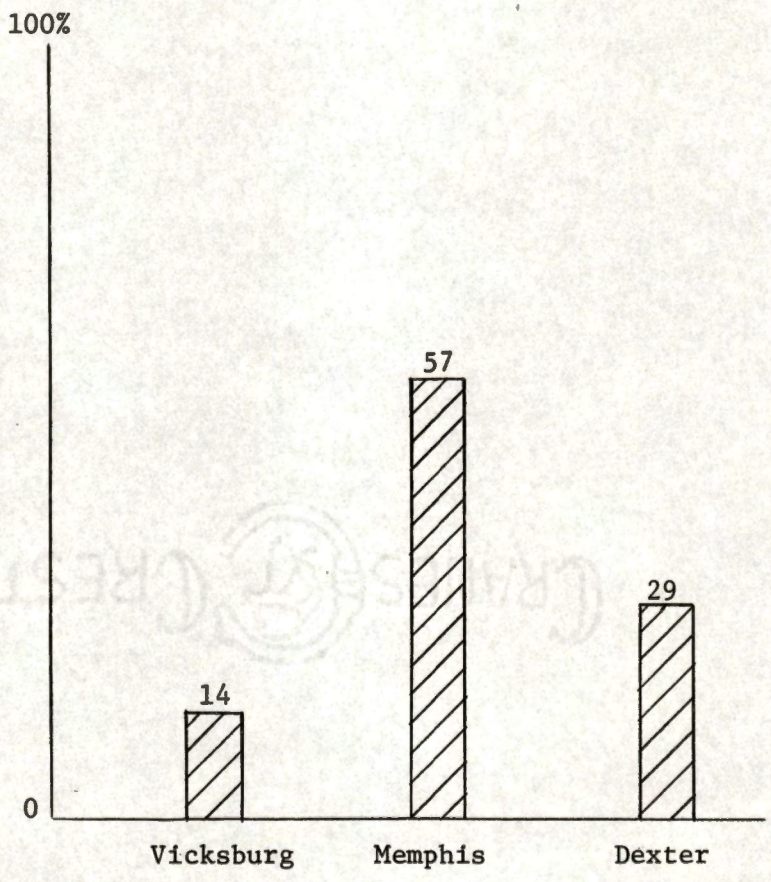

FIGURE 19. Incidence of P. irregulare in each of three soil types as a percentage of the total number of isolates of P. irregulare.

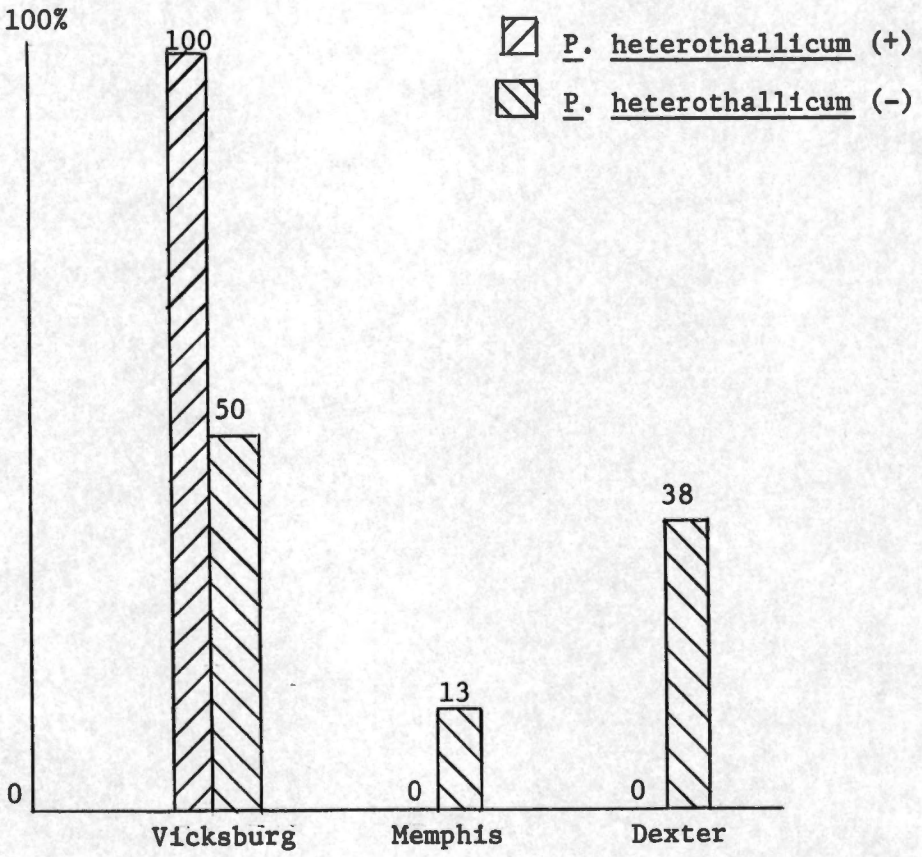

FIGURE 20. Incidence of P. heterothallicum in each of three soil types as a percentage of the total number of isolates of P. heterothallicum.

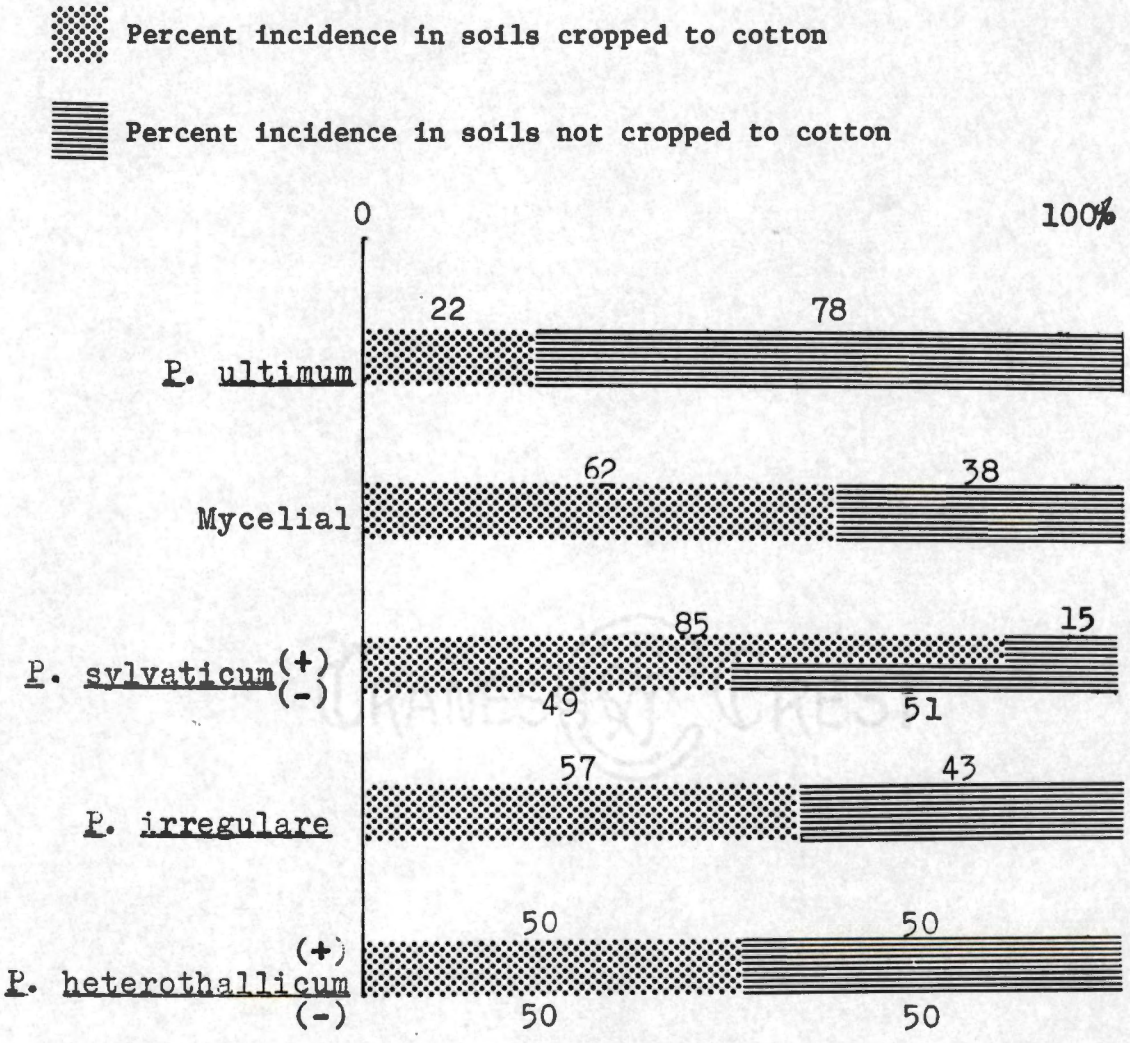

FIGURE 21. Incidence of species of Pythium in soils of different crop histories.

TABLE II

PERCENT NECROTIC HYPOCOTYLS AND pH
FOR TWELVE SAMPLE LOCATIONS

Sample Number	Soil	Percent Necrotic Hypocotyls	pH
1	Vicksburg Fine Sandy Loam	7	5.5
2	Vicksburg Fine Sandy Loam	10	5.5
3	Vicksburg Fine Sandy Loam	12	5.9
4	Vicksburg Fine Sandy Loam	9	6.1
5	Memphis Silt Loam	3	4.7
6	Memphis Silt Loam	7	5.8
7	Memphis Silt Loam	12	5.2
8	Memphis Silt Loam	9	6.0
9	Dexter Loam	7	5.1
10	Dexter Loam	7	5.7
11	Dexter Loam	8	5.9
12	Dexter Loam	10	4.7

CHAPTER IV

DISCUSSION AND SUMMARY

From the results of this experiment it appears that Pythium is the primary pathogen of seedling disease in these three soils, followed by Fusarium, Rhizoctonia, and Thielaviopsis.

Vicksburg soil had the greatest number of pathogens followed by Dexter and Memphis.

Both Pythium and Fusarium were found to have significant seasonal fluctuations. Undoubtedly numerous interactions occur among these pathogens as well as with other nonpathogenic concomitant micro-organisms. For example, Pythium and Rhizoctonia, but not Fusarium can be parasitized by Thrichoderma viride as reported by Lang (37). It is probable that these results do not represent actual populations but rather are skewed by competion for infection of the hypocotyls, although they do give some indication of actual populations, and are most useful as a practical measurement of disease incidence.

When only those pathogens from soils cropped to cotton for at least three years are considered, Pythium is still the major pathogen (Figure 22). Fusarium is still the second most prevalent. Rhizoctonia and Thielaviopsis although found less often than Pythium and Fusarium, occurred more frequently in soils cropped to cotton. Of the species of Pythium, P. ultimum and the mycelial form which failed to produce sexual spores are still the major pathogenic species and are about equal in importance.

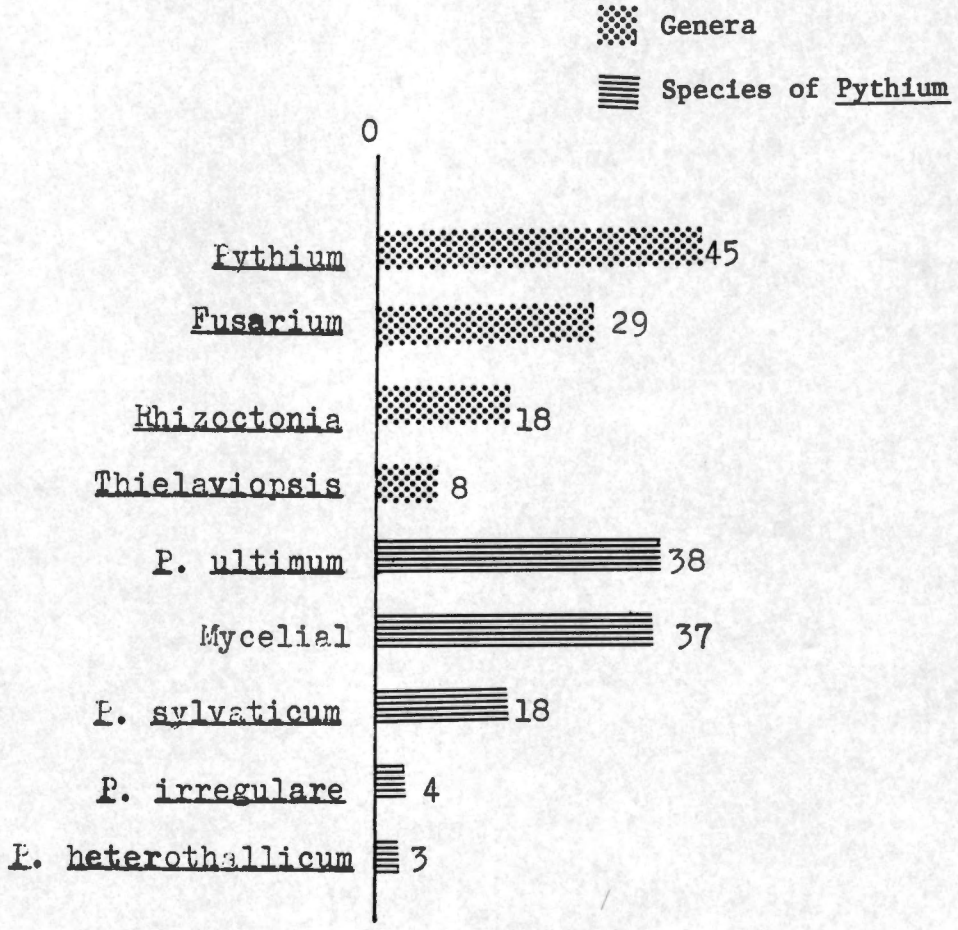

FIGURE 22. Relative distribution of those pathogens recovered from soils cropped to cotton for at least three years previously. The stippled bars show the percent for each genus of all pathogens found. The striped bars show the percent of each species of Pythium found.

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