To the Graduate Council:

I am submitting herewith a thesis written by Joyce Holladay Doyle entitled "Physical, Chemical, and Biological Characteristics of Cotton Soils and Their Relationship to Seedling Disease of Cotton." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

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PHYSICAL, CHEMICAL, AND BIOLOGICAL CHARACTERISTICS OF COTTON SOILS AND THEIR RELATIONSHIP TO SEEDLING DISEASE OF COTTON

A Thesis

Presented for the

Master of Science

Degree

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Joyce Holladay Doyle

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DEDICATION

This thesis is dedicated to my father, Wendell Holladay, whose belief in the power of education has been an inspiration to me.

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ABSTRACT

Cotton (Gossypium hirsutum) seedling disease is responsible for considerable reductions of cotton yields in Tennessee each year. The complex of causal organisms, in order of pathogenicity, includes species of Pythium, Rhizoctonia, Thielaviopsis, and Fusarium. Attempts to control the disease have been mostly unsuccessful, especially during cool, wet periods which favor disease development. Because of the lack of a good control, a bioassay technique was tested for accuracy in predicting occurrence and severity of cotton seedling disease. It was hoped that by using this technique better recommendations could be made on selecting fields for cotton culture. A quantitative method for isolating Pythium from soil on a selective medium was compared to the bioassay technique for effectiveness in predicting the disease.

Forty-five soil samples were collected at 15 sites in West Tennessee in the fall of 1982 and again in the fall of 1983. The bioassay procedure consisted of planting Stoneville 213 cotton seeds in pots filled with each soil sample. The seedlings that emerged were each evaluated for disease severity and the causal organism was isolated from each diseased plant. A soil assay for Pythium was performed on each soil sample using gallic

acid medium. In addition, each soil sample was analyzed for pH, texture, and organic matter content. A field test was conducted in 1984 which consisted of planting Stoneville 213 cotton seed at the sites where soil samples were taken the previous fall. Emerged seedlings were evaluated for disease severity as in the bioassay procedure. Percent stand and percent post-emergence mortality were also determined.

Correlation coefficients were calculated each year between all the variables that were evaluated. There was a definite negative relationship between the percent clay in the soil and the disease severity of seedlings grown in the field. Disease severity of the seedlings grown during the bioassay procedure and numbers of seedlings infected with Pythium and/or Rhizoctonia did not correlate with the disease severity of the seedlings in the field. Also, disease severity correlated positively with soil pH.

Significant correlations occurred both years between percent clay and the disease indexes from the bioassay, the number of Fusarium isolates and disease indexes from the bioassay, pH and the number of Rhizoctonia isolates, and percent clay and the number of Fusarium isolates. There was no correlation between the numbers of Pythium colonies isolated on gallic acid medium and any of the other variables either year.

TABLE OF CONTENTS

CHAPTER		PAGE
INTRODUCTION		1
I.	LITERATURE REVIEW	4
	Seedling Disease Complex	4
	Seedling Disease in Tennessee	5
	Control of Cotton Seedling Disease	6
	Prediction of Cotton Seedling Disease Occurence	7
II.	MATERIALS AND METHODS	9
	Collection of Soil Samples	9
	Bioassay Technique	9
	Isolation of *Pythium* and *Rhizoctonia* on Selective Media	12
	Soil Analysis	13
	Field Test	13
	Seed Germination Test	14
III.	RESULTS	15
	Physical and Chemical Characteristics of Soil Samples	15
	Seed Germination Test	15
	Biological Characteristics of Soil Samples	15
	Statistical Analysis of Data	26
IV.	DISCUSSION	36
LITERATURE CITED		41
VITA		45

LIST OF TABLES

TABLE		PAGE
1.	Location and Sites of Soil Samples Collected in 1982 and 1983	10
2.	Physical and Chemical Characteristics of Soil Samples From Tennessee Cotton Fields Collected in November 1982	16
3.	Physical and Chemical Characteristics of Soil Samples From Tennessee Cotton Fields Collected in October 1983	18
4.	Biological Characteristics of Soil Samples From Tennessee Cotton Fields Collected in November 1982	20
5.	Biological Characteristics of Soil Samples From Tennessee Cotton Fields Collected in October 1983	23
6.	Disease Severity of Cotton Seedlings in Field Plots (Planted May 1984, Data Were Collected June 1984)	27
7.	Significant Correlations Between Biological Variables and Physical and Chemical Characteristics of Soil Samples From Cotton Fields	30
8.	Significant Correlations Between Disease Index (Bioassay) and Numbers of Organisms Isolated From Soil Samples Collected From Tennessee Cotton Fields	31
9.	Significant Correlations Between Bioassay Variables of Soil Samples Collected in October 1983 and Field Data Variables From Cotton Plantings in May 1984	31

INTRODUCTION

Each year seedling pathogens are responsible for considerable reduction of cotton yields. Estimates of losses in cotton in the United States have averaged 2.8% per year over the last 10 years (33). In 1982, the estimated reduction in yield due to seedling disease was 283,736 bales. In Tennessee annual losses averaged 11% from 1978-1982, which is by far the highest of the cotton producing states (2,3,4,5,16).

In cotton, the complex of causal agents of seedling disease includes Pythium spp., Fusarium spp., Rhizoctonia solani Kuehn, and Thielaviopsis basicola (Berk. and Br.) Ferr. Any one of these fungal soil inhabitants, or several acting together, may produce the disease (33). Isolates of Pythium caused the most severe disease, followed by isolates of Rhizoctonia, Thielaviopsis, and Fusarium in pathogenicity tests of fungi from diseased cotton hypocotyls grown in Tennessee soils (21). R. solani and Pythium spp. are apparently the major pathogens of cotton seedlings in Tennessee (20).

Seedling disease of cotton is characterized by pre-germination decay of seed, pre- and post-emergence damping-off, stem canker (sore shin), and seedling root rot. Seed decay and damping-off can cause partial loss of stands (33). This phenomenon is especially common in

Tennesse due to low temperatures and high soil moisture levels which frequently occur after cotton has been planted. The incidence and severity of the disease is directly linked with the occurrence of these environmental conditions.

Attempts to control cotton seedling disease consist mainly of the use of fungicides. Control by selection of disease resistant cultivars has not been emphasized in the development of most commercial cottons (24). Since most soils are infested with several seedling pathogens, a combination of several fungicides to control seedling disease is often necessary (22). Applications may be expensive and control may be variable or unsatisfactory during periods when environmental conditions are favorable for disease development.

Because of the lack of a good control for severe cases of cotton seedling disease, there is a need for a method that can be used to predict the occurrence and severity of the disease. Thus, growers could be informed on the feasibility of using proctectant fungicides. Several quantitative methods have been developed for selective isolation of Pythium spp. and Rhizoctonia solani from soil (13,14,23,25,27,29,31,32,34). None of these techniques can be used to successfully predict disease occurrence or severity on cotton since non-

pathogenic strains of the organisms are isolated as well as pathogenic strains. The purpose of this study was to test a specific bioassay technique for accuracy in predicting the occurrence and severity of cotton seedling disease in cotton fields of West Tennessee.

CHAPTER I

LITERATURE REVIEW

Seedling Disease Complex

In 1892, Atkinson (1) described soreshin symptoms on cotton seedlings caused by Rhizoctonia solani. In 1925 and 1926, Shapovalov (28) isolated Fusarium spp. from diseased cotton seedlings. These and other findings gave rise to the concept of cotton seedling disease being caused by a complex of organisms in which one or several organisms may cause typical disease symptoms. Brinkerhoff et al. (11) isolated Pythium spp. from diseased cotton seedlings grown during periods of cold, damp weather and Staffeldt (30) found Thielaviopsis basicola (Berk. and Br.) Ferr. also to be a part of the cotton disease complex. R. solani, Fusarium spp., and Pythium spp. have been reported to be the primary pathogens in the seedling disease complex of cotton (26).

R. solani alone or in conjunction with other pathogens may produce all the symptoms of cotton seedling disease (33). Fusarium spp. and R. solani are the major causes of seed rot. Pythium spp. and R. solani are the major causes of pre- and post-emergence damping-off. Thielaviopsis basicola may also cause post-emergence damping-off. This pathogen is encountered most in the

Southwestern United States but is occasionally found in the Southeast (33).

Pythium spp. isolated from and pathogenic to cotton include P. aphanidermatum (Eds.) Fitz., P. debaryanum Hesse, P. heterothallicum Campbell and Hendrix, P. irregulare Buisman, P. polytylum Drechs., P. splendens Braun, P. sylvaticum Campbell and Hendrix, and P. ultimum Trow. P. ultimum is the most prevelant and pathogenic of these species. The species of Fusarium which have been identified in diseased seedlings include F. moniliforme Sheldon, F. oxysporum Schlecht, and F. roseum Link (33).

Seedling Disease in Tennessee

In Tennessee, the most frequently isolated fungi from diseased cotton hypocotyls are Pythium spp. followed by Fusarium spp., Rhizoctonia solani, and Thielaviopsis basicola. Pythium spp. also causes the most severe disease on cotton seedlings grown in Tennessee soils followed by isolates of Rhizoctonia, Thielaviopsis, and Fusarium. The Pythium spp. isolated in order of their frequency are P. ultimum, P. sylvaticum, P. irregulare, and P. heterothallicum (21).

Johnson et al. (20) found that in years when average soil moisture content was high and the average post-planting soil temperature was 13°C or lower, Pythium was more prevalent. When soil temperatures averaged 20°C or

higher, R. solani was more prevalent. When soil temperatures averaged 14-19°C, isolation frequencies of both organisms were similar.

Control of Cotton Seedling Disease

Control of cotton seedling disease includes use of cultural methods, resistant cultivars, and fungicides. According to Minton and Garber (24), one of the most important factors affecting seedling disease losses is planting depth. Seed should be planted only as deeply as necessary to insure adequate moisture for seed germination and seedling growth, and to decrease exposure to cooler soil temperatures and pathogen attack. Selection of high quality seed is also important. Exposure of seed to high moisture and heat decreases seed quality (33).

In the past, breeding of cotton for resistance has played only a small part in control of seedling disease. Recently, Bird (6) selected multi-adversity-resistant (MAR) cultivars which are more resistant to mold growth and rot of the seed than most commercial cultivars. The seedlings of MAR cultivars are resistant to many pathogens and have high cold tolerance. In 1983, three MAR cultivars were tested in California (24) in a field heavily infested with P. ultimum, R. solani, and T. basicola. The number of surviving seedlings was significantly higher with the MAR cultivars than with

Acalas developed locally and of a Mississippi commercial cultivar.

The use of fungicides for seed treatments and in-furrow applications is one of the most common methods of controlling cotton seedling disease. The diversity of the potential pathogens warrants the use of a combination of several fungicides to control the disease but with severe outbreaks even this may not be effective. Chambers (12) reported that of eleven soil fungicide formulations and four application methods tested for control of cotton seedling disease in two experiments during 1982, none increased yields significantly.

In tests performed over several years, some fungicides for control of P. ultimum and R. solani have been effective. Metalaxyl (Apron, Ridomil) has given excellent control of Pythium in greenhouse and field trials and BAS 389 and CGA-64251 effectively controlled R. solani. The latter fungicide was also effective against T. basicola (24).

Prediction of Cotton Seedling Disease Occurence

Only one study has been made relating a quantitative isolation method of soil-borne organisms to the amount of disease occurrence (13). This study was done in the San Joaquin Valley of California to examine the relationship between soil populations of several Pythium species

isolated on a selective medium and the survival of cotton seedlings. It was found in six field tests that the survival of cotton seedlings was directly related to the concentration of soil-borne propagules of P. ultimum. Other species of Pythium were also present in the soil samples but in relatively low concentrations. Pathogenicity of the isolates was not determined.

CHAPTER II

MATERIALS AND METHODS

Collection of Soil Samples

Surface soil samples were collected from 15 sites in cotton fields in West Tennessee in November 1982 and again in October 1983 (Table 1). Ten of these sites were common for both collections. Each site consisted of a 9.15 m row along the border of a field cropped to cotton. Each site was divided into three 3.05 m subsites, designated A,B, and C. Soil samples from each subsite consisted of 15 or more 10-15 cm deep "trowel" samples. The samples were placed in heavy duty plastic bags to prevent drying and brought back to the laboratory where they were mixed to insure uniformity and stored at room temperature.

Bioassay Technique

A bioassay technique similar to that of Johnson *et al*. (21) was performed on each soil sample. Soil from each sample was placed in a 10 cm diameter plastic pot. Seven Stoneville 213 cotton seeds were planted in each pot which were then placed in a constant temperature growth chamber at 26°C. The pots of soil were watered periodically to insure germination and growth of seedlings. After eight days the temperature was reduced to

Table 1. Location and Sites of Soil Samples Collected in 1982 and 1983.

Site No.	1982		1983	
	Location	Soil	Location	Soil
1	Ames[a]	Loring Silt Loam		Same as 1982
2	Ames	Loring Silt Loam		Same as 1982
3	Ames	Calloway Silt Loam		Same as 1982
4	Ames	Memphis Silt Loam		Same as 1982
5	Ames	Memphis Silt Loam		Same as 1982
6	Milan[b]	Falaya Silt Loam		Same as 1982
7	Milan	Collins Silt Loam (Field 1)	Milan	Collins Silt Loam (Field 2)
8	Milan	Vicksburg Silt Loam		Same as 1982
9	Milan	Collins Silt Loam	Milan	Vicksburg Sandy Loam
10	Milan	Memphis Silt Loam	Jackson	Memphis Silt Loam
11	Jackson[c]	Dexter Loam		Same as 1982
12	Jackson	Loring Silt Loam (Field 1)	Jackson	Loring Silt Loam (Field 2)
13	Jackson	Grenada Silt Loam		Same as 1982
14	Jackson	Loring Silt Loam		Same as 1982
15	Jackson	Dexter Loam	Jackson	Vicksburg Silt Loam

[a]Ames Plantation, Grand Junction, Tennessee.

[b]Milan Agricultural Experiment Station, Milan Tennessee.

[c]West Tennessee Agricultural Experiment Station, Jackson, Tennessee.

17°C and the seedlings were thinned to four per pot. Thirteen days later the seedlings were removed from the soil, washed, and rated according to the following rating system:

1 - healthy, no disease symptoms;

2 - slight, pinpoint, necrotic lesions or a tan diffused area on the hypocotyl;

3 - definite necrotic hypocotyl lesion, dark, not necessarily sunken, less than 1/2 cm in length;

4 - necrotic hypocotyl lesion more than 1/2 cm in length;

5 - plant wilted;

6 - plant emerged, but dead;

7 - plant not emerged.

After the seedlings were rated, 1/2 cm of the diseased portion of the hypocotyl was cut from each plant, placed in screw cap vials, and washed in five changes of water. The first two washes contained Tween-20 at a concentration of one drop per 100 ml distilled water. The last three washes were plain sterile distilled water. After washing, the hypocotyls were blotted on sterile filter paper and placed on 2% water agar in petri dishes. Four days after incubation at 25°C, the fungus or fungi growing from each hypocotyl segment were identified to genus. If no growth was observed, the plates were incubated a few days longer. This procedure was repeated four times on the soil

samples collected in 1982 and five times on the samples collected in 1983.

Isolation of Pythium and Rhizoctonia on Selective Media

An assay for Pythium spp. was performed on each soil sample with a selective gallic acid medium developed by Flowers and Hendrix (14). This gallic acid medium was stored for at least 24 hours at 5°C before using. It was then "seeded" with a 1:25 soil dilution prepared by adding 4 g of a soil sample to 100 ml of cooled 0.25% water agar. One ml of the soil-agar suspension was spread over the surface of the medium in a petri dish which was then stored at 20°C. After 24 hours, the soil was washed off the surface of the agar with slowly running tap water. The plates were stored again at 20°C for 36-48 hours. Developing Pythium spp. colonies were then counted and recorded. For the soil samples taken in 1982, two plates were seeded for each sample. For the soil samples taken in 1983, three plates were seeded for each sample.

Two techniques were used to isolate R. solani from the samples. The first was the use of a selective medium devised by Summer and Bell (32) which contained metalaxyl and benomyl as inhibitors of fungi other than Rhizoctonia spp. and neomycin sulfate for bacterial inhibition. The other method was described by Weinhold (34) and consisted

of removing organic particles from the soil samples by sieving. These particles were dispersed in 1% water agar in petri dishes and incubated 18-24 hours at room temperature.

Soil Analysis

Percent organic matter was determined for each soil sample using the Walkey-Black method (7). This procedure was duplicated for each sample. Soil texture of each sample was determined with the Bouyoucos hydrometer method (10). Soil pH was determined for 25 g of each sample with a Fisher ion analyzer.

Field Test

On May 9, 1983 and May 17, 1984, Stoneville 213 cotton seed was planted at the sites where soil samples were taken the previous fall. Furrows were made with a hand plow at each site. One hundred seeds were planted in each 3.05 m site in the furrow. Approximately four weeks after planting, emerged seedlings were lifted from the soil, washed, and rated on the same rating scale as previously described. Percent stand and post-emergence mortality were also determined. In 1983, extensive flooding washed out many seeds and prevented estimation of mortality and disease severity. Weather conditions were mostly favorable in 1984 and percent stand, post-

emergence mortality, and disease severity data were obtained on all plots except for sites 8A and 13C, which were low, flooded areas.

Seed Germination Test

Each year, the germination of the Stoneville 213 cotton seed used in these studies was tested by planting 10 seeds each in 10 pots filled with sterile sand. The pots were placed in a constant temperature growth chamber at 25°C and incubated for nine days. The pots were watered periodically to insure germination. The germinated seeds were counted and percent germination was determined.

CHAPTER III

RESULTS

Physical and Chemical Characteristics of Soil Samples

Percent organic matter, pH, percent sand, silt, and clay of the soil samples collected in 1982 and 1983 are listed in Tables 2 and 3, respectively. The figures given for percent organic matter are mean values of two determinations.

Seed Germination Test

Viability of the Stoneville 213 cotton seeds used in both the 1983 and 1984 bioassay procedures and field experiments decreased during the 2-year period. Seventy-nine percent of the seed germinated in 1982, but only 72% germinated in 1983.

Biological Characteristics of Soil Samples

Average disease indexes of cotton plants and the number of seedling hypocotyls from which the organisms, Pythium spp., Rhizoctonia spp., Fusarium spp., Thielaviopsis spp., and others were isolated in 1982 are listed in Table 4. Also listed are the average numbers of Pythium colonies isolated on gallic acid medium from soil samples collected that year. Corresponding data for soil samples collected in 1983 are given in Table 5.

Table 2. Physical and Chemical Characteristics of Soil Samples From Tennessee Cotton Fields Collected in November 1982.

Site No.	Organic Matter (%)	pH	Mineral Fraction		
			Sand (%)	Silt (%)	Clay (%)
1A	1.16	4.2	10.8	64.4	25.2
1B	0.84	4.4	0	70.8	29.2
1C	1.16	4.6	0	72.8	27.2
2A	1.20	3.9	0	83.8	16.2
2B	0.99	4.4	0	74.8	25.2
2C	1.11	4.3	0	81.6	18.4
3A	0.93	5.2	0	81.6	18.4
3B	1.14	5.2	0	86.6	14.4
3C	1.03	5.1	3.2	84.0	12.8
4A	1.07	6.2	1.6	93.0	5.4
4B	1.18	6.7	0	91.6	8.4
4C	1.13	6.5	0	89.6	10.4
5A	0.81	5.6	0	89.6	10.4
5B	0.84	6.6	0	88.8	11.2
5C	0.99	6.3	0.8	88.8	11.2
6A	1.19	5.3	0.8	91.0	8.2
6B	1.29	5.3	1.6	91.0	7.4
6C	1.21	4.9	0	91.6	8.4
7A	0.81	5.5	23.6	68.0	8.4
7B	0.99	5.3	17.6	73.0	9.4
7C	1.02	5.3	8.8	82.0	9.2
8A	1.14	4.8	50.8	40.0	9.2
8B	0.56	5.2	36.8	55.0	8.2
8C	0.50	4.8	44.8	46.0	9.2
9A	1.15	5.7	5.6	86.0	8.4
9B	1.08	6.1	3.6	89.0	7.4
9C	1.12	5.7	6.8	85.0	8.2
10A	1.32	6.2	2.8	82.0	8.2
10B	1.45	5.8	1.6	84.0	14.4
10C	1.46	6.1	3.6	82.0	14.4
11A	1.21	4.7	7.6	74.0	18.4
11B	1.12	4.9	7.6	74.0	18.4
11C	1.10	4.8	11.6	70.0	18.4
12A	1.25	6.5	0	91.6	8.4
12B	1.24	6.4	1.6	89.0	4.4
12C	1.35	6.1	1.2	90.0	8.8
13A	1.44	6.5	3.2	87.0	9.8
13B	1.29	6.5	1.6	88.0	10.4
13C	1.16	6.5	1.6	88.0	10.4

Table 2 (Continued)

Site No.	Organic Matter (%)	pH	Mineral Fraction		
			Sand (%)	Silt (%)	Clay (%)
14A	0.71	6.0	4.8	88.8	7.2
14B	0.82	5.8	8,8	83.0	8.2
14C	0.82	6.2	6.6	83.0	10.4
15A	0.84	5.7	1.6	90.0	8.4
15B	0.87	5.1	0	89.6	10.4
15C	0.81	5.4	0	88.2	11.8

Table 3. Physical and Chemical Characteristics of Soil Samples From Tennessee Cotton Fields Collected in October 1983.

Site No.	Organic Matter (%)	pH	Mineral Fraction		
			Sand (%)	Silt (%)	Clay (%)
1A	0.83	4.7	0	75.2	24.8
1B	0.75	4.5	0	77.4	22.6
1C	0.75	4.2	0.8	77.0	22.2
2A	1.08	4.6	3.6	82.0	14.4
2B	1.03	4.5	3.6	80.0	16.4
2C	1.00	4.4	1.6	81.0	17.4
3A	0.99	4.4	0.4	87.0	12.6
3B	1.03	4.3	0	87.4	12.6
3C	0.97	4.4	1.6	85.0	13.4
4A	1.52	5.0	2.0	88.0	10.0
4B	1.23	5.0	4.0	85.0	11.0
4C	1.17	5.2	2.4	90.0	7.6
5A	0.80	4.7	1.2	89.0	9.8
5B	0.74	4.7	1.2	88.0	10.8
5C	0.79	4.7	0.6	88.0	11.4
6A	0.89	4.9	6.8	88.0	5.2
6B	0.84	4.9	11.6	82.0	6.4
6C	0.88	5.4	9.6	82.0	8.4
7A	0.95	5.0	16.4	75.0	8.6
7B	0.82	4.9	14.4	79.0	6.6
7C	0.85	5.1	14.2	78.0	7.8
8A	0.92	5.2	16.2	72.0	11.8
8B	0.82	5.1	23.2	66.0	10.8
8C	0.80	4.8	22.8	67.0	10.2
9A	0.89	4.9	14.4	75.0	10.6
9B	1.02	5.0	15.4	75.0	9.6
9C	1.08	4.8	14.4	76.0	9.6
10A	0.89	5.3	18.4	67.0	14.6
10B	0.88	5.2	13.2	72.0	14.8
10C	0.88	5.4	17.2	66.0	16.8
11A	0.86	4.8	15.2	70.0	14.8
11B	0.91	5.1	12.8	71.0	16.2
11C	0.90	4.9	13.6	70.0	16.4
12A	0.73	5.8	12.6	71.0	16.4
12B	0.85	6.0	12.4	72.0	15.6
12C	0.83	6.0	10.8	74.0	15.2
13A	1.21	6.2	5.6	85.0	9.4
13B	1.10	6.1	3.6	82.0	14.4
13C	1.10	6.2	0.8	84.0	15.2

Table 3 (Continued)

Site No.	Organic Matter (%)	pH	Mineral Fraction		
			Sand (%)	Silt (%)	Clay (%)
14A	0.70	5.3	8.2	81.0	10.8
14B	0.55	5.7	21.6	74.0	4.4
14C	0.64	5.5	9.6	82.0	8.4
15A	0.91	6.3	5.8	81.0	13.2
15B	0.89	6.3	6.6	80.0	13.4
15C	0.84	6.4	6.2	81.0	12.8

Table 4. Biological Characteristics of Soil Samples From Tennessee Cotton Fields Collected in November 1982.

Site No.	Disease Index[b]	Organism Isolated[a]					
		Pythium	Rhizoctonia	Fusarium	Thielaviopsis	Other[c]	Pythium (Selective Medium)[d]
1A	2.3	2	0	14	0	0	28
1B	2.1	0	1	15	0	0	19
1C	2.7	1	1	12	0	0	23
2A	2.5	5	1	11	0	0	31
2B	3.3	4	3	8	0	0	4
2C	2.8	3	0	11	0	0	33
3A	2.3	4	0	11	0	0	9
3B	3.3	2	1	10	0	0	11
3C	3.3	5	1	7	0	0	27
4A	3.1	2	5	7	0	1	18
4B	2.5	3	2	9	0	0	0
4C	3.9	4	3	4	0	0	14
5A	2.4	1	10	2	0	1	9
5B	2.3	3	7	5	0	1	11
5C	2.7	3	13	2	0	0	22
6A	3.9	8	1	6	1	0	41
6B	3.9	3	4	9	1	0	23
6C	4.6	2	4	5	1	2	26
7A	3.9	4	8	2	0	0	6
7B	5.1	4	8	2	0	0	9
7C	3.8	7	2	5	0	1	17
8A	2.8	1	2	5	0	5	5
8B	3.9	4	2	10	0	0	17
8C	2.3	3	1	7	2	1	27

Table 4 (Continued)

Site No.	Disease Index[b]	Organism Isolated[a]					
		Pythium	Rhizoctonia	Fusarium	Thielaviopsis	Other[c]	Pythium (Selective Medium)[d]
9A	4.9	5	6	2	0	0	29
9B	4.7	6	3	2	0	0	34
9C	4.6	5	2	3	0	1	14
10A	2.4	3	3	10	0	0	7
10B	2.7	5	3	5	0	2	14
10C	2.0	0	2	13	1	0	29
11A	2.8	3	1	9	0	0	11
11B	2.4	6	0	7	0	2	32
11C	2.2	5	0	8	0	1	23
12A	4.7	7	1	5	0	1	10
12B	4.7	9	0	7	0	0	25
12C	4.4	9	0	4	1	0	27
13A	4.6	6	1	8	0	0	8
13B	3.8	6	5	4	0	0	10
13C	2.1	2	6	3	0	2	34
14A	3.3	5	1	8	0	1	7
14B	4.3	9	1	3	0	1	9
14C	3.4	5	1	8	0	0	40
15A	5.3	6	3	1	0	0	14
15B	5.2	8	1	2	0	0	15
15C	4.1	12	1	2	0	0	30

[a]Number of seedling hypocotyls from which organisms were isolated. A total of 16 plants were grown in soil from each site and assayed.

Table 4 (Continued)

[b]Disease index is a measure of disease severity based on symptoms where 1 = no symptoms, 6 = dead plant, and 7 = plant not emerged. Each index value is the mean of 16 replicate hypocotyls.

[c]Other genera isolated include *Penicillium*, *Rhizopus*, *Alternaria*, and *Trichoderma*.

[d]Number of *Pythium* colonies isolated on gallic acid medium. Numbers given are mean values of two plates each seeded with 0.04g soil from each site.

Table 5. Biological Characteristics of Soil Samples From Tennessee Cotton Fields Collected in October 1983.

Site No.	Disease Index[b]	Organism Isolated[a]					
		Pythium	Rhizoctonia	Fusarium	Thielaviopsis	Other[c]	Pythium (Selective Medium)[d]
1A	2.9	3	1	13	0	0	16
1B	2.5	2	0	18	0	1	12
1C	2.4	2	1	17	0	0	12
2A	2.2	2	2	17	0	0	20
2B	3.6	2	1	12	0	0	15
2C	2.8	7	0	15	0	0	21
3A	5.1	5	0	5	0	0	19
3B	4.4	4	0	8	0	0	14
3C	3.9	2	1	11	0	0	11
4A	2.1	2	1	14	0	0	12
4B	4.3	3	0	9	0	0	4
4C	3.3	6	1	11	0	1	12
5A	5.1	4	0	3	0	0	10
5B	5.3	4	1	3	0	0	10
5C	6.8	1	0	1	0	0	13
6A	5.9	2	0	2	0	1	7
6B	6.4	2	0	0	1	0	9
6C	6.4	3	0	1	0	0	22
7A	5.0	2	2	3	0	0	19
7B	5.2	1	2	7	0	0	20
7C	5.4	4	3	7	0	0	25
8A	6.3	1	2	2	0	0	5
8B	5.0	1	0	8	0	0	3
8C	5.9	4	2	4	0	0	8

Table 5 (Continued)

Site No.	Disease Index[b]	Organism Isolated[a]					
		Pythium	Rhizoctonia	Fusarium	Thielaviopsis	Other[c]	Pythium (Selective Medium)[d]
9A	4.2	2	0	9	2	2	19
9B	3.1	2	0	11	0	1	11
9C	4.7	6	0	5	0	0	4
10A	5.3	5	1	4	0	1	2
10B	4.4	3	2	9	0	0	3
10C	4.4	7	1	6	0	0	5
11A	4.5	2	1	5	0	1	8
11B	4.0	4	0	7	0	1	13
11C	4.4	8	1	2	0	1	14
12A	2.9	3	1	10	0	1	5
12B	2.2	1	0	13	0	2	10
12C	1.9	0	1	15	0	2	6
13A	3.4	5	2	10	0	0	10
13B	4.3	3	1	8	0	0	7
13C	4.4	4	1	8	0	1	12
14A	5.3	3	2	3	0	0	9
14B	5.5	4	2	3	0	0	7
14C	4.6	3	0	2	0	1	7
15A	2.3	1	5	12	0	0	14
15B	4.8	3	2	5	0	0	21
15C	3.1	0	1	12	0	1	23

[a]Number of seedling hypocotyls from which organisms were isolated. A total of 20 plants were grown in soil from each site and assayed.

Table 5 (Continued)

[b]Disease index is a measure of disease severity based on symptoms where 1 = no symptoms, 6 = dead plant, and 7 = plant not emerged. Each index value is the mean of 20 replicate hypocotyls.

[c]Other genera isolated include *Penicillium*, *Rhizopus*, *Alternaria*, and *Trichoderma*.

[d]Number of *Pythium* colonies isolated on gallic acid medium. Numbers given are mean values of three plates each seeded with 0.04g soil from each site.

Bioassay data were collected from 16 plants per sample in 1982 and from 20 plants in 1983.

Rhizoctonia solani did not occur on any of the plates of the tannic acid selective medium designed to isolate this fungus (32). Also, R. solani was not isolated with Weinhold's (34) method which consisted of dispersing organic particles, sieved from soil, in 1% water agar.

The results of the field test performed in 1984 are given in Table 6. The disease indexes given are mean values of 100 seeds planted per site. All the data in this table were adjusted to reflect 72% seed germination.

Statistical Analysis of Data

All statistical analyses of the data collected were made with the Statistical Analysis System (SAS 82). Correlation coefficients (r) were calculated with data of the following variables in 1983: the number of Pythium colonies isolated on gallic acid medium, percent clay, total numbers of Pythium, Rhizoctonia, and Thielaviopsis isolates, number of Fusarium isolates, number of Rhizoctonia isolates, number of Pythium isolates, disease index, pH, and percent organic matter. The correlation coefficients were calculated with data of corresponding variables collected in 1984 plus percent stand, percent post-emergence mortality, and average disease indexes

Table 6. Disease Severity of Cotton Seedlings in Field Plots (Planted May 1984, Data Were Collected June 1984).[a]

Site No.	Stand[b] (%)	Postemergence Mortality[c] (%)	Disease Index[d]
1A	70.7	8.7	3.7
1B	68.5	7.6	3.9
1C	71.7	7.6	3.9
2A	7.4	33.3	6.3
2B	56.3	4.6	4.6
2C	76.6	7.4	3.7
3A	15.6	6.5	6.3
3B	19.5	2.6	6.2
3C	30.9	9.9	5.6
4A	33.7	25.6	5.4
4B	43.7	19.5	5.0
4C	54.0	6.9	4.7
5A	53.4	12.5	4.5
5B	56.8	7.9	4.5
5C	60.7	7.9	4.3
6A	5.3	9.3	6.7
6B	25.6	15.9	5.9
6C	17.7	15.2	6.2
7A	22.8	8.9	5.9
7B	23.8	13.8	5.8
7C	16.7	12.8	6.2
8A	-	-	-
8B	30.6	22.3	5.5
8C	60.2	5.7	4.3
9A	35.3	18.8	5.4
9B	40.7	16.3	5.1
9C	43.8	22.5	4.8
10A	20.5	6.4	6.0
10B	20.8	3.9	6.1
10C	20.5	5.1	6.1
11A	27.2	11.1	5.7
11B	25.6	3.8	5.8
11C	25.3	6.3	5.9
12A	17.1	1.3	6.3
12B	27.8	5.1	5.8
12C	18.2	3.9	6.2
13A	35.7	16.7	5.3
13B	40.9	4.8	5.1
13C	-	-	-

Table 6 (Continued)

Site No.	Stand[b] (%)	Postemergence Mortality[c] (%)	Disease Index[d]
14A	28.8	7.5	5.8
14B	16.7	12.8	6.1
14C	23.8	13.8	5.8
15A	30.0	7.5	5.7
15B	30.1	9.6	5.6
15C	26.6	6.3	5.7

[a]Data were adjusted to reflect 72% seed germination.

[b]Living, emerged plants.

[c]Emerged plants that died.

[d]Disease index is a measure of disease severity based on symptoms where 1 = no symptoms, 6 = dead plant, and 7 = plant not emerged.

from the field test (Tables 7,8,9). Significant correlations obtained for both years were between percent clay and the disease indexes from the bioassay, number of Fusarium isolates and disease indexes from the bioassay, pH and the number of Rhizoctonia isolates, and percent clay and the number of Fusarium isolates. A significant negative correlation not listed in the tables occurred in 1983 between percent clay and pH (r= -0.637**).

In 1984, disease index and post-emergence mortality were correlated negatively with percent clay. Percent clay was correlated positively with percent stand. Post-emergence mortality was correlated positively with percent organic matter. Both percent stand and disease index were correlated positively with soil pH. Only one bioassay variable was correlated significantly with field data: numbers of hypocotyls from which Fusarium was isolated correlated positively with percent stand and negatively with disease index.

Numbers of Pythium colonies isolated on gallic medium did not correlate with any of the other variables either year.

Linear regression was calculated for the pairs of variables that had a high correlation coefficient and whose relationships were significant to this study. These are illustrated in Figures 1,2,3, and 4.

Table 7. Significant Correlations Between Biological Variables and Physical and Chemical Characteristics of Soil Samples From Cotton Fields.

Biological Variable	Soil Characteristic Variable	Correlation Coefficient (r) 1983	1984
Bioassay Data			
Disease Index	% Clay	-0.516**[a]	-0.560**
	% Organic Matter		-0.305*
Pythium (no.)	% Clay	-0.346*	
Rhizoctonia (no.)	pH	0.346*	0.372*
Fusarium (no.)	% Clay	0.629**	0.581**
	% Organic Matter		0.307*
	pH	-0.432**	
Total *Pythium*, *Rhizoctonia*, and *Thielaviopsis* (no.)	% Clay	-0.588**	
	pH	0.467**	
Field Data[b]			
% Post-emergence Mortality	% Clay	-	-0.371*
	% Organic Matter	-	0.376*
% Stand	% Clay	-	0.421**
	pH	-	0.368*
Disease Index	% Clay	-	-0.411**
	pH	-	0.371*

[a] * = Significant at 5% level of probability; ** = Significant at 1% level of probability.

[b] Field data for 1983 not available for correlation.

Table 8. Significant Correlations Between Disease Index (Bioassay) and Numbers of Organisms Isolated From Soil Samples Collected From Tennessee Cotton Fields.

Organism (Number)	Disease Index Correlation Coefficient (r)	
	1983	1984
Pythium	0.609**	
Fusarium	-0.586**	-0.912**
Total *Pythium*, *Rhizoctonia*, and *Thielaviopsis*	0.486**	

** Significant at 1% level of probability.

Table 9. Significant Correlations Between Bioassay Variables of Soil Samples Collected in October 1983 and Field Data Variables From Cotton Plantings in May 1984.

Bioassay Variable	Field Variable	Correlation Coefficient (r)
Fusarium (no.)	% Stand	0.313*
	Disease Index	-0.322*

* Significant at 5% level of probability.

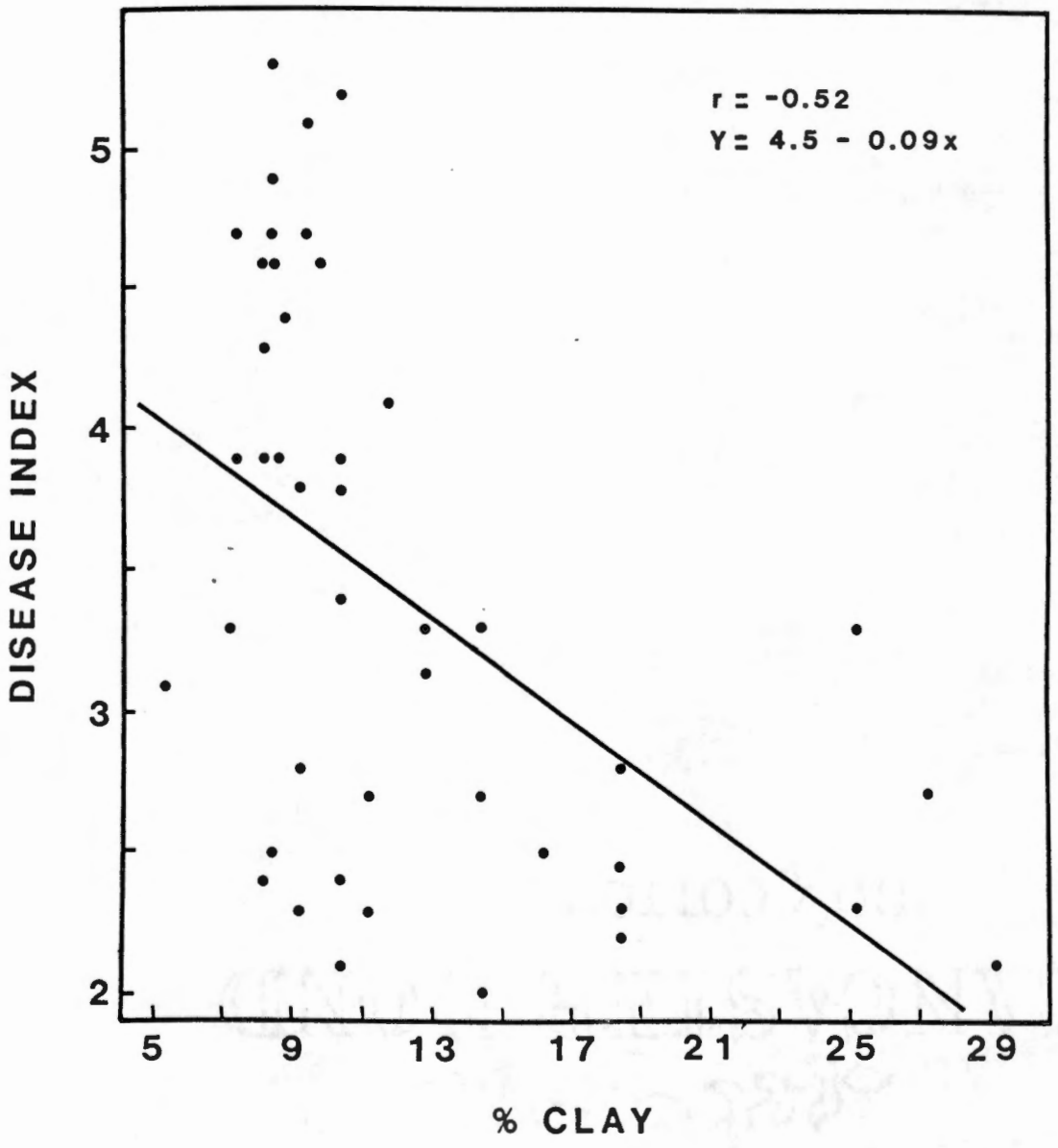

Figure 1. Relationship of Clay Content of Soil Samples to Disease Severity of Cotton Seedlings Grown in the Soil Samples in Plant Growth Chambers in 1983.

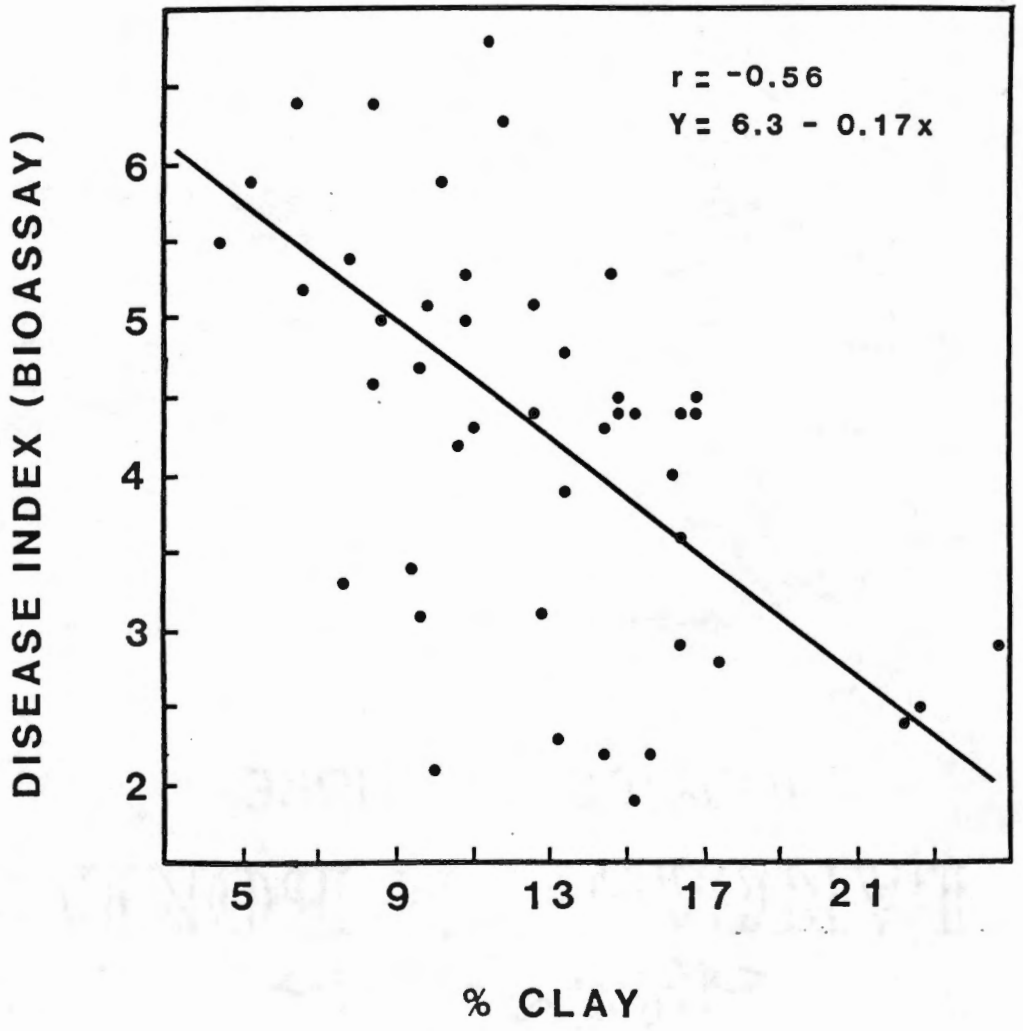

Figure 2. Relationship of Clay Content of Soil Samples to Disease Severity of Cotton Seedlings Grown in the Soil Samples in Plant Growth Chambers in 1984.

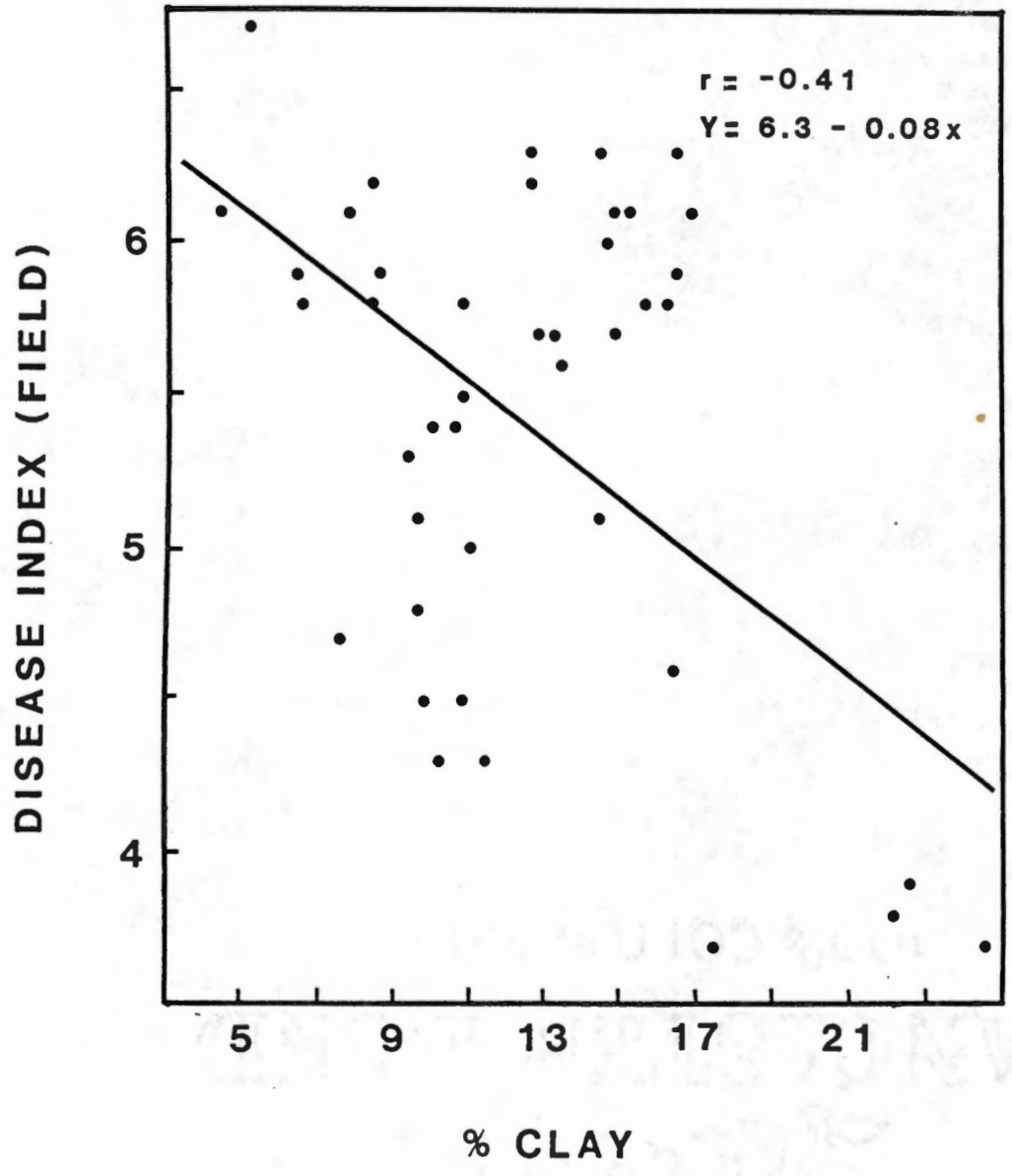

Figure 3. Relationship of Clay Content of Soils to Disease Severity of Cotton Seedlings Grown in These Soils in 1984.

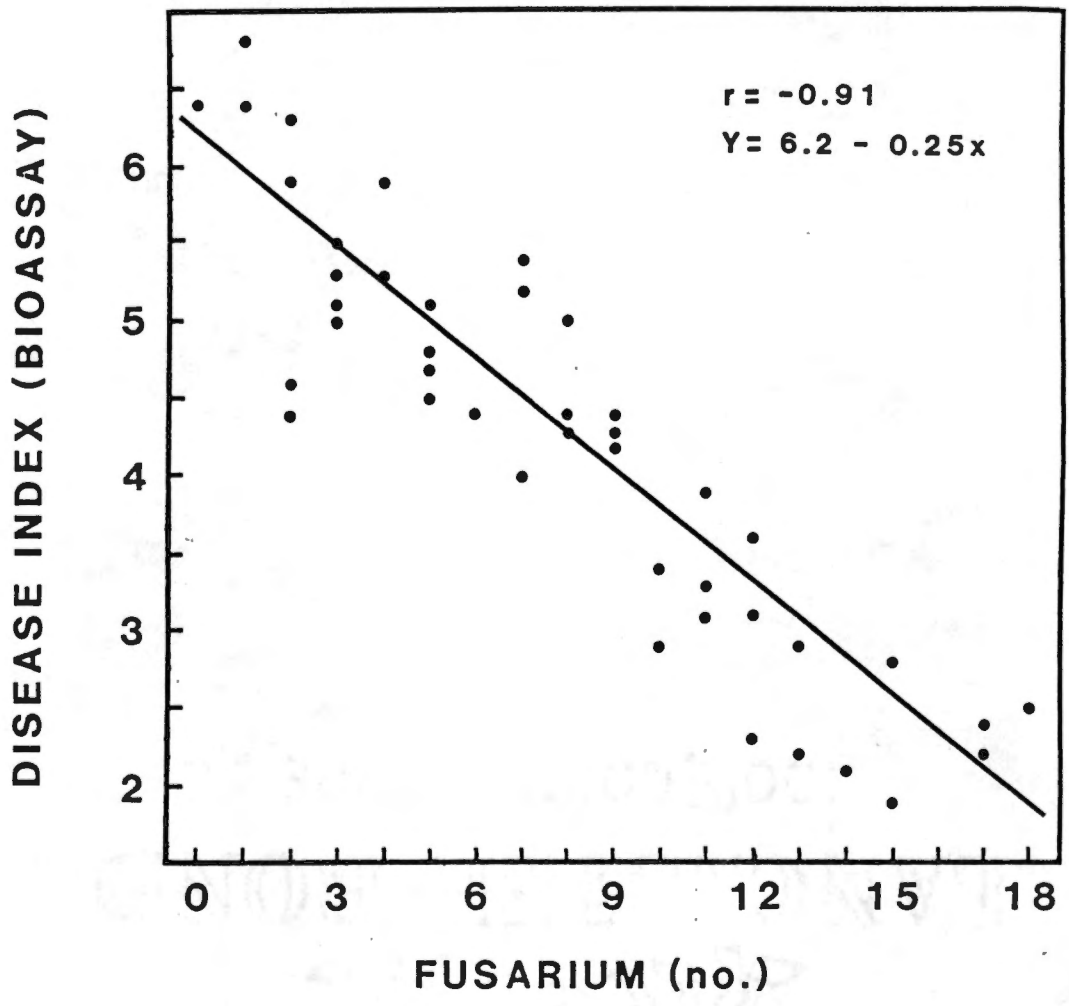

Figure 4. Relationship of Cotton Seedling Disease Severity to the Number of Isolates/Soil Sample of Fusarium Obtained in a Bioassay Procedure of 45 Soil Samples in 1984

CHAPTER IV

DISCUSSION

The primary purpose of this study was to test a bioassay technique for predicting cotton seedling disease occurrence and severity in the field. The disease indexes obtained from the bioassay technique were not correlated with the disease indexes of the cotton seedlings grown in the field. Also, numbers of seedlings infected with Pythium and/or Rhizoctonia spp., the two major pathogens of cotton seedlings, were not correlated with disease severity in the field. The reason for this lack of correlation could be that the regulated conditions of seedlings grown in a growth chamber did not accurately simulate natural field conditions. In the field, temperature and moisture conditions of the soil fluctuate considerable more and in different ways than those in growth chambers.

However, an analysis of other variables of the bioassay technique revealed some interesting relationships with disease severity. For example, numbers of Fusarium isolates had a very high negative correlation with disease severity as measured with the bioassay technique and also with disease severity of cotton seedlings grown in the field. This finding supports the conclusion of Johnson et al. (21) that Fusarium isolates

are the least pathogenic of the organisms involved in the seedling disease complex and therefore cause minimal damage.

The numbers of Pythium isolates and total numbers of Pythium, Rhizoctonia, and Thielaviopsis isolates both were correlated with disease severity as determined with the bioassay in 1983 but not in 1984. This discrepancy is probably the result of a procedural deficiency. For each growth chamber test seven seeds per pot were planted and after eight days they were thinned to four per pot. In 1984, many of the pots contained less than four emerged plants and isolation data were taken only on emerged plants. Thus the data were probably biased towards post-emergence seedling disease severity.

Jakob (19) reported that in greenhouse experiments 59% of cotton seedlings that died prior to emergence were killed by Rhizoctonia, 17% by Fusarium, and 15% by Pythium. Pre-emergence death of the seedlings was high in 1984 and data were not taken on isolations of Rhizoctonia of these dead seedlings. This also could explain the lack of a significant correlation of Rhizoctonia in 1983 and 1984 with disease index and the non-correlation of the total of the three pathogens in 1984 with disease index.

There was a positive relationship between the number of Fusarium isolates and percent clay in the soil. The

percentage of clay in the soil also appears to have a strong negative relationship with disease severity as measured with the bioassay technique and with disease severity in the field. From the results of this study it appears that disease severity is greater in soils with relatively low clay content. Garrett (15) concluded that relatively few soil-borne diseases appear to be favored by soils of heavy texture. Hancock (17) also reported that suppressiveness to Pythium ultimum was associated with certain finely textured soils in California's San Joaquin Valley.

There was a positive correlation between numbers of Rhizoctonia isolates and the pH of the soil. Bloom and Couch (9) reported that Rhizoctonia brown patch of bent grass caused by Rhizoctonia solani was less at pH 4.0 than at pH 5.6 and 9.0 when nitrogen levels were high. Hearn (18) found that R. solani occurs in soils with pH 6.0 to 8.2 with pH 7.0 being optimum for growth. Blair (8) observed that Rhizoctonia grew within the range of pH 5.8 to 8.1, and that a neutral soil pH appeared optimal. When the soil pH was high in the present study, disease severity of the seedlings grown in the field was high and stand density was low. These results could be due to high densities of Rhizoctonia which appears to thrive at a neutral pH.

A secondary purpose of this study was to compare the

quantitative isolation of Pythium on a selective medium to the bioassay technique used in this study for effectiveness in predicting cotton seedling disease severity. Numbers of Pythium colonies isolated on gallic acid medium in this study were not correlated with any of the other variables either year. These results conflict with the findings of DeVay et al. (13). These workers compared populations of Pythium spp. isolated from soil samples on pimaricin-vancomycin medium, to survival of cotton seedlings planted in those soils. They found that survival of the seedlings was directly related to the concentration of soil-borne propagules of Pythium ultimum as determined with selective medium. Their study was conducted in six field tests in the San Joaquin Valley of California. Although a different selective medium was used in the present study, there is no evidence that the pimaricin-vancomycin medium is better than the gallic acid medium for selecting Pythium isolates from the soil. The difference in results could be due to differences in soils and climatic conditions in California and Tennessee.

Although this study did not give positive results for the aspect of the bioassay technique (i.e. disease severity) expected to be helpful in predicting cotton seedling disease severity in the field, other aspects of this study are promising as a possible means for

forecasting the disease. Three factors appear to have a definite relationship to seedling disease severity in the field - percent clay in the soil, pH of the soil, and numbers of Fusarium isolates as determined with the bioassay technique. Of these three factors, percent clay had the highest correlation with disease severity of seedlings grown in the field. By determining percent clay in a soil sample a prediction of disease severity in the field can be made by applying that value into the regression equation given in Figure 3. This model could be used as a tool for predicting cotton seedling disease severity in a particular soil and could result in better decisions for selecting fields for cotton culture.

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