Relationships of Seedling Disease of Cotton to Characteristics of Loessial Soils in Tennessee

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ABSTRACT

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Forty-five samples of loessial soils in western Tennessee were collected at 15 sites in the fall of 1982 and again in 1983. Fungi were isolated from discolored or necrotic hypocotyls of cotton seedlings grown in the soil samples at 17 C, and a disease severity index value for each sample was determined. The most frequently isolated fungi were *Fusarium* spp., *Pythium* spp., *Rhizoctonia solani*, and *Thielaviopsis basicola*. The soil samples also were characterized as to textural class, pH, and organic matter content. Cotton was planted in 1984 at the sites from which samples were taken previously, and seedling disease determinations were made. Isolations of *Pythium* spp. and disease indices obtained in the bioassay procedure were positively correlated with disease severity in the field plantings. A significant negative correlation was obtained between isolations of *Fusarium* spp. and disease severity. Soil clay content was inversely correlated with disease severity. Models were developed with a stepwise regression analysis to describe significant relationships of disease severity with soil biological and physical characteristics.

Major pathogens of cotton (*Gossypium hirsutum* L.) seedlings in Tennessee include *Rhizoctonia solani* Kühn, *Thielaviopsis basicola* (Berk. & Br.) Ferr., and at least four species of *Pythium*. Species most often isolated from diseased hypocotyls or roots (in order of decreasing frequency) are *Pythium ultimum* Trow, *R. solani*, and *Pythium sylvaticum* Campbell and Hendrix (11,12). These fungi cause a variety of symptoms including seed rot, pre- and postemergence death of seedlings, root rot, and hypocotyl lesions (11,14,22). Seedling disease is severe when postplanting temperatures are low and soil moisture levels are high. Because of the frequent occurrence of these adverse postplanting weather conditions in Tennessee (13) and the diverse nature of the fungi that cause the disease, fungicide applications often do not result in satisfactory control.

Studies to relate disease incidence and severity of cotton seedlings with quantity of pathogens in soil have not been definitive. High populations of *Pythium* (as measured by isolations on a selective medium) were found in certain California soils in which severe seedling disease occurred, but in other soils, no such relationship with inoculum density of *Pythium* and disease severity could be demonstrated (7). In a more recent study (5), survival of seedlings was correlated negatively with concentration of propagules of *P. ultimum* in six California soils. It is not known whether such correlations occur in Tennessee soils where other pathogens often contribute to seedling damage. *R. solani* was shown to be the major pathogen of cotton seedlings in 3 yr of a 7-yr study (13). Moreover, cotton soils in Tennessee were found to differ considerably in composition of seedling pathogens (12). Interactions of cotton seedling pathogens with each other and with physical and chemical soil characteristics have not been studied. If such interactions are significant, crop and soil management practices for predicting and reducing seedling disease severity could be improved.

The objectives of this study were to define certain physical, chemical, and biological characteristics of a number of soils on which cotton is grown and to determine relationships of these characteristics to cotton seedling pathogens and to seedling disease severity.

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MATERIALS AND METHODS

Fifteen field sites in western Tennessee on which cotton had been grown for 5 yr or more were selected for study. Fields were located on the Ames Plantation at Grand Junction, TN, and on the Agricultural Experiment Stations at Jackson and Milan, TN. Sites at Grand Junction were approximately 113 km distant from those at Milan. Each site consisted of a 9.15-m row and was divided into three equal subsites. Soil samples were collected from each subsite in November, 1982, and again in October, 1983. Thirty of the subsites were common to both collections. Samples from each subsite consisted of 15 or more 8- to 12-cm-deep cores of soil taken with a hand trowel. Cores were bulked, placed in plastic bags, and stored at room temperature.

Organic matter content was determined in duplicate for each soil sample with a chromic acid titration method (1). Particle size analyses were made with the Bouyoucos hydrometer method (3) and soil texture designations were assigned according to Luntz (16). Measurements of pH were made with 1:1 (w:v) water solutions of the samples with an Accumet 750 selective ion analyzer (Fisher Scientific, Atlanta, GA) fitted with a combination glass electrode.

Bioassay. The bioassay procedure was similar to that previously described (11). Soil from each of the 45 samples was placed in a 10-cm-diameter plastic pot. Seven cotton seeds (cultivar Stoneville 213), delinted, but not treated with fungicides, were planted in each pot and incubated at 26 C in a plant growth chamber with continuous illumination. After 8 days, emerged seedlings were thinned to four per pot and the temperature was reduced to 17 C, a temperature previously found suitable for infection of seedlings by several pathogens (11). Thirteen days later, seedlings were removed from the soil, washed, and rated for disease severity as follows: 1 = no visible symptoms; 2 = from one to a few pinpoint dark spots or a faint diffused discolored area on the hypocotyl; 3 = a distinct necrotic lesion on the hypocotyl, usually sunken but less than 0.5 cm in length; 4 = hypocotyl lesion 0.5 cm or longer in length; 5 = plant wilted with cotyledons drooping; 6 = plant emerged, but dead; and 7 = plant not emerged. Discolored or necrotic portions (0.5 cm each) were cut from the hypocotyls, placed in screw-cap vials, and

washed in five changes of sterile distilled water; the first two washes contained Tween-20 (one drop per 100 ml of water). After washing, hypocotyl segments were blotted on sterile filter paper, and placed on 2% water agar in a petri dish. After 4–10 days of incubation at room temperature, fungi that emerged from each segment were identified to genus. This procedure was repeated four times with soil samples collected in 1982 and five times with those collected in 1983. In addition to this bioassay procedure, *Pythium* spp. were isolated from the soil samples on a selective gallic acid agar medium (6).

Field plantings. On 9 May 1983 and 17 May 1984, Stoneville 213 cotton seeds were planted at the sites where soils were sampled each previous fall. One hundred seeds were planted in a furrow in each 3.05-m site. Approximately 4 wk after planting, emerged seedlings were removed from the soil, washed, and rated with the same rating scale as previously described for the bioassay procedure. Also, data were collected on percent emergence and postemergence mortality.

Seed germination tests and data analyses. Germination of the seed used in these studies was tested by planting 10 each in 10 pots containing autoclaved sand. The pots were placed in a constant temperature growth chamber at 25 C for 9 days and percent germination was determined. Data on emergence in both field and bioassay plantings were adjusted to reflect percentage of viable seed. Data were analyzed with the general linear model of the Statistical Analysis System (SAS 1982, SAS Institute Inc., Cary, NC). Statistics to determine differences or correlations among the variables were applied according to regression analyses ($P = 0.05$). Data were also analyzed by stepwise multiple regression procedures ($P = 0.15$).

RESULTS

All of the soil samples were high in silt content; the lowest contained 40% silt (Table 1). High silt and low clay contents are characteristics of these loessial soils. For the 30 subsites that were common to both collection years, pH, organic matter content, and textural class values obtained in 1983 were consistent with those obtained the previous year.

Disease index and preemergence mortality values were higher in 1983 soil collections than in the 1982 collections (Table 2). This can be explained in part in that 15 sites were sampled in 1983 that were not sampled in 1982. Seeds for both years were taken from one lot of Stoneville 213. Seed viability decreased from 79% in 1982 to 72% in 1983. Data were adjusted to reflect 100% viability for each year, but decrease in seed quality (22) not detected in seed germination tests could have resulted in increased disease severity in 1983.

In the 1983 field plantings, extensive postplanting floods washed the seeds from several of the plots and disease severity data were not taken that year. In 1984, percent stand, postemergence mortality, and disease index values were obtained on all plots except two, which were in low, flooded areas. Disease severity was relatively high. An average of only 34% of seedlings survived 4 wk after planting. This was probably related to decreased seed quality and to postplanting weather conditions. The mean daily minimum temperature 5–15 days after planting at Milan was 12.2 C. Measurable amounts of rain occurred on 6 of those 11 days with a total of 8.8 cm. Temperature and rainfall at the other two locations were similar (8). These weather conditions have previously been associated with severe seedling disease (13).

Percent clay was the only variable which was correlated significantly with biological variables in both experimental years and in means of sites common to both years (Table 3). The correlation between percent clay and the disease index was negative, and correlation between percent clay and percent of seedlings from which *Fusarium* spp. was isolated was positive. Other significant correlations were not consistently obtained for both sampling years. However, in means of sample sites common to both years, soil pH was correlated highly with both the percent of seedlings from which *Pythium* spp. was isolated and with numbers of colonies of *Pythium* on the selective medium. Also, a high correlation was obtained between pH and disease index, and a negative correlation between pH and percent of seedlings from which *Fusarium* spp. was isolated. The linear regression slope of the bioassay disease index over percent clay was inverse (Fig. 1). Values are means of the 10 fields (sites) comprising 28 sampling subsites common to both sampling years. Locations and soil textural classes of these 10 field sites were as follows: Sites 1, 2, 3, 4, and 5 were at Grand Junction and the soils were Loring silt loam, Loring silt loam, Callaway silt loam, Memphis silt, and Memphis silt, respectively. Sites 6 and 8 were at Milan and the soils were Falaya silt and Vicksburg silt loam, respectively. Sites 11, 13, and 14 were at Jackson and the soils were Dexter silt loam, Granada silt, and Loring silt, respectively.

Percent clay, measured in samples collected in 1983, and means of measurements of sample sites common to both sampling years were correlated positively with emergence of seedlings in the field planting and negatively with the field disease index (Table 4). Percentage of seedlings from which *Fusarium* spp. was isolated in the bioassay procedure was correlated negatively with the field disease index. Significant correlations that were obtained with the means of sites common to both years were a negative correlation between percentage of seedlings from which *Pythium* spp. was isolated and percent emergence, a positive correlation between *Pythium* spp. and the field disease index, and a negative and positive correlation of the bioassay disease index with field emergence and field disease index, respectively.

TABLE 1. Some chemical and physical characteristics among loessial soils in western Tennessee selected for evaluating cotton seedling disease[a]

Variable	1982 soil collection			1983 soil collection		
	Low	High	Mean	Low	High	Mean
pH[b]	4.2	6.7	4.9	4.2	6.4	4.9
Organic matter	0.5	1.5	1.1	0.6	1.5	0.9
Mineral fraction						
Sand (%)	0.0	50.8	6.3	0.0	23.0	8.8
Silt (%)	40.0	91.8	81.4	66.0	90.0	79.0
Clay (%)	5.4	29.2	12.1	4.4	24.8	14.9

[a] Values in table represent data from 45 subsites in 15 field sites.
[b] Mean pH was determined by calculating the mean H^+ ion concentration converted to pH.

TABLE 2. Characteristics of some biological variables among loessial soils in western Tennessee selected for evaluating cotton seedling disease[a]

Variable	1982 soil collection ($n = 45$)			1983 soil collection ($n = 43$)		
	Low	High	Mean	Low	High	Mean
Soil bioassay						
Disease index[b]	2.0	5.3	3.4	1.9	6.8	4.2
Preemergence mortality (%)	0	38	13	0	90	39
Isolation frequency (%)						
Pythium spp.	0	75	28	0	40	15
Rhizoctonia solani	0	69	16	0	25	5
Fusarium spp.	6	94	41	0	90	40
Thielaviopsis basicola	0	13	2	0	10	0.1
Selective medium (propagules per gram)						
Pythium spp.	0	1,025	488	50	625	297
Field planting[c]						
Disease index	...	...	...	3.7	6.7	5.4
Emergence (%)[d]	...	...	...	5	77	34
Postemergence mortality	...	...	...	1	33	11

[a] Values represent data from 45 subsites in 1982 or 43 subsites in 1983 in 15 field sites.
[b] Measure of disease severity based on symptoms; 1 = no symptoms, and 7 = plant not emerged.
[c] Cotton seeds planted in the spring of 1984 at the sites ($n = 43$) where soil collections were made the previous fall.
[d] Percentage of plants that emerged and were living 4 wk after planting.

From these and additional analyses, three variables of the bioassay were found to be correlated positively with the field disease index: percentage of seedlings from which *Pythium* spp. was isolated ($P = 0.02$); percentage of seedlings from which *T. basicola* was isolated ($P = 0.15$); and percentage that did not emerge ($P = 0.01$). Conversely, two bioassay variables were correlated negatively with the field disease index: percentage of seedlings from which *R. solani* was isolated ($P = 0.15$) and percentage from which *Fusarium* spp. was isolated ($P = 0.05$). The three variables that were correlated positively with the field disease index were combined and designated PTD in Tables 4 and 5. This combined variable was correlated positively with the field disease index, and negatively with percent emergence of the field planting.

Analyses of the data by stepwise multiple regression yielded information on models accounting for 60% or more of the variation. From the stepwise analyses (Table 5), low soil clay content, low percentage of seedlings from which *R. solani* was isolated, a high value of the three combined variables (PTD), and a high soil organic matter content were associated with severe seedling disease.

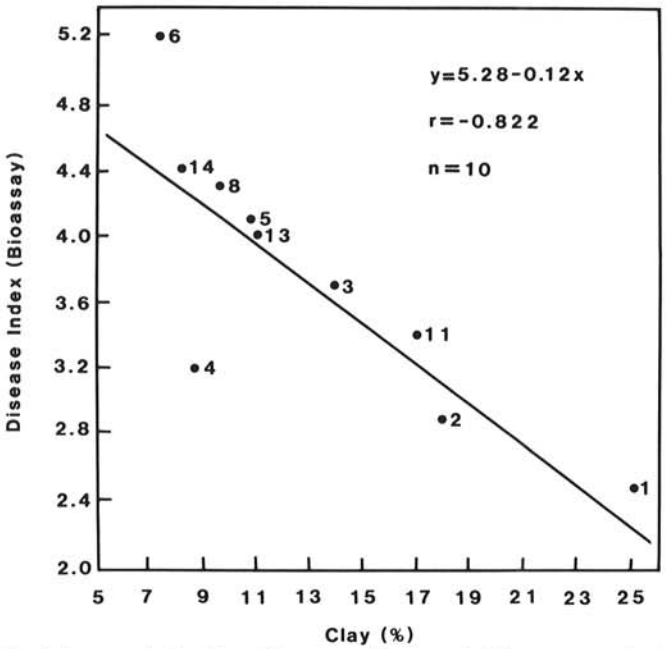

Fig. 1. Inverse relationship of disease severity (from the bioassay procedure) to clay content in soil samples from 10 cotton fields. Numbers at data points (mean values of subsites within a field) refer to site locations listed in the text.

TABLE 3. Statistically significant relations between biological variables determined by bioassay procedures and chemical or physical characteristics of soils selected for evaluating cotton seedling disease

Soil bioassay variable	Chemical or physical variable	Correlation coefficient (r) 1982 ($n = 45$)	1983 ($n = 43$)	Mean ($n = 28$)[a]
Disease index[b]	pH	···	···	0.50**
	Organic matter (%)	···	−0.34*	···
	Clay (%)	−0.51**	−0.53**	−0.72**
	Silt (%)	0.31*	0.57**	0.72**
	Sand (%)	···	0.32*	···
Isolation frequency (%)				
Pythium spp.	pH	···	···	0.44**
	Clay (%)	−0.33*	···	···
	Silt (%)	0.31*	···	···
R. solani	pH	0.37*	0.39*	···
	Clay (%)	−0.30*	···	···
	Silt (%)	···	···	0.38*
Fusarium spp.	pH	−0.43**	···	−0.51**
	Organic matter (%)	···	0.33*	···
	Clay (%)	0.63**	0.57**	0.72**
	Sand (%)	···	−0.37*	···
Selective medium (propagules per gram)				
Pythium spp.	pH	···	···	0.58**
	Sand (%)	···	−0.30*	−0.39*

[a] Mean of 28 subsites common to both years.
[b] Measure of disease severity based on symptoms; 1 = no symptoms, and 7 = plant not emerged. Asterisks * and ** indicate statistically significant correlation, $P = 0.05$ and $P = 0.01$, respectively.

DISCUSSION

Most of the cotton in western Tennessee and Mississippi is grown on Peorian loess, a band of wind-deposited soil east of the Mississippi River, which extends into eastern Kentucky and south through western Tennessee, Mississippi, and into southeastern Louisiana. Loessial soils selected for this study are about 1.2 to 2.1 m deep (18,21) and contain high silt (40–92%) and low clay contents (5–25%). Since Peorian loessial soils are rather unique in their formation and textural characteristics, results of this study may apply only to them and not to soils found elsewhere in the cotton belt.

Fusarium spp. almost always grew from discolored or necrotic hypocotyl segments on water agar plates whenever *Pythium* spp. or *R. solani* did not appear. Occasionally, *Pythium* spp., *R. solani*, or *T. basicola* grew along with *Fusarium* spp. from the same hypocotyl segment. Although often isolated from discolored or necrotic hypocotyls, *Fusarium* spp. are only mildly pathogenic (11). This was verified by the inverse correlation of isolations of

TABLE 4. Statistically significant correlations between chemical, physical, or biological characteristics of soil samples and seedling disease severity of cotton planted at corresponding sampling sites

Soil sample variable	Field planting variable (1984)	Correlation coefficient (r) 1983 ($n = 43$)	Mean ($n = 28$)[a]
Chemical			
pH	Emergence (%)[b]	−0.37*	···
	Disease index[c]	0.37*	···
Organic matter (%)	Postemergence mortality (%)	0.38*	···
Physical			
Clay (%)	Emergence (%)	0.44**	0.51**
	Postemergence mortality (%)	−0.36	···
	Disease index	−0.43**	−0.52**
Sand (%)	Emergence (%)	−0.42**	···
	Disease index	0.40**	···
Bioassay			
Disease index	Emergence (%)	···	−0.43*
	Disease index	···	0.46*
Isolation frequency (%)			
Pythium spp.	Emergence (%)	···	−0.42*
	Disease index	···	0.43*
Fusarium spp.	Emergence (%)	0.31*	···
	Disease index	−0.32*	−0.38*
PTD[d]	Emergence	···	0.56**
	Disease index	···	0.59**

[a] Mean of 28 sites common to both 1982 and 1983 sampling years.
[b] Emerged living plants.
[c] Measure of disease severity based on symptoms; 1 = no symptoms, and 7 = plant not emerged.
[d] Percentage of seedlings in the bioassay with preemergence mortality, plus seedlings from which *Pythium* spp. and *T. basicola* were isolated. Asterisks * and ** indicate statistically significant correlation, $P = 0.05$ and $P = 0.01$, respectively.

Fusarium spp. with disease severity. Apparently species of *Fusarium* are not important etiological agents in the cotton seedling disease complex.

The role of *R. solani* in the seedling disease complex was not very definitive in this study. Although bioassay isolations of *R. solani* were correlated inversely with field disease severity only slightly ($P = 0.15$), negative correlations of *R. solani* with disease severity appeared in the best two-, three-, and four-variable models generated through stepwise regression analysis. In a previous study (13), *R. solani* was most often isolated from field-grown cotton seedlings during periods when the mean minimum soil temperature was 17 C or above. Conversely, species of *Pythium* were isolated most often when mean minimum soil temperatures were below 17 C. The temperature during seedling incubation in the bioassay procedure was maintained at a constant 17 C and could have been more selective for *Pythium* spp. than for *R. solani*. Moreover, daily minimum air temperatures during 5–15 days after planting in the field in 1984 averaged 12.2 C, a temperature which probably predisposed the seedlings for pathogenesis by *Pythium*.

DeVay et al (5) isolated species of *Pythium* from six soils with a pimaricin-vancomycin selective medium and identified those that were *P. ultimum*. Survival of cotton seedlings in the six soils was related directly to the concentration of propagules of *P. ultimum*. In our study, total numbers of colonies of *Pythium* isolated on gallic acid medium were not correlated significantly with any of the biological variables of the bioassay or field plantings. Apparently, colonies of *Pythium* obtained on the selective medium consisted of pathogenic and nonpathogenic strains and, as a group, did not relate to disease severity. Numbers of colonies of *Pythium* on gallic acid medium were correlated inversely with soil pH. Percentages of seedlings from which *Pythium* (and also *R. solani*) was isolated, as determined with the bioassay procedure, were related directly with soil pH. These results are in agreement with those of Lumsden and Ayers (17) for *P. ultimum* and with Bloom and Couch (2) for *R. solani*.

There are conflicting reports in the literature on the effect of soil texture on severity of diseases caused by *Pythium* spp. and *R. solani*. McKeen (19) isolated *P. ultimum* and *R. solani* more often from sugar beet roots grown in clay soil than in sandy loam or marl soil. Root rot of sugarcane caused by *P. arrhenomanes* Dresch was more severe in clay soils than in sandy soils (20). There was no apparent relationship between severity of cotton seedling disease caused by *P. ultimum* and soil type in six California soils (5). Hancock (10) found that after oospores of *P. ultimum* were added, there was no difference in survival (for 180 days) in two soils, a clay and sandy loam, but in another study all of the more coarsely textured soils that he investigated were conducive to *P. ultimum* (9). Johnson et al (11) isolated *P. ultimum* and *R. solani* more often from cotton seedlings grown in a fine sandy loam than from seedlings grown in silt loam soils. Carter (4) obtained more severe disease of cotton seedlings caused by *R. solani* in soil with 8% clay than in soils with 15 or 22% clay. Lewis (15) found that *R. solani* survived longer in a sandy loam than in a silty clay loam soil. The results obtained in the present study were in agreement with these latter researchers (4,9,11,15). Soil clay content was correlated inversely with both disease severity values obtained in the bioassay and in the field plantings. From stepwise regression analysis, clay content was the most significant soil physical variable contributing to cotton seedling disease severity (Table 5).

From the results of this study we believe that prediction of severity of seedling disease of cotton can be made with reasonable accuracy. Although there were significant correlations of several of the variables of the bioassay with field emergence and disease index, it appears that the most important bioassay variable was the mean value for the three combined variables (PTD in Tables 4 and 5). It is suggested that risk factors can be estimated for these wind-deposited soils by use of the bioassay procedure along with determinations of soil clay and organic matter content.

TABLE 5. Stepwise regression models for dependent variables of seedling disease severity of cotton planted at 28 subsites in 10 fields of loessial soils in western Tennessee[a]

Field planting variable	Best models[b]			
	1-Variable	2-Variable	3-Variable	4-Variable
Emergence (%)[c]	−PTD[d] ($r^2 = 0.31$)	−PTD *R. solani*[e] ($r^2 = 0.44$)	−PTD *R. solani* Clay (%) ($r^2 = 0.53$)	
Disease index[f]	PTD ($r^2 = 0.35$)	PTD −*R. solani* ($r^2 = 0.48$)	PTD −*R. solani* −Clay (%) ($r^2 = 0.57$)	PTD −*R. solani* −Clay (%) Organic matter (%) ($r^2 = 0.61$)
Postemergence mortality (%)	−Clay (%) ($r^2 = 0.11$)	−Clay (%) *Fusarium* spp.[e] ($r^2 = 0.44$)		

[a] Models were derived from the stepwise regression analysis, SAS 1982 program ($P = 0.15$).
[b] Variables of means of 28 sites common to both sampling years. A negative correlation is indicated by a minus (−) preceding the variable name.
[c] Emerged, living plants.
[d] Percentage of seedlings in the bioassay with preemergence mortality plus seedlings from which *Pythium* spp. and *T. basicola* were isolated.
[e] Percentage of seedlings in the bioassay from which this fungus was isolated. Measure of disease severity based on symptoms; 1 = no symptoms, and 7 = plant not emerged.

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