Fungi Associated with Postemergence Seedling Disease of Cotton in Three Soils

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ABSTRACT

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Soil samples were collected from 12 sites of three soils (Vicksburg fine sandy loam, Memphis silt loam, and Dexter loam) during a 12-mo period. Fungi were isolated from discolored or necrotic hypocotyls of cotton seedlings grown in the three soils at 19 C. More diseased hopocotyls occurred in soils not cropped to cotton than in soils cropped to cotton for 4 yr. The most frequently isolated fungi were *Pythium* spp., followed in descending order of frequency by *Fusarium* spp., *Rhizoctonia solani*, and *Thielaviopsis basicola*. The *Pythium* spp. isolated, in order of their frequency, were *P. ultimum*, nonsporulating "mycelial" isolates, *P. sylvaticum*, *P. irregulare*, and *P. heterothallicum*. In pathogenicity tests, isolates of *Pythium* caused the most severe disease on cotton hypocotyls, followed by isolates of *Rhizoctonia*, *Thielaviopsis*, and *Fusarium*. Fewer diseased hypocotyls occurred on cotton grown in soils collected in February than in June, August, or November. In November, *Pythium* spp. were most prevalent; *Fusarium* spp., *R. solani*, and *T. basicola* were more prevalent in June and August than in February or November. *Rhizoctonia solani* and *T. basicola* were more prevalent in the Vicksburg fine sandy loam than in the Memphis or Dexter soils. *Pythium* spp. and *Fusarium* spp. were isolated more frequently from plants grown in soils not cropped to cotton and *R. solani* and *T. basicola* in soils cropped to cotton.

Additional key words: cotton seedling blight, cotton damping-off.

Species of *Pythium*, *Rhizoctonia solani*, *Fusarium* spp., *Glomerella gossypii*, and *Thielaviopsis basicola* (3, 5, 6, 9, 10) have been associated with seedling blight of cotton. The disease often is severe and results in stand failures and reduced yield (2, 5). Incidence and severity of seedling blight have been shown to be affected by soil temperature and moisture content (2, 4, 5, 8), but little is known about how other natural soil or environmental conditions affect disease severity or the specific pathogen or pathogens involved. Since the disease often is caused by a complex involving more than one pathogen, it is possible that environmental conditions such as soil type or cropping history might have a differential effect on the quantitative and qualitative distribution of the pathogens.

This 12-mo ecological study was initiated to determine the distribution of cotton seedling pathogens in three soils in western Tennessee cropped to cotton for 3 yr or more, and in plots of the same three soils not cropped to cotton.

MATERIALS AND METHODS

Bulk samples of surface soil were collected from 12 sites in western Tennessee. Each bulk sample consisted of 50 subsamples collected from each 12 × 12 m site. Collections were made from three soils: Vicksburg fine sandy loam, Memphis silt loam, and Dexter loam. Cotton had been grown for 4 yr or more on two of the sites of each soil. On the two additional sites of each soil, crops other than cotton had been grown for 10 yr or more. The soil samples were passed through screens with 6-mm openings and then placed in 15-cm diameter pots. Seven pots from each sample were prepared in this manner. Seven seeds from a mechanically mixed composite of 32 cotton cultivars were planted in each pot, which then were placed in a constant temperature growth chamber at 27 C. After 9 days, the seedlings were thinned to 48 plants per soil sample and transferred to a growth chamber with a constant temperature of 19 C, a temperature which is favorable for several species of fungi to infect cotton hypocotyls (5, 6). After 12 days, all plants were removed from the soil and washed. Segments (0.5-1.0 cm each) of discolored or necrotic hypocotyls were cut from the plants, washed in flowing tap water for 24 hr, and then were washed in three successive changes of sterile water and blotted with sterile filter paper. Each hypocotyl segment was placed on the surface of 2% agar containing 10 mg aureomycin /liter. After 3-5 days of incubation at 24 C, hyphal tips of fungi growing from the segments were transferred to plates of potato-dextrose agar (PDA) which also contained 10 mg aureomycin/liter. The

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resulting colonies were identified to genus. Isolates of *Pythium* were transferred to hemp-seed agar (1) to induce formation of sexual structures for identification to species; those that did not sporulate on hemp-seed agar were crossed (6) with heterothallic plus and minus strains of *P. sylvaticum* and *P. heterothallicum*. Incompatible isolates that did not produce sexual structures were labeled "mycelial" isolates of *Pythium*.

Isolates of each genus, selected at random for pathogenicity tests, were grown on potato-dextrose agar in petri dishes. Disks, 4 mm in diameter cut with a cork borer from 4-day-old cultures were used as inoculum on 8-day-old seedlings grown in sterilized sand at 27 C. A small hole in the soil adjacent to each hypocotyl was made with a jet of sterile water from a plastic wash bottle. An agar disk of the fungus was placed against the hypocotyl in the hole and the sand was pressed gently against it to hold it in place. In this manner eight plants were inoculated with each isolate tested. Inoculated plants were incubated for 7 days at 19 C and then disease severity ratings were made.

Soil sample collections and isolations of fungi from hypocotyls were made from the 12 sites four times during 1974-75: November (1974), and February, June, and August (1975). After collection, bulk samples were stored in polyethylene bags for not more than 2 wk before being processed as described.

RESULTS

During the 12-mo experimental period, a total of 2,304 cotton plants were grown in samples of the three soils and examined for diseased hypocotyls; 51% (1,174) developed discolored or necrotic hypocotyls. The soils with their respective crop histories and number of diseased hypocotyls are listed in Table 1. Fewer diseased hypocotyls developed in soils cropped to cotton for 4 yr or more than in soils cropped to other plants. Twenty-four

TABLE 1. Postemergence cotton seedling disease in samples of three soils at four sites in western Tennessee, two cropped to cotton and two with different crop histories[a]

Soil series and site no.	Crop history[a]	pH[b]	Discolored or necrotic hypocotyls[c] (no.)
Vicksburg fine sandy loam			
Site 1	Cotton (+5)	5.5	72
Site 2	Cotton (+5)	5.5	118
Site 3	Corn (+3)	5.9	145
Site 4	Soybean (+5)	6.1	110
Memphis silt loam			
Site 1	Cotton (+3)	4.7	47
Site 2	Cotton (+3)	5.8	96
Site 3	Corn (+2) Soybeans (+2)	5.2	125
Site 4	Tomatoes (+4)	6.0	90
Dexter loam			
Site 1	Cotton (+3)	5.1	71
Site 2	Cotton (+3)	5.7	72
Site 3	Wheat (+1) Barley (+3)	5.9	103
Site 4	Tomatoes (+4)	4.7	125

[a]Previous crop and number of years grown (in parentheses).
[b]Measurements made with August soil collections [1:1 (v/v) dilution of soil to water].
[c]A total of 192 seedlings were grown in soil samples collected from each site.

TABLE 2. Pathogenicity of fungi isolated from necrotic cotton seedling hypocytyls[a]

Genus	Isolates in infection class[b]										Class mean
	1 (no.)	2 (no.)	3 (no.)	4 (no.)	5 (no.)	6 (no.)	7 (no.)	8 (no.)	9 (no.)	10 (no.)	
Pythium			4	4	10	6	2	1	1		5.2
Fusarium	2	5	14	8	1						3.0
Rhizoctonia			3	4		3	1	1			4.8
Thielaviopsis		3	2		2	5	1				4.5

[a]Eight cotton plants were inoculated with each isolate.
[b]Infection classes of 1 - 10 based on severity, where 1 = no symptoms and 10 = dead plant.

percent (or 47) of the plants developed necrotic hypocotyls when grown in soil from site 1 of Memphis silt loam, a cotton soil; but 76% (or 145) became diseased in soil from site 3 of Vicksburg fine sandy loam, a noncotton soil. There does not appear to be a significant relationship of disease with soil pH.

The most frequently isolated fungi were *Pythium* spp., followed by species of *Fusarium*, *Rhizoctonia solani* Kuhn., and *Thielaviopsis basicola* (Berk. & Br.) Ferr. Other fungi obtained were one isolate of *Alternaria* and six isolates of a nonconidial septate fungus with intercalary chlamydospores. The *Pythium* spp. isolated were *P. ultimum* Trow., *P. sylvaticum* Campbell and Hendrix, *P. irregulare* Buisman, and *P. heterothallicum* Campbell and Hendrix. One-hundred twenty nine *Pythium*-like isolates did not produce sexual structures when plated with plus or minus strains of *P. sylvaticum* or *P. heterothallicum*.

Of the 83 isolates selected at random, only two isolates of *Fusarium* did not cause discolored or necrotic lesions on hypocotyls when tested for pathogenicity (Table 2). Most isolates of *Fusarium* spp. caused discolored areas on the hypocotyls, but some produced distinct but small necrotic areas on the cotton hypocotyls (infection classes 4 and 5). As a group, isolates of *Pythium* were most pathogenic, followed by *Rhizoctonia solani*, *Thielaviopsis basicola*, and *Fusarium*. The most highly virulent isolate was a culture of *P. sylvaticum* which caused wilting or death of all eight plants tested. However, as groups, species and mycelial isolates of *Pythium* did not differ appreciably in pathogencity.

Significantly ($P = 0.01$) fewer hypocotyls were diseased in soil samples collected in February than in the other collection months (Table 3). Numbers of isolates of all four genera were low in February soil collections. Significantly more isolates of *Pythium* ($P = 0.01$) were obtained from samples collected in November. Isolates of all *Pythium* spp. except *P. heterothallicum* were more prevalent in November samples. Sig ·ficantly more isolates of *Fusarium*, *R. solani*, and *T. basicola* were obtained ($P = 0.05$) from soils collected during June and August.

TABLE 3. Frequency of isolation of fungi from necrotic cotton seedling hypocotyls grown in soil samples collected during a 12-mo period

Fungus	Isolates obtained[a] in February	June	August	November	Total
Pythium spp. (total)	99	122	93	268	582
(*P. ultimum*)	(57)	(92)	(45)	(174)	(368)
("mycelial" isolates)	(19)	(14)	(40)	(56)	(129)
(*P. sylvaticum*)	(17)	(12)	(8)	(24)	(61)
(*P. irregulare*)	(1)	(2)	(0)	(11)	(14)
(*P. heterothallicum*)	(5)	(2)	(0)	(3)	(10)
Fusarium spp.	48	154	165	34	401
Rhizoctonia solani	14	41	60	25	140
Thielaviopsis basicola	3	12	21	4	40
Total isolates	164	329	339	331	1,163

[a]Each figure in the table is the total number of isolates obtained from 576 plants grown in soil samples collected during the indicated month. Enclosure in parentheses indicates subtotals within the *Pythium* spp. total.

TABLE 4. Frequency of isolation of fungi from necrotic cotton hypocotyls from plants grown in three soils with different crop histories[a]

Fungus	Vicksburg		Memphis		Dexter	
	Cotton[b]	Other[c]	Cotton	Other	Cotton	Other
Pythium spp. (total)	90	156	57	99	66	114
(*P. ultimum*)	(32)	(120)	(28)	(90)	(19)	(79)
("mycelial" isolates)	(39)	(24)	(6)	(0)	(36)	(24)
(*P. sylvaticum*)	(14)	(9)	(17)	(6)	(7)	(8)
(*P. irregulare*)	(1)	(1)	(6)	(2)	(3)	(1)
(*P. heterothallicum*)	(4)	(2)	(0)	(1)	(1)	(2)
Fusarium spp.	32	60	52	101	54	102
Rhizoctonia solani	47	29	20	14	19	11
Thielaviopsis basicola	21	2	13	0	4	0
Total isolates	190	247	142	214	143	227

[a]Each figure in the table is the total number of isolates obtained from 384 plants grown in soil samples collected during a 12-mo period. Enclosure in parentheses indicates subtotals within the *Pythium* spp. total.

[b]Soil samples from sites in which cotton had been grown 4 yr or more.

[c]Soil samples from sites in which crops other than cotton had been grown 10 yr or more.

There was no significant difference in total numbers of diseased plants grown in the three soils. However, significantly more isolates ($P = 0.05$) of *R. solani* and *T. basicola* were obtained from hypocotyl lesions of cotton in the Vicksburg, Memphis, or Dexter soils (Table 4). Also, more isolates of *Pythium* were obtained from the Vicksburg soils, but the difference was not significant. More diseased cotton plants ($P = 0.01$) occurred in soils not cropped to cotton than in soils cropped to cotton. *Pythium ultimum* and *Fusarium* spp., were isolated much more frequently from plants in noncotton soils. Conversely, significantly more ($P = 0.01$) isolates of *R. solani* and *T. basicola* were isolated from plants grown in soils that had been cropped to cotton.

DISCUSSION

It appears that certain ecological similarities may exist between *R. solani* and *T. basicola* associated with necrotic cotton hypocotyls. Both were more prevalent in soils collected during the summer months, both occurred more frequently in the Vicksburg soils, and both were isolated more frequently from hypocotyls of cotton grown in soils cropped to cotton than from those grown in noncotton soils. The populations of these two pathogens apparently were increased, or at least maintained at a high level, with continuous cotton culture.

The distribution of *Pythium* spp. was quite different. It was not associated with a particular soil and was more prevalent in soils collected in November. A similar increase in populations of *Pythium* spp. in November was reported by Lumsden et al. (7). In addition, more *Pythium* isolates were obtained from cotton grown in soils not cropped to cotton than in soils cropped to cotton. This finding was unexpected since *Pythium* spp. include major pathogens of cotton in Tennessee (5, 6), and in the present study, *Pythium* spp. were isolated much more often than the other genera. An examination of the data revealed no particular noncotton crop associated with populations of *Pythium* spp. in the three soils. Further studies will have to be made for an understanding of this phenomenon.

The distribution of *Fusarium* spp. was similar to that of *Pythium* spp. It was isolated more often from cotton grown in soils not cropped to cotton than in cotton soils. Although some isolates of *Fusarium* were found to cause distinct necrotic lesions on cotton hypocotyls, *Fusarium* isolates as a group were the least pathogenic. Thus, it is assumed that damage caused by *Fusarium* spp. in cotton fields is minimal.

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