STUDIES OF THE VARIABILITY OF RHIZOCTONIA SOLANI ON COTTON

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STUDIES OF THE VARIABILITY OF RHIZOCTONIA SOLANI ON COTTON

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

INTRODUCTION

Extensive studies have been carried on over a number of years to determine the importance of the various seedling diseases of cotton. Soreshin (Rhizoctonia solani Kuhn) has been recognized as one of the most important of the seedling diseases because of its wide distribution and the virulence of the causal organism.

Up to the present time no adequate measures have been found for controlling soreshin, but methods have been developed to reduce the severity of the disease. One factor that has complicated the development of an adequate control measure is the lack of knowledge of the variability of the causal organism. A knowledge of the extent of this variability would be an aid in the development of adequate control measures.

The purpose of this study was to measure possible variations in pathogenicity and cultural characteristics of isolates of R. solani found in Oklahoma. The isolates used in this study were obtained from cotton seedlings grown in soil from a number of locations, and from fields that had been used for different crops the previous season. Studies were also made to find out whether or not a number of isolates of R. solani showed differences in tolerance to soil applied fungicides.

LITERATURE REVIEW

Extensive studies have been made of the role of Rhizoctonia solani Kuhn in the seedling disease complex of many plants. The knowledge of damping-off or soreshin of cotton in which R. solani is the causal organism dates back to the work of Atkinson (3), who was able to isolate the organism from diseased cotton seedlings in 1892. He described the causal organism of soreshin as a "sterile," damping-off fungus. The organism was first described by Kuhn in 1858, when he was studying a disease of potatoes, Duggar (7) gave a very complete description of the organism in 1915.

Importance of Rhizoctonia in the Seedling Disease Complex

R. solani is world wide in its distribution. Duggar (7) reported that it was known throughout the United States, Canada, and parts of the far East. Miller (15) made a survey on the prevalence and distribution of the various fungi associated with damping-off of cotton in the states on the Southeast and Gulf coasts and part of western Tennessee. He found that Glomerella gossypii Edj. was most prevalent, Fusarium moniliforme Sheld. was next, and R. solani was third in prevalence. Miller (15) concluded R. solani played

a more important role as a pathogen in Mississippi and Louisiana than in the Southeastern States. The findings of Weindling *et al.* (28) substantiated those of Miller. Arndt (1) found that *R. solani* was less prevalent than *G. gossypii*, *Fusarium* sp. and *Pythium ultimum* Trow. in South Carolina. Walker (26) concluded that soreshin was the most common cause of poor stands in Florida.

Ray and McLaughlin (20) conducted an extensive survey of the fungi associated with diseased cotton seedings of Oklahoma. They found that *F. moniliforme* was most common, and *R. solani* was the second most commonly isolated fungus from cotton seedlings in this area. They also tested the virulence of the fungi isolated, and found that *R. solani* was more pathogenic than all the organisms tested except *Glomerella*. The tests showed that *G. gossypii* had a high degree of pathogenicity, but it was not very prevalent in Oklahoma. Weindling *et al.* (28) did not find *G. gossypii* to be prevalent in Texas or Oklahoma. *R. solani*, because of its high degree of virulence and its frequency in Oklahoma soils, was considered by Ray and McLaughlin (20) to be the most important fungus involved in diseases of cotton seedlings.

Symptoms of Soreshin

The characteristics of soreshin are very similar to symptoms of damping-off in young seedlings of many plants.

Duggar (7) described damping-off of delicate plants as a translucent appearance near the base of the stem, which is followed by shrinkage of the tissue and a weakness of the stem. The plants may topple over, or more robust seedlings may exhibit characteristic lesions. The following description was given by Atkinson (4) of the effect of the soreshin fungus on cotton: The fungus grows into superficial tissues of the stem near the ground and disintegrates them before it passes to the deeper tissues; in other words, it kills as it goes and the tissues become brown and depressed. Walker (25) described soreshin of cotton as being characterized by a breaking over of young plants at the soil level, with the leaves becoming limp, and a dark brown canker being noticeable just below the soil level.

Action of Rhizoctonia on Cotton Seedlings

Kernkamp *et al.* (11) present an up-to-date literature review, and also some pertinent information on the pathological histology of soreshin. They found in studies of peas and soybeans that entrance through the epidermis was accomplished by a peglike structure which penetrated directly, probably with the assistance of enzymes. Nakayama (16) studied the infection of cotton seedlings by *R. solani* in Petri dishes, and found that the principal mode of penetration of the hypocotyl is by means of "infection cushions" of hyphae. He showed that *R. solani* could enter roots,

hypocotyl, and cotyledons, and that the root tip is very susceptible to infection. He also found that the fungus penetrated the epidermis of the root tip and branched out intercellularly and intracellularly into the endodermal region, and that penetration of the root was likewise accomplished through the natural injuries associated with the extrusion of secondary roots from the primary root. Kernkamp et al. (11) state that there is considerable deterioration of host tissues 8 to 10 cell layers ahead of the invading hyphae of R. solani. This is in agreement with the original work of Atkinson.

According to Duggar (7) R. solani exists in the soil as a saprophyte. Walker (26) states that the fungus lives from year to year in the soil on decaying plant material and under favorable conditions it is capable of attacking a great many different kinds of plants when they are young.

Gilchrist (9) showed that the susceptible region of many plants is deficient or lacking cuticle, and that variety differences in susceptibility may be due to the degree of cuticularization. During a study of a number of varieties of cotton, Brinkerhoff et al.[1] found varietal differences in susceptibility to R. solani and other seedling diseases. Some varieties showed tolerance to R. solani, but none of them showed enough resistance to withstand the

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[1]Brinkerhoff, L. A., R. P. Pfeifer, and John M. Green. Unpublished data.

disease when temperature and moisture conditions were especially favorable for infection.

Cultural and Pathogenic Variation of Rhizoctonia Solani

A number of papers have been published on the cultural variation of R. solani. This work was reviewed by LeClerg (12) in 1934, and was brought up to date by Kernkamp et al. in 1952. Kernkamp et al. (11) studied a number of cultural races that differ in color, zonation, growth rate, type of sclerotia, width of hyphae or enzyme production. Other workers have shown that different races respond differently to temperature, nutrients, hydrogen-ion concentration, and to toxic chemicals. Houston (10) studied the effect of temperature upon the growth rate of a number of isolates and found that the majority had an optimum temperature of 28° C.

Numerous workers (10, 11, 20, 23, 25, 27) have concentrated their efforts on testing the pathogenicity of isolates of R. solani. Houston (10) sums up most of the knowledge of the pathogenicity of the organism with this statement: "Two isolates having the same degree of pathogenicity on one host are not necessarily equally virulent in their attack upon another host." Weindling (27) has found that R. solani is not adversely affected by variations of pH of the soil. He found that R. solani grows well over the whole range of pH values occuring in natural soils, even at

a pH of 3.0. Walker (26) found that pathogenicity is correlated with the temperature of the soil, and that the fungus was capable of attacking plants below 80° F. He stated that the greatest injury occurs during damp, cool periods, and that the optimum temperature for killing of seedlings lies between 17° C. and 23° C. Walker (26) stated that all soil moistures at which cotton would germinate and grow are sufficient to allow R. solani to attack. The organism seemed to grow better when more moisture was present, but high soil moisture was not necessary. Schwegmann (23) found that isolates of R. solani differed in their tolerance to temperature. His results showed that some isolates can show a high degree of pathogenicity on cotton at temperatures as high as 30° C. while others were injurious only at temperatures below 18° C.

Control Measures

Numerous workers (6, 13, 14, 17, 18, 19) have found that seed treatment with various fungicides helps to control seed and seedling infections in which R. solani is the causal organism. Miles (14) found that the use of Cerasan and New Improved Cerasan (organic mercury dusts) gave increases in emergence and yield. Both chemicals gave significant increases over non-treated seed with New Improved Cerasan giving the better results. Nettles (17) reported that only 16% of the plants grown from seed treated with Cerasan were

affected with soreshin, while 60% of the plants grown from non-treated seed were affected. However, Lehman (13) found that in inoculated soil the increase in disease-free seedlings resulting from seed treatment with New Improved Cerasan was not significant. He stated that it appeared so far as final stands are concerned, that organic mercury dust used on the seed may be of little value in control of R. solani.

Ray (18) reported that Spergon (tetrachloro-para-benzoquinone) was equal to the best organic mercury dusts, and that seeds dusted with the various agents gave a significantly greater emergence than the non-dusted seed. However, he also concluded that so far as final stand was concerned, dusted seed had little value as a protectant against R. solani. Ray (19) reported that cotton seedling diseases caused by seed-borne organisms, and not soil-borne organisms such as R. solani, could be controlled by seed treatment with organic mercury dusts. Felix (8) reported that Spergon treatment of machine delinted cotton seed effectively controlled damping-off in flats of Mississippi cotton soil. Staten (22) reported that Spergon was more effective than New Improved Cerasan or Cerasan in greenhouse tests. Plants from Spergon treated seeds had about 60% survival, those from New Improved Cerasan treated seeds had about 40%, and those from Cerasan treated seed had about 33%. In tests using a number of seed treatments,

Brinkerhoff *et al.* (6) have found that seed treatment probably has considerable value in protecting cotton from seed rot and pre-emergence injury, but does not give adequate protection to insure a good stand when the inoculum level is high or the environment is favorable for post-emergence injury.

Arndt (2) has evaluated a number of chemicals as seed and seedling protectants. The chemicals were placed in sand surrounding the seed. Under controlled experimental conditions he was able to get good protection of cotton seedlings from infection by *R. solani* with the use of pentachloronitrobenzene, and tetrachlorophenol and trichlorophenol derivatives. Brinkerhoff *et al.* (5) have found that pentachloronitrobenzene, thiram, and zineb mixed with the soil in the seed row were effective in greenhouse tests in which the soil was artificially infested with *R. solani*.

Another type of control measure that has been tried is the use of other microorganisms that are parasitic on *Rhizoctonia*. Weindling (27) reported that good control of damping-off of citrus seedlings due to *R. solani* had been obtained in sterilized soil by adding viable spores of the fungus *Trichoderma*. This action has not been tried on cotton.

MATERIALS AND METHODS, AND RESULTS

Tests to Determine Seedling Disease Potential in Field Soil

Soil samples were collected in 20 lb. paper sacks from fields in various parts of Oklahoma at different times during the fall and winter of 1952-53. These samples were screened through a 1/4 inch mesh wire, and placed in wooden flats, 16 X 24 inches, that had been previously washed and surface sterilized in a sodium hypochlorite (Clorox) solution. Each of the flats was planted with 50 seeds of each of two varieties of cotton, Stoneville 62-1-84 and CR-1. Previous investigations[1] have shown that the former shows a high degree of susceptibility to the soreshin fungus, R. solani, and the latter shows some tolerance to the organism.

The soil samples were taken from fields in various locations that had been used for many crops, including cotton. The locations selected were mostly in the Washita valley in central Oklahoma. Samples were also taken near Stillwater.

A small portion of each soil sample of Test 1 was used to get a measurement of pH. One gm. of each sample was placed in a beaker, and 5 ml. of distilled water were added.

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[1] Brinkerhoff, L. A., R. P. Pfeifer, and John M. Green. Unpublished data.

The mixture was agitated, and then allowed to stand until the solid particles had settled. The liquid portion was decanted, and a measurement of its pH was made on a Beckman pH meter.

The following procedure was used in isolating R. solani and other fungi from the diseased seedlings of these tests. The lesion or diseased area was cut from the stem, and placed in a Petri dish of 95% ethyl alcohol for about 10 seconds. The piece was then placed in a 0.5% solution of sodium hypochlorite for 1 to 3 minutes, and then transferred to a Petri plate of acidified potato-dextrose agar. The agar was acidified to a pH of about 4 with lactic acid to inhibit growth of bacteria that are found in the stems along with seedling disease fungi. Transfers were made to ordinary potato-dextrose agar of any fungus cultures that grew from the stems.

Isolates of R. solani were grown on sterilized grain sorghum. The grain was placed in prescription bottles or in Ehrlenmeyer flasks, water was added to it, and it was steam sterilized at 15 pounds pressure for 1 hour. The grain served as a good growth medium, and was in a convenient form to be used for inoculum in greenhouse and field tests.

Test 1. Greenhouse test using flats of field soil from different locations following different crops

The flats used in Test 1 were planted and watered, and then placed in the cool section of the greenhouse for 4

days. Then they were moved to the warm section. The temperatures ranged from 65° F. to 75° F. in the cool section, and from 70° F. to 92° F. in the warm section.

Results. The emergence and survival of seedlings of Stoneville 62-1-84 was much less in all the soil samples from the field than in flats of sterilized soil. This was also true for CR-1 in some of the samples. These reductions in emergence in nonsterilized soil indicate the presence of organisms harmful to cotton seeds and seedlings.

The data obtained from Test 1 are shown in Table 1. The total emerged and surviving seedlings was much less for Stoneville 62-1-84 than for CR-1. There was a great deal of variation within each variety, between samples from different locations used for the same crop, and also between samples from the same location used for different crops.

A correlation was computed between the pH readings and the emergence of Stoneville 62-1-84. The calculations indicated that there was no correlation between the two groups.

Test 3. Greenhouse test using flats of field soil from one location following different crops.

The flats used in Test 3 were planted, watered and then placed in the cool section of the greenhouse for 6 days. Then they were moved to the warm section. The temperatures ranged from 65° F. to 85° F. in the cool section, and from 70° F. to 95° F. in the warm section.

Table 1. Determination of seedling disease potential of soil from 6 locations following various crops

Code	Location	Previous crop	pH	Cotton seedlings after 14 days (%)[a] Sto. 62-1-84 Emerged	Sto. 62-1-84 Survived	CR-1 Emerged	CR-1 Survived
1	Newcastle	Cotton	6.6	26	21	47	46
2[c]	"	Corn	6.6	31	29	45	44
3[b]	"	Alfalfa	6.8	37	37	39	39
4	"	Wheat	6.7	34	33	42	38
5	Chickasha	Virgin prairie	6.8	17	17	38	38
6[b]	"	Alfalfa	6.9	40	39	39	36
7	"	Cotton	6.7	27	25	47	46
8[b,c]	"	"	6.7	26	24	49	49
9[c]	Anadarko	"	7.0	23	11	34	20
10[b]	"	Pasture	7.1	4	4	46	46
11	"	Alfalfa	6.9	26	26	43	43
12	"	Corn	7.5	38	38	48	48
13	Verdin	Cotton	7.4	39	36	45	45
14	"	Alfalfa	6.7	29	28	45	43
15	"	Cotton	6.5	41	17	47	45
16	Chickasha	Grain sorghum	6.6	33	22	44	32
17	"	Cotton	6.3	29	22	39	30
18[b]	Perkins	"	6.2	27	24	30	28
19	"	"	6.0	24	24	49	49
20	"	Corn	6.1	30	30	47	47
21[b]	"	Grain sorghum	6.3	21	20	33	33
22[b,c]	"	Rye & vetch	6.1	5	4	27	20
23[c]	"	Alfalfa	6.1	15	14	15	13
24	Stillwater	Corn	6.2	28	27	27	22
25[b]	"	Cotton	6.2	34	31	42	42
26	"	"	6.2	39	37	41	41
27	"	Alfalfa	6.2	29	29	47	47
28	"	Grain sorghum	6.3	20	18	42	42
29	"	Sterilized	6.3	44	41	47	47
30	"	"	6.3	43	43	47	47
31	"	"	6.3	46	46	48	48
			Total	905	817	1279	1214
			Mean	29.2	26.4	41.3	39.2

[a] Based on percent of seeds planted
[b] Isolates of R. solani obtained from these treatments were used in Test 2.
[c] Isolates of R. solani obtained from these treatments were used in Test 6.

Results. The emergence of both varieties was much less in all field soil samples than in sterilized soil. This is similar to the results obtained in Test 1. The emergence was much less for Stoneville 62-1-84 than for CR-1, the difference being significant at the 1% level. The soil samples were all from Chickasha, Oklahoma, but the difference between samples that had been used for different crops was significant at the 1% level. The data obtained from Test 3 are shown in Table 2.

Test 4. Greenhouse test using flats of field soil from one location following different crops.

The flats used in Test 4 were planted and watered, and placed in the greenhouse. Temperatures ranged from 70° F. to 90 or 95° F.

The emergence in Test 4 was similar to that obtained in the two previous tests. Stoneville 62-1-84 showed much less emergence, and both varieties showed less emergence in the samples than in sterilized soil. The samples were all from Perkins, Oklahoma. The difference between varieties was significant at the 5% level, and the difference between samples that had been used for different crops was significant at the 1% level. The data obtained from Test 4 are shown in Table 3.

Table 2. Determination of seedling disease potential in soil from Chickasha, Oklahoma, following different crops

Soil sample Code	Previous crop	Cotton seedlings emerged (after 14 days) Mean of 4 replications (%) Sto. 62-1-84	CR-1
1 /a	Alfalfa	37.0	73.0
2	Cotton	51.0	80.0
3 /a	Corn	44.4	85.4
4	Wheat	51.4	83.0
5	Steam sterilized	82.0	92.0
	L.S.D. (1%) = 22.6		

Stand counts from replications

	Sto. 62-1-84					CR-1				
Code	1	2	3	4	Mean	1	2	3	4	Mean
1	12	17	23	22	18.5	37	37	40	32	36.5
2	27	26	27	22	25.5	46	35	40	39	40.0
3	21	23	17	28	22.2	43	45	36	47	42.7
4	22	25	23	33	25.7	46	40	39	41	41.5
5	35	44	44	41	41.0	43	48	47	46	46.0
	L.S.D. (1%) = 11.3									

Analysis of variance of Table 2

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	39	4039.0		
Replications	3	21.1		
Previous crop	4	581.9	145.5	4.2**
Varieties	1	2363.3	2363.3	68.3**
Error	31	1073.7	34.6	

/a Isolate obtained from this treatment was used in Test 6.

Table 3. Determination of seedling disease potential in soil from Perkins, Oklahoma, following different crops

Soil sample Code	Previous crop	Cotton seedlings emerged (after 14 days) Mean of 4 replications (%) Sto. 62-1-84	CR-1
1 [a]	Rye & vetch	46.4	46.0
2	Corn	63.0	77.4
3	Cotton	59.4	76.0
4	Alfalfa	67.0	83.0
5	Steam sterilized	87.4	94.4
	L.S.D. (1%) = 14.8		

Stand counts from replications

	Sto. 62-1-84					CR-1				
	1	2	3	4	Mean	1	2	3	4	Mean
Code										
1	26	20	26	21	23.2	28	23	25	16	23.0
2	31	36	28	31	31.5	42	39	43	31	37.7
3	35	38	27	19	29.7	36	37	40	39	38.0
4	38	37	30	29	33.5	43	43	42	38	41.5
5	42	43	48	42	43.7	47	47	47	48	47.2
	L.S.D. (1%) = 7.4									

[a] Isolate obtined from this treatment was used in Test 6.

Analysis of variance of Table 3

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	39	3031.0		
Replications	3	182.6		
Previous crop	4	973.1	243.2	4.69**
Varieties	1	270.2	270.2	5.21*
Error	31	1605.1	51.8	

Tests to Determine The Relative Pathogenicity of Different Isolates of R. solani

Isolates of R. solani were obtained from diseased cotton seedlings in the previous tests using field soil, and from seedlings in samples from cotton fields not used in the previous tests. Those soil samples from which isolates of R. solani were obtained for use in these tests are designated in Tables 1, 2, and 3. The remaining isolates were all obtained from soil samples from fields used for cotton, and the various locations are given in each of the tables in this section. Isolates of R. solani were obtained from most of the samples of soil used in the previous tests, but only those that were secured in a pure culture were used in these tests. Other fungi, mostly Fusarium sp., were also obtained, but not as frequently as R. solani.

Several tests were set up in the greenhouse using 8 inch glazed crocks. Randomized block designs were used with either 3 or 4 replications. The crocks were filled with soil, and steam sterilized for one hour at 15 pounds pressure. After being sterilized the crocks were kept covered with paper sacks to reduce contamination.

The inoculum for the greenhouse tests was prepared from grain sorghum cultures that had grown in an icebox at 20° C. for 7 to 10 days. A small amount of the culture was weighed out on a piece of sterile filter paper by means of a laboratory balance. This was placed in a mortar, and tap water

was slowly added while the inoculum was being ground to a thin paste. This paste was diluted with water and poured over the surface of the soil in each crock or else dropped into each crock with a 10 ml. pipette. After each culture was ground up, the mortar and pestle was washed with soap and water and dried with 95% ethyl alcohol.

The crocks were inoculated and watered, and then allowed to stand for 1 to 3 days before they were planted. This was done to allow the cultures of R. solani to grow in the soil and build up an infestation similar to what would be expected in the field. After the inoculum had been allowed to grow, the crocks were planted with cotton seeds. The seeds used in all greenhouse and field tests were from lots that were acid delinted and gravity graded, the floating fraction being discarded. The seeds were all from field grown lots, no greenhouse grown seeds were used. In Test 2 the same varieties of cotton were used as in the previous tests with field soil, Stoneville 62-1-84 and CR-1. In Tests 7 and 8 the variety Mebane 6801 was used.

Two field tests were set up during May, 1953. Inoculum was put in the row along with the seeds. Isolates of R. solani were grown on grain sorghum as for previous tests, and ground in a food blender instead of in a mortar. The amount of grain sorghum culture was measured by volume, and placed in the food blender with some water. The mixture was ground to a pulpy consistency, and then diluted with

additional water and added to vermiculite soil conditioner. Each seed was coated with this mixture of inoculum and vermiculite prior to planting.

The field Tests were set up in rows 33 feet long, and 40 inches apart. The furrows were opened with a garden plow, and hills 1 foot apart were marked by means of lugs on the wheel of the plow. The seeds and inoculum were placed in these hills by hand, using 100 seeds per row. Each row was covered over with a Planet Jr. hand planter with the opening device removed. Both Stoneville 62-1-84 and CR-1 were used in Test 5, but only Stoneville 62-1-84 was used in Test 6.

Test 2. Greenhouse test of isolates of R. solani

The crocks used in Test 2 were placed in the cool section of the greenhouse, inoculated and allowed to stand for 3 days. The inoculum for each crock consisted of 4 ml. of a suspension of a grain sorghum culture prepared by grinding 1 gm. of culture with 20 ml. of water. Three days after inoculation each crock was planted with 20 seeds of each variety, and left in the cool section of the greenhouse for 5 days. The temperatures ranged from 65° F. to 80° F. in the cool section. On the fifth day after planting, the crocks were moved to the warm section of the greenhouse, where the temperatures ranged from 64° F. to 92° F.

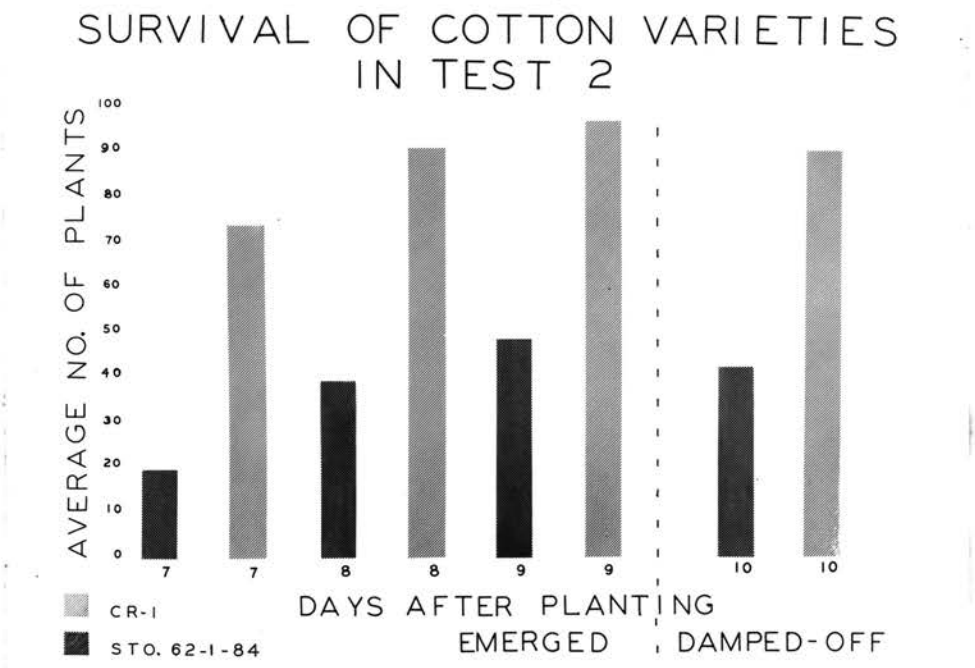

Figure 1. Daily stand counts of seedlings in Test 2.

Results. The data obtained from Test 2 are shown in Table 4, and in Figure 1. Table 4 shows the amount of emergence after 12 days, whereas Figure 1 shows daily counts of emergence and a final count of damping off. The differences between the various isolates, and between the varieties used, were significant at the 1% level.

Test 5. Field test of isolates of R. solani

Test 5 was planted on May 23, 1953, at the Agronomy Farm west of Stillwater. The inoculum for each treatment consisted of a suspension of grain sorghum culture and vermiculite placed around each seed. Fifty ml. of grain

Table 4. Test of isolates of R. solani from soil from several locations, following different crops.

Isolates Code	Source	Previous crop	Cotton seedlings emerged (after 12 days) Mean of 3 replications (%) Sto. 62-1-84	CR-1
1	Newcastle	Alfalfa	0	15.0
2	Chickasha	"	0	1.5
3	"	Cotton	11.5	23.5
4	Anadarko	Pasture	10.0	13.5
5	Verdin	Alfalfa	26.5	68.5
6	Perkins	Cotton	5.0	13.5
7	"	Grain sorghum	16.5	45.0
8	"	Rye & vetch	5.0	25.0
9	Stillwater	Cotton	46.5	95.0
10	Temple	"	10.0	33.5
11	Webbers Falls	"	16.5	28.5
12	Checotah	"	28.5	6.5
13	Council Hill	"	13.5	18.5
14	Steam sterilized	"	56.5	88.5
	L.S.D. (1%) = 20.1			

Stand counts from replications

	Sto. 62-1-84				CR-1			
	1	2	3	Mean	1	2	3	Mean
Code								
1	0	0	0	0	6	2	1	3.0
2	0	0	0	0	0	0	1	0.3
3	2	3	2	2.3	3	4	7	4.7
4	1	3	2	2.0	4	4	0	2.7
5	9	3	4	5.3	17	16	8	13.7
6	0	1	2	1.0	2	2	4	2.7
7	4	1	5	3.3	8	13	6	9.0
8	2	0	1	1.0	6	5	4	5.0
9	11	8	9	9.3	20	19	18	19.0
10	1	2	3	2.0	9	6	5	6.7
11	3	3	4	3.3	6	8	3	5.7
12	2	5	10	5.7	0	0	4	1.3
13	0	8	0	2.7	4	5	2	3.7
14	13	14	7	11.3	17	18	18	17.7
	L.S.D. (1%) = 4.02							

Analysis of variance of Table 4

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	83	2359.0		
Replications	2	11.0		
Isolates	13	997.5	76.7	21.3**
Varieties	1	1109.2	1109.2	308.1**
Error	67	241.3	3.6	

sorghum culture was ground up along with 150 ml. of water, and then added to 50 gm. of vermiculite. This mixture was then used to cover 140 gm. of seed, which was enough to plant all the replications of one treatment.

From the time that Test 5 was planted to the time the counts of surviving seedlings were made, the maximum air temperature was 96° F., the minimum was 64° F., and the average temperature was about 86° F. There was no rainfall during this period.

Results. The data obtained from Test 5 are shown in Table 5. The difference between the two isolates was not significant, but the difference between the two varieties was significant at the 5% level. The weather was unusually warm at the time the test was put in, and this did not favor disease development. This fact probably accounts for the good emergence, and the lack of a significant difference between the isolates.

Test 6. Field test of isolates of R. solani

Test 6 was planted on May 26, 1953, at the Cotton Research Station, Chickasha, Oklahoma. The inoculum was prepared as in Test 5, except a higher rate was used. Seventy-five ml. of a grain sorghum culture was ground up along with 125 ml. of water. One hundred-fifty ml. of this suspension was added to 50 gm. of vermiculite. This mixture was used to cover 90 gm. of seed, which was enough to plant all the replications of one treatment.

Table 5. Field test with cotton seeds infested with isolates of R. solani from Checotah and Chickasha, Oklahoma

Isolates			Cotton seedlings surviving (after 11 days) Mean of 9 replications (%)	
Code	Source	Previous crop	Sto. 62-1-84	CR-1
1	Checotah	Cotton	61.6	78.6
2	Chickasha	"	64.6	69.9
3	No inoculum		68.6	88.4
	L.S.D. (5%) = 17.9			

Stand counts from replications

Sto. 62-1-84

Code	1	2	3	4	5	6	7	8	9	Mean
1	64	56	60	59	57	58	75	62	63	61.6
2	72	83	87	41	48	38	69	70	72	64.6
3	57	63	64	60	60	78	91	69	75	68.6

L.S.D. (5%) = 17.9

CR-1

Code	1	2	3	4	5	6	7	8	9	Mean
1	81	82	84	68	87	89	53	80	83	78.6
2	63	48	71	68	80	67	79	79	74	69.9
3	100	83	89	86	94	85	87	87	85	88.4

L.S.D. (5%) = 17.9

Analysis of variance of Table 5

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	53	10,297.0		
Replications	8	796.0	99.5	
Isolates	1	7.5	7.5	0.038
Varieties	1	1,000.0	1,000.0	5.06*
Error	43	8,493.5	197.5	

From the time that Test 6 was planted to the time that the counts of surviving seedlings were taken, the maximum air temperature was 98° F., the minimum was 63° F., and the average temperature was about 85° F. There was no rainfall during this period.

Results. The data obtained from Test 6 are shown in Table 6. The table shows the emergence after 8 days and the yields of lint cotton obtained from each treatment in the fall of 1953. The differences between the various isolates were significant at the 1% level. Only one variety of cotton was used in the test. A view of part of Test 6 is shown in Figure 2.

Figure 2. Photograph of Test 6 taken on September 23, 1953. Rows 1, 2, and 3 are treatments 6, 8, and 5 of replicate 3.

Table 6. Field test with cotton seeds infested with isolates of R. solani from soil from several locations, used for several crops

Isolates Code	Source	Previous crop	Cotton seedlings surviving (after 8 days) Mean of 6 replications (%) Sto. 62-1-84	Yield (lint) Pounds/acre
1	Chickasha	Alfalfa	72.0	750
2	Perkins	"	66.8	683
3	Anadarko	Cotton	54.3	545
4	Chickasha	"	36.2	459
5	"	Corn	68.1	754
6	Newcastle	"	69.8	654
7	Perkins	Rye & vetch	72.0	677
8	"	" " "	30.5	439
9	No inoculum		72.7	678

L.S.D. (1%) = 11.6 (For seedlings only)

Stand counts from replications Sto. 62-1-84

Code	1	2	3	4	5	6	Mean
1	74	79	74	67	64	64	72.0
2	75	71	84	78	74	59	66.8
3	54	63	62	41	51	55	54.3
4	22	41	50	34	39	31	36.2
5	71	73	75	87	67	76	68.1
6	73	64	70	75	65	72	69.8
7	71	69	69	74	67	82	72.0
8	22	51	30	37	16	27	30.5
9	63	66	78	79	72	78	72.7

L.S.D. (1%) = 11.6

Analysis of variance of Table 6

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	53	17,086.0		
Replications	5	515.0	103.0	
Isolates	8	14,339.0	1,792.4	32.12**
Error	40	2,232.0	55.8	

Test 7. Greenhouse test of isolates of R. solani

The flats used in Test 7 were placed in the Agronomy Department's cotton testing laboratory. Each flat was filled with plant bands containing sterilized soil. The inoculum for each treatment consisted of 5 ml. of a hyphal suspension of a grain sorghum culture prepared by grinding 1 gm. of culture along with 100 ml. of water. Each treatment comprised 14 plant bands with 3 seeds being planted in each band.

The flats were inoculated, allowed to stand for 1 day, and then planted. The temperature in the laboratory was 78° F. and the humidity was 64% throughout the time the flats were there. The flats were removed after 7 days and placed outside along the north side of the greenhouse. The temperatures outsided ranged from 72° F. to 95° F.

Results. The data obtained from Test 7 are shown in Table 7. The differences between the various isolates were not significant. There was very little evidence of disease development in the test. This was probably due to the fact that lower temperatures could not be obtained.

Test 8. Greenhouse test of isolates of R. solani

Test 8 was essentially a repetition of Test 7. Two of the isolates were omitted and a stock culture of R. solani was included. The test was set up in crocks and these were placed in temperature-controlled boxes which are described

Table 7. Test of isolates of R. solani from soil from various locations used for cotton

Isolates Code	Source	Cotton seedlings surviving (after 10 days) Mean of 4 replications (%) Mebane 6801
1	Tipton	69.5
2	Caddo	88.5
3	Hobart	80.9
4	Temple	83.3
5	Webbers Falls	66.6
6	Checotah	79.7
7	Council Hill	51.6
8	No inoculum	90.4
	L.S.D. (5%) Not sig.	

	Stand counts from replications Mebane 6801				
Code	1	2	3	4	Mean
1	31	39	17	30	29.2
2	37	39	37	36	37.2
3	38	35	31	32	34.0
4	31	35	37	37	35.0
5	16	36	29	31	28.0
6	34	35	35	30	33.5
7	27	19	21	20	21.7
8	37	40	39	36	38.0
	L.S.D. (5%) Not sig.				

Analysis of variance of Table 7

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	31	1421		
Replications	3	78	26.0	
Treatments	7	592	84.6	2.37
Error	21	751	35.7	

in the following section. The inoculum for each crock consisted of 20 ml. of a suspension of a grain sorghum culture prepared by grinding 1 gm. of culture along with 100 ml. of water.

The crocks were inoculated and allowed to stand at room temperature for 1 day, and then planted with 40 seeds per crock. Then the crocks were placed in the constant temperature boxes and left for 3 days. After 3 days the crocks were moved to the cool section of the greenhouse. The temperature in the controlled boxes varied from 63° F. to 65° F., and the temperatures in the cool section of the greenhouse ranged from 68° F. to 95° F.

Results. The data obtained from Test 8 are shown in Table 8. The differences between the isolates were significant at the 1% level.

Tests to Show the Action of Several Soil Fungicides As Seed and Seedling Protectants Against Different Isolates of R. Solani

Tests were set up using a modification of the method proposed by Arndt (2) to evaluate chemicals as seed and seedling protectants. The general procedure was similar to the previous tests for the determination of the relative pathogenicity of isolates of R. solani except that fungicides were mixed with the soil around the seed. Pentachloronitrobenzene was used in Test 9, and thiram was used in Test 10.

Table 8. Test of isolates of R. solani from soil from various locations used for cotton

Isolates Code	Source	Cotton seedlings emerged (after 10 days) Mean of 4 replications (%) Mebane 6801
1	Tipton	5.0
2	Hobart	33.7
3	Temple	7.5
4/[a]	Webbers Falls	87.5
5	Checotah	57.5
6	Chicasha	26.7
7/[b]	No inoculum	93.0
	L.S.D. (1%) = 18.7	

	Stand counts from replications Mebane 6801				
	1	2	3	4	Mean
Code					
1	2	2	2	2	2.0
2	15	10	10	19	13.5
3	0	1	9	2	3.0
4/[a]	37	35	34	34	35.0
5	26	18	28	20	23.0
6	8	8	15	12	10.7
7/[b]	36	40	37	37	37.2
	L.S.D. (1%) = 7.5				

/[a] This treatment was omitted from the analysis because the isolate was non-pathogenic in this test.

/[b] This treatment was omitted from the analysis.

Analysis of variance of Table 8

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	19	1385.0		
Replications	3	64.6		
Treatments	4	1175.0	293.7	24.3**
Error	12	145.4	12.1	

The inoculum for these tests was whole grain, placed in the center of each pot. Four kernels were used to inoculate each pot. The pots were inoculated, and watered, and then allowed to stand at least 1 day before being planted. After being planted, the crocks were placed in temperature-controlled boxes which were maintained at 63 to 65° F. These controlled boxes consist of old refrigerators equipped with an adjustable heating unit. The boxes were placed in a large walk-in refrigerator that is operated at temperatures below 60° F., thus making it possible to maintain any temperature above 60° F. by means of the heating unit in each box.

In each of the tests the planted crocks were incubated in the temperature-controlled boxes for 3 days and then moved to the warm section of the greenhouse.

The technique used in planting the crocks was as follows: A wooden plate with 30 holes 12 mm. in diameter was placed on the surface of the soil in each crock. The holes in the plate were in concentric rings, 12 in each of the outer rings and 6 in the inner ring, plus four smaller holes 5 mm. in diameter near the center of the plate. A piece of glass tubing which was fixed so as to protrude through the plate 20 mm. was used to make 4 small holes near the center for inoculum. The plate was removed from the crock, and one grain of inoculum was placed in each of the 4

small holes. Then each hole was covered with sterile soil, and the crock was watered.

One day later the plate was again placed on the crock and the 30 large holes were made by using a piece of large glass tubing. The glass tubing was fixed so as to protrude through the plate 32 mm. The plate was then removed and the holes were filled half full with a soil and fungicide mixture, or plain sterile soil in the case of a non-treated check. One seed was placed in each hole, and then the hole was filled completely. The crock was then watered and placed in one of the temperature-controlled boxes.

Test 9. Greenhouse test using Mathieson 275 (PCNB) in the soil

The crocks used in Test 9 were inoculated and allowed to stand for 3 days at room temperature. The crocks were then planted with 30 seeds per crock, using a mixture of pentachloronitrobenzene and sterile soil around the seeds. After being planted, the crocks were placed in the temperature-controlled boxes for 4 days, and then moved to the warm section of the greenhouse. The temperature in the boxes was 65° F., and the temperatures in the greenhouse ranged from 70° F. to 92° F.

Results. The data obtained from Test 9 are shown in Table 9. The differences between the isolates in the presence of the fungicide were not significant. Since the differences between these isolates were significant in Test

Table 9. Test showing the action of pentachloronitrobenzene as a seed and seedling protectant against isolates of R. solani

Treatments /a Code	Source of isolate	Cotton seedlings emerged (after 12 days) Mean of 4 replications (%) Sto. 62-1-84
1	Tipton	81.5
2	Hobart	90.7
3	Temple	84.8
4	Webbers Falls	91.4
5	Checotah	85.8
6	No inoculum	90.7
	L.S.D. (5%) Not sig.	

	Stand counts from replications Sto. 62-1-84				
	1	2	3	4	Mean
Code					
1	24	27	25	23	24.7
2	28	26	28	28	27.5
3	26	27	25	25	25.7
4	27	28	28	28	27.7
5	22	28	27	27	26.0
6	28	28	28	26	27.5
	L.S.D. (5%) Not sig.				

/a Pentachloronitrobenzene (Mathieson 275, 50%) used at the rate of 1 part of active chemical to 20,000 parts of soil in all treatments.

Analysis of variance of Table 9

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	23	70		
Replications	3	8		
Treatments	5	29	5.8	2.02
Error	15	43	2.86	

8, this would indicate that pentachloronitrobenzene controlled all the isolates to the same degree. There was very little post-emergence damping-off and almost all seedlings examined did not have lesions at the soil line. However, in a number of the seedlings the primary root tips appeared infected. Some of these root tips were plated out on potato-destrose agar, and cultures of R. solani were obtained from them

Test 10. Greenhouse test using thiram in the soil

Test 10 was set up in the same manner as Test 9, except that thiram was used in place of pentachloronitrobenzene, and the stock culture of R. solani was used in place of one of the isolates. The temperature in the boxes was 64° F., and the temperatures in the greenhouse ranged from 68° F. to 92° F., except during one period of several hours when the temperature was over 100° F.

Results. The data obtained from Test 10 are shown in Table 10. The differences between the isolates in the presence of the fungicide were not significant at the 5% level, but there was a significant difference between the treatment without the fungicide and all the treatments with a fungicide. This would indicate that thiram did control all the isolates to the same degree, and was of value in protecting the seedlings. There was more post-emergence injury in Test 10 than in Test 9.

Table 10. Test showing the action of tetramethylthiuramidisulphide (thiram) as a seed and seedling protectant against isolates of R. solani

Treatments [a] Code	Source of isolate	Rate of thiram	Cotton seedlings emerged (after 10 days) Mean of 4 replications (%) Sto. 62-1-84
1	Tipton	1:20,000	51.8
2	Hobart	"	54.4
3	Temple	"	46.8
4	Checotah	"	54.4
5	Chickasha	"	61.7
6	Chickasha	None	26.4
	L.S.D. (5%) = 16.5		

	Stand counts from replications Sto. 62-1-84				
	1	2	3	4	Mean
Code					
1	11	12	18	22	15.7
2	17	15	19	15	16.5
3	12	14	14	17	14.2
4	16	16	17	17	16.5
5	15	22	18	20	18.7
6	6	8	8	10	8.0
	L.S.D. (5%) = 5.0				

[a] Tetramethylthiuramidisulphide (thiram) used at the rate of 1 part of active chemical to 20,000 parts of soil.

Analysis of variance of Table 10

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	23	415.0		
Replications	3	52.5		
Treatments	5	194.0	38.8	3.46*
Error	15	168.5	11.2	

Histological Studies to Determine The Action of R. Solani on Roots of Cotton Seedlings

Observations of cotton seedlings in tests showing the action of fungicides as seed and seedling protectants indicated that R. solani may cause a stunting of young seedlings. In Test 9 an excellent emergence was obtained when pentachloronitrobenzene was mixed with the soil around the seed. However, the seedlings were smaller in inoculated crocks than in non-inoculated checks. This is shown in Figure 3. The fungicide was used at the same rate in all 6 of the crocks. These crocks were observed for 3 weeks and the stunted seedlings did not increase in size more than 1/2, whereas the seedlings in the non-inoculated crock more than doubled their size. A study was made to determine the cause of this stunting.

Figure 3. Stunting of seedlings by R. solani. Crocks 1 through 5 were inoculated, 6 was non-inoculated.

When some of the stunted seedlings were pulled up, and examined, it was evident that only a very few of them had characteristic lesions in the hypocotyl area. Additional seedlings were removed from the soil by washing the soil from the roots with water so as to keep the entire root system intact. It was noted that the tip of the primary root was brown and shriveled in most of the seedlings. R. solani was obtained from these root tips in the previous tests. Since most of the primary roots were infected, and only a very few seedlings in all the crocks of Test 9 showed any stem lesions, it was assumed that the locus of the trouble was in the primary root.

Root tips of stunted seedlings were cut from the stem, and killed and fixed in Craf III (21) solution. Then they were processed through the dioxan series, and embedded in wax. Microtomed sections 14 to 16 microns in thickness were made of the root tips. Both cross and longitudinal sections were made using a rotary microtome. The sections were stained using various combinations of stains. The staining series that was most satisfactory consisted of iron hematoxylin, tannic acid and ferric chloride, safranin O, and fast green.

Photomicrographs were taken of a number of the slides using a phase microscope with a 15X wide field ocular. The objectives used were of 10X, 20X and 43X magnification. A Leitz Micam photomicrographic attachment and a Bausch and Lomb Model K photomicrographic camera were used to take the photomicrographs.

Results. It was found that the root tips contained fungus hyphae. Since the seedling were grown in sterilized soil infested with R. solani, it is evident that the fungus is R. solani. The tissues of the primary root were badly disorganized and shrunken in the area adjacent to the hyphae in many of the sections. An example of this is shown in Figure 4. Many of the root tips were so completely destroyed that only a thin outer layer of cells remained intact, leaving a hollow shell.

In the sections in which branching could be observed, the hyphae branched intercellularly in the parenchyma. This is shown in Figure 5. The hyphal strand seemed to grow longitudinally in the parenchyma tissue adjacent to a xylem element. After the hyphae had grown up the primary root for about 5 mm. it seemed to branch out laterally, growing radially from the center toward the outermost tissue of the root.

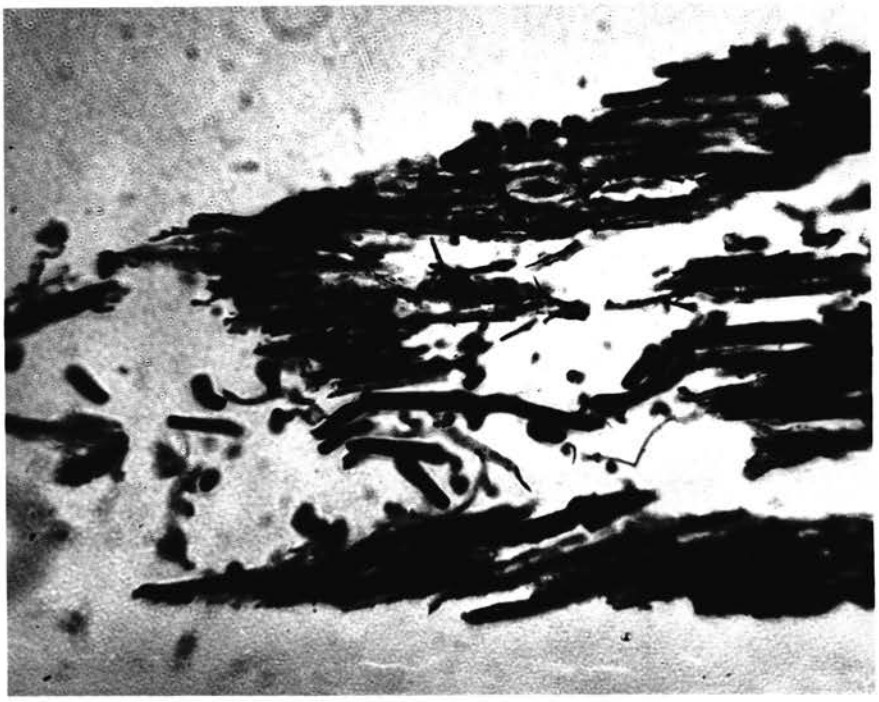

Figure 4. Longitudinal section of an infected primary root. (450X.)

Some of the sections showed that the hyphae of R. solani will destroy parenchyma tissue, and not affect xylem tissue to any extent. This is shown in Figure 6. The xylem

elements in this primary root were surrounded by fungus hyphae, and yet they were intact even after the adjacent parenchyma tissue was almost completely destroyed.

Figure 5. Longitudinal section of an infected primary root. The dark areas are composed of hyphal strands, and the broken fragment between the strands is the remains of a xylem vessel. (600X.)

Figure 6. Longitudinal section of an infected primary root. Fragments of hyphae are shown in the parenchyma tissue. (850X.)

It was noticed in a number of sections that considerable necrosis can be caused by R. solani even though the fungus has not been able to penetrate the root very extensively. This is shown in Figure 7. The darkened area appeared to be a reaction of the cotton tissue to the invading hyphae.

Figure 7. Longitudinal section of a primary root tip showing necrosis due to R. solani. (200X.)

Tests to Determine The Rates of Growth of Various Isolates of R. solani

A study was made to determine the relative rates of growth of 10 isolates of R. solani. The isolates were grown at temperatures ranging from 50° F. to 90° F. The isolates were grown on grain sorghum for several weeks, and then one kernel of grain was placed in the center of a Petri plate of potato-dextrose agar. The Petri plate cultures were placed in incubators or in the constant temperature boxes previously described, or in a refrigerator with a constant temperature unit. Measurements were made of the diameter of each colony up until one of the cultures in the series grew to the edge of the plate.

Information about the 10 isolates used in the growth rate studies is given in Table 11. The isolates were all obtained from diseased cotton seedlings except number 10 which was obtained from diseased sugar beets.

Table 11. Specifications on the isolates of R. solani used in the growth rate studies

Code	Source of isolate	Previous crop	Date isolated
1	Tipton	Cotton	October, 1952
2	Hobart	Cotton	October, 1952
3	Temple	Cotton	October, 1952
4	Checotah	Cotton	October, 1952
5	Chickasha	Cotton	May, 1950
6	Perkins	Alfalfa	January, 1953
7	Newcastle	Corn	January, 1953
8	Chickasha	Alfalfa	March, 1953
9	Perkins	Rye & vetch	March, 1953
10 [a]	Belle Fourche, S. Dakota	Sugar beet	August, 1951

[a] Isolate was obtained from Dr. R. H. Converse, Plant Pathologist, U.S.D.A. and Botany and Plant Pathology Dept., Okla. A. & M. College.

Results. The data from the growth rate studies are presented in Figure 8. The optimum temperature for growth for all the isolates seemed to be near 80° F. There was less variation in the rate of growth between the isolates from cotton fields (1 through 5) than between the isolates from fields used for other crops (6 through 10). Isolate 10 showed a slower rate of growth than any of the other isolates at all temperatures but 90° F. Isolates 8 and 10 both seemed to be favored by higher temperatures, and retarded by lower temperatures.

Isolate 10 seemed to vary from the average rate of growth more than any other isolate, and also had a much more sparse growth of mycelium. This is shown in Figure 9. Isolate 10 also showed a varied type of sclerotia production,

being less compact than the other types. There was a great deal of variation in the size and structure of sclerotia produced by the various isolates. This is shown in Figure 10.

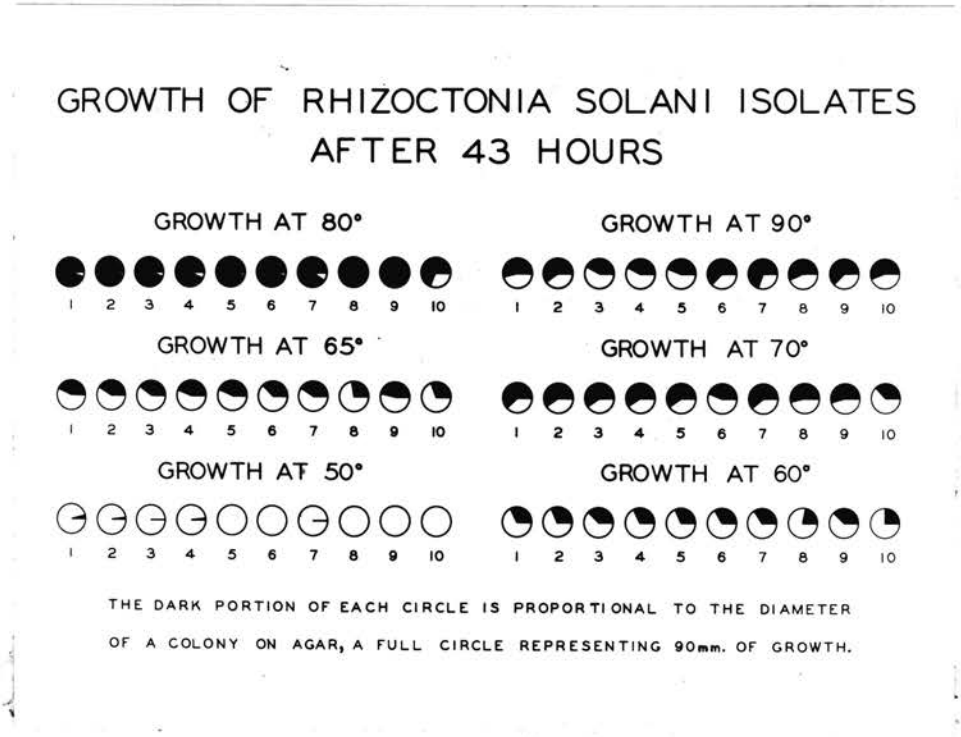

Figure 8. Measurements of the growth of 10 isolates of R. solani at various temperatures at the time that one or more of the isolates grew to the edge of its dish.

Isolate 10 is the only isolate that was found to be non-pathogenic to cotton seedlings. The hyphae of isolate 10 were able to penetrate the hypocotyl of cotton seedlings, but no damping-off was obtained under conditions favorable for post emergence injury. Variability in pathogenicity of the other 9 isolates is shown in Tests 2, 5, 6, 7, and 8.

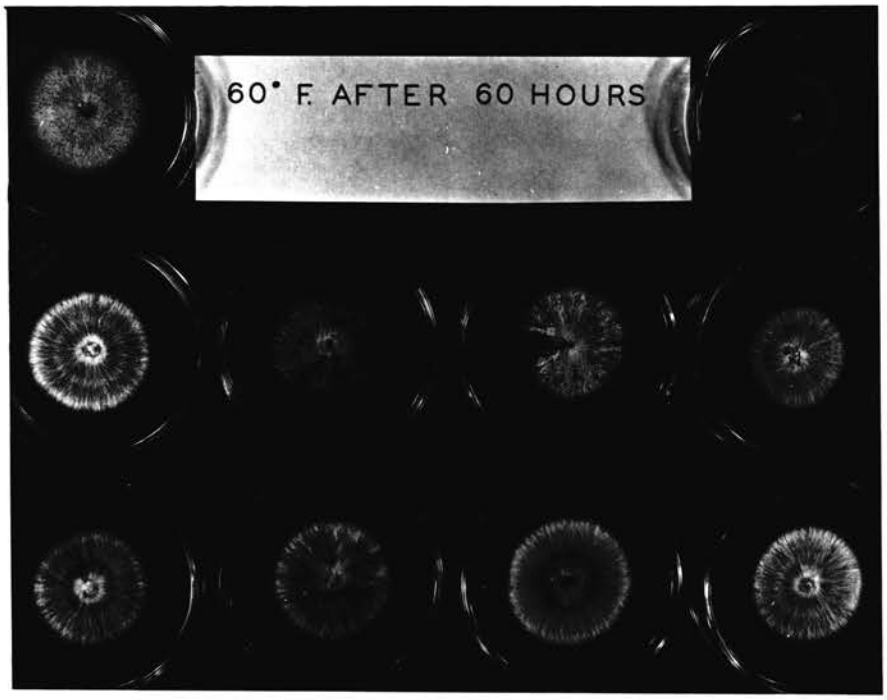

Figure 9. Growth of 10 isolate of R. solani at 60° F. after 60 hours. Isolates are in order, starting with number 1 in the lower left corner, and going from left to right, ending with number 10 in the upper right corner.

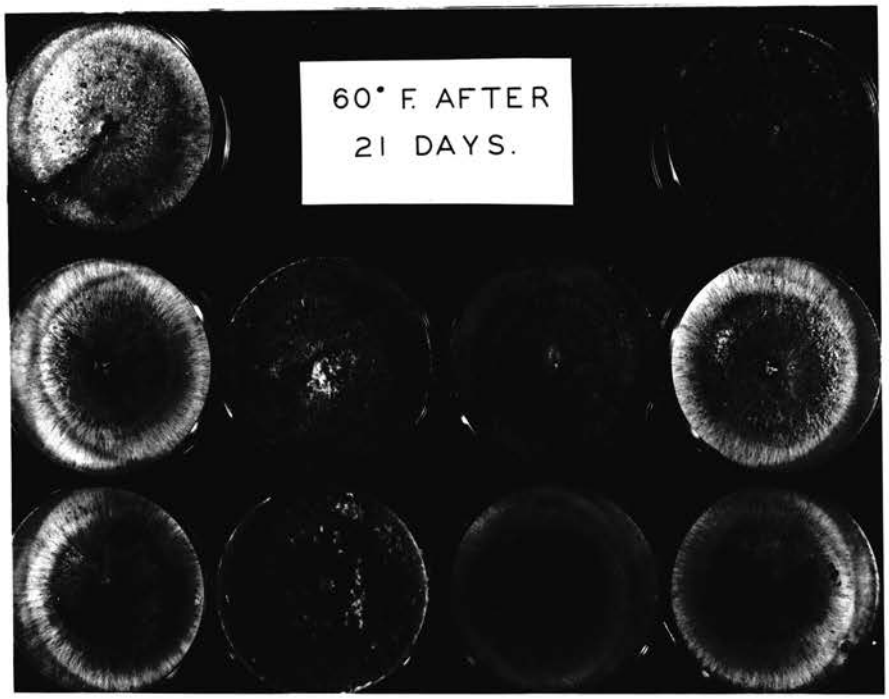

Figure 10. Growth of 10 isolates of R. solani at 60° F. after 21 days showing varied types of sclerotia. Isolates are in order, starting with number 1 in the lower left corner, and going from left to right, ending with number 10 in the upper right corner.

DISCUSSION

Studies of the seedling disease potential in samples of field soil from various parts of Oklahoma substantiates the work of previous investigators who found that R. solani was very widespread in this area. In fact, R. solani was isolated from infected cotton seedlings grown in all samples of soil, provided that the environmental conditions were favorable for infection. There is the possibility of R. solani being spread by aerial means, but it is more likely to be present in each soil sample than to be spread from one sample to the next in the greenhouse. About 3/4 of all the cultures obtained from diseased cotton seedlings were R. solani.

The isolates tested in these studies showed a great deal of variation in pathogenicity. The significance of this variation is shown in differences in yields obtained in a replicated field test in which the seeds were inoculated with different isolates of R. solani. Yields varied as much as 300 pounds per acre between treatments in this test. A reduction in yield of cotton at the end of the growing season is the most important consequence, economically, of seedling diseases.

There was essentially no difference between the reaction of the various isolates tested to the different

varieties of cotton used. The variety that was most susceptible to one isolate seemed to be most susceptible to all the isolates. This knowledge would be essential to anyone who might be working on a program of breeding for resistance to seedling diseases.

There was very little difference in the reaction the various isolates to chemical treatments in the soil. Although the various isolates showed much variation in pathogenicity, they all seemed to react alike as far as tolerance to chemicals was concerned. Further work along this line would be an aid in evaluating new fungicides. If there are differences in the reaction of isolates of R. solani to any fungicides, this should be known before the fungicide is used commercially.

Previous investigators have considered the hypocotyl region between the original seed level and the soil line as the susceptible region for attack by R. solani. The present studies have indicated that cotton seedlings can be stunted due to infection of the primary root by R. solani. Further work to determine the relative importance of this stunting would be valuable. The relation of infection of the primary root to the type of root system that is developed by the plant is of especial importance. If infection of the primary root is of importance, consideration should be given to this fact when protectants are used with cotton seedlings so that all of the susceptible region of the plant is protected.

SUMMARY

Studies using field soil samples indicated that cotton seed and seedling disease pathogens were present in all fields from which samples were collected. This study included representatives of two major cotton producing areas of the state, and also included fields used for the following crops: cotton, corn, alfalfa, wheat, virgin prairie, pasture, grain sorghum, and rye and vetch.

Tests of the relative pathogenicity of a number of ioslates indicated that there is a great deal of pathogenic variation of R. solani within Oklahoma. Although there were highly significant differences in pathogenicity between isolates obtained from infected cotton seedlings, the more virulent isolates did not seem to be from any certain location or from fields used for any one crop.

Preliminary tests involving the tolerance of isolates of R. solani to soil fungicides indicated that isolates showed little difference in pathogenicity in the presence of a seed and seedling protectant. Pentachloronitrobenzene was more effective against the isolates used than thiram.

Histological studies of cotton roots indicated that R. solani can infect the primary root and cause considerable damage to the tissues. Cotton seedlings with chemical

protectants were found to be stunted by the disease although they were free of lesions at the soil line. This may be one of the factors that reduces the benefit of seed and seedling protectants.

Studies of cultural variation of R. solani showed that the cultures used varied in growth rate, density of growth, and type of sclerotia produced. The isolates all seemed to have about the same optimum temperature for growth, but all isolates showed a great deal of variation at all temperatures.

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