ISOLATION AND INFECTION TESTS WITH

SEED-BORNE COTTON PATHOGENS

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By

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J. H. Mc.

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INTRODUCTION

Cotton is the most important crop in the Southern States. Throughout the area where cotton is grown the plants are attacked by various organisms causing plant disease and crop losses. Many of these organisms are known to live in the soil from year to year while others are seed-borne and are reintroduced into the soil each year with the cotton seed. These organisms are frequently so destructive in the germinating and seedling stages of the cotton plant that it is not uncommon for the cotton grower to use a seeding rate 10 or more times in excess of the expected stand.[7]

Seedling diseases are important to the cotton grower because of: (a) poor stands, (b) necessity of replanting, (c) decrease in production because of frequent skips in rows, (d) excess amount of cotton seed required, (e) the many seedling diseases which later develop into lint- and boll-destroying diseases, (f) the lessened value and amount of lint cotton produced per unit area, (g) weakening of the plants, making it much easier for secondary organisms to infect the cotton.

Damping off and other seedling diseases of the cotton plant may be caused and accelerated by a number of organisms. Arndt[2] in a study of the etiology of damping off of cotton seedlings, in South Carolina in 1935 isolated the following organisms from diseased seedlings: Glomerella gossypii, Fusarium sp., Pythium ultimum, Rhizoctonia sp., bacteria of various types, and several species of nematodes. In 1937 Arndt[3] found that the presence of the nematodes increased stunting of the plants by Fusarium moniliforme;

that typical damping off was produced by Glomerella gossypii; and that the presence of Fusarium sp. reduced germination and produced lesions on the hypocotyl but did not cause typical damping off. Lehman[10] tested 35 seed lots of the 1936 cotton crop from 19 counties in North Carolina. He found that 81 per cent of the seed germinated; 40 per cent of the seed in 31 of the lots were infected with Glomerella gossypii and 31.4 per cent of the seed of all lots were infected with various species of Fusarium among which F. moniliforme was frequently recognized. Shaw[15] also reporting on cotton diseases from the 1936 North Carolina crop, found F. vasinfectum present in the soils of an extremely large number of farms. Shaw stated that for the season of 1936, damping off caused a reduction in yield of approximately 133,650,000 pounds of seed cotton valued at $7,056,720. Camp et. al.[6] report from a survey in Florida the diseases most common during the first 2 months of the season to be sore shin caused by several organisms particularly Rhizoctonia solani; angular leaf spot (Bacterium malvacearum); and wilt(Fusarium vasinfectum). They also found a large proportion of Fusarium moniliforme to be present.

Miller[12] collected and received samples (25 to 50 seedlings per sample) of the 1938 cotton plantings from Virginia, North Carolina, South Carolina, Georgia, Alabama, Mississippi, Louisiana, Texas and Tennessee. These samples were cultured and the fungi associated with the diseased seedlings identified by Ullstrup of the South Carolina Experiment Station. Of the 344 cultures examined, Glomerella gossypii was found 283 times; Fusarium moniliforme, 239 times; Rhizoctonia solani, 44 times; Fusarium sp. 161 times; Pythium

sp. 15 times; Diplodia gossypina, 11 times; Rhizoctonia (Sclerotium) bataticola, 15 times; Fusarium vasinfectum, 4 times; Sclerotium rolfsii, 3 times and Aspergillus sp., once. Glomerella gossypii was found to be the predominant organism in 240 culturings; Fusarium moniliforme, 14 culturings; Rhizoctonia solani, 11 culturings and the others (organisms as listed above) 79 culturings. Glomerella gossypii was found to be the predominant organism in culturings from every state except Texas. These survey data are important because they show the predominance of G. gossypii throughout the Cotton Belt east of the Mississippi River reaching an apparent westward limit in Texas.

Miller and Weindling[13] collected and received samples of the 1939 cotton plantings from Alabama, Arkansas, Georgia, Kentucky, Louisiana, Mississippi, Missouri, North Carolina, Oklahoma, South Carolina, Tennessee, Texas and Virginia. A total of 462 samples were cultured of which 413 (89.4 per cent) yielded Glomerella gossypii; 407 (88.1 per cent) Fusarium moniliforme; 61 (13.2 per cent) Rhizoctonia solani; 191 (41.3 per cent) Fusarium sp. (of which F. vasinfectum made up 70 to 80 per cent); 21 (4.5 per cent) Rhizoctonia (Sclerotium) bataticola; 10 (2.2 per cent) Diplodia gossypina; and 256 (55.4 per cent) other fungi including Alternaria sp.; Penicillium sp.; Aspergillus sp. and other unidentified species. Here again G. gossypii is the predominant organism east of the Mississippi River and absent in Texas and Oklahoma.

The foregoing references give ample proof of the presence, variety, and destructiveness of seed-borne organisms of cotton.

Table I. Fungi causing cotton seedling diseases ranked according to predominance in the samples examined.

State	Predominance of Organism			
	Rank I	Rank II	Rank III	Rank IV
Alabama	G.g.	F.m.	Rh.s	F.sp.
Arkansas	G.g.	F.m.	F.sp.	Rh.s.
Georgia	G.g.	F.m.	F.sp.	Rh.s.
Kentucky	G.g.	F.m.	F.sp.	---
Louisiana	G.g.	F.m.	F.sp.	Rh.s.
Mississippi	G.g.	F.m.	F.sp.	Rh.s.
Missouri	G.g.	F.m.	F.sp.	---
North Carolina	G.g.	F.m.	F.sp.	Rh.bat.
South Carolina	G.g.	F.m.	F.sp.	Rh.s.
Tennessee	G.g.	F.m.	F.sp.	---
Virginia	G.g.	F.m.	F.sp.	Rh.bat.
Oklahoma	F.m.	F.sp.	Rh.bat.	Dip.gos.
Texas	F.sp.	---	---	---

Abbreviations: G.g.: Glomerella gossypii; F.m.: Fusarium moniliforme F.sp.: Fusarium species; Rh.s.: Rhizoctonia solani; Rh. bat. Rhizoctonia (Sclerotium) bataticola; Dip.gos.: Diplodia gossypina. Arranged from data given by Miller[15].

Examination of Table 1 shows G. gossypii to be the predominant organism causing seedling disease in all of the states sampled except the two mentioned. The absence of G. gossypii from Oklahoma and Texas may be explained by the fact that moisture in the soil gradually becomes less as one comes westward from the Mississippi River. As regards the presence of G. gossypii in Oklahoma, Gordon[9] in 1937 reported that surveys in 1929 showed heavy infestation in Pittsburg county and light infestation in 1931 and 1932, while surveys in 1935 and 1937 showed no infestation in that county.

Almost without exception the above investigators have either stated that the pathogenicity of the organisms encountered was not fully known or that there were questions as to their pathogenicity. Obviously, when such a list of organisms is encountered so closely related to the problem of damping off and seedling diseases of

cotton it is desirable that information be available on the specific pathogenicity of these organisms in their relationship to the seedling cotton plants. The purpose of this paper is to present the results obtained by the writer in soil inoculation experiments with many of these organisms.

MATERIALS AND METHODS

The cotton seed used in this experiment consisted of three varieties namely: Oklahoma Acala seed from the 1938 crop grown in Oklahoma; Texas Paymaster seed from the 1939 crop grown in Texas; and D. & P. L. 11A seed grown near Hattiesburg, Mississippi in 1938. The Oklahoma Acala and Texas Paymaster seed were portions of the seed used in the uniform seed treatment tests of the Cotton Disease Council (Test A) in 1938.

Experiments were conducted to test the pathogenicity of the various organisms by: (a) inoculation of the organisms into sterile soil and subsequent planting of sterile cotton seed, and (b) inoculating cotton seedlings grown in test tubes on water agar. It was planned to grow the plants in the soil under as many varying conditions of soil acidity, temperature, moisture, and light as feasible.

The cotton seed were delinted with concentrated sulphuric acid and graded to remove internally infected seed, then surface sterilized by immersing in 10 per cent calcium hypochlorite for a period of 5 minutes immediately before planting in the soil. The seed were planted in soil which consisted of 2 parts loam,

1 part sand and 1 part sewer sludge. The soil was thoroughly mixed, screened to remove foreign material, and sterilized in an autoclave at 15 pounds pressure for 50 minutes. The soil was placed in 2¼ inch clay pots, each of which had been sterilized by immersing in boiling water for a period of 5 minutes. The inoculum, young cultures of the organisms to be tested, was used at the rate of 5 tubes (approximately 50 cc. of agar) per 20 pots of soil. The organisms were cultured on potato dextrose agar for a period of 10 days to 2 weeks. The organisms were removed from the tubes with sterile instruments and mixed with clean sterile sand in a mortar. The fungus-sand mixture was then added to the proper amount of sterile soil. Care was exercised to keep water which might contaminate the cultures from being splashed on the growing plants. Water was boiled, then cooled before being used to water any of the pots.

The organisms used in the experiments are listed in Table II.

Table II. List of organisms used in soil and test tube inoculation experiments.

Culture No.	Organism	Isolated by	Location	Isolated from	Date	Identified by
18	Fusarium moniliforme Sheld.	C.H. Arndt	S.Car.	cotton seed	1938	C.H. Arndt
115	Fusarium moniliforme Sheld.	C.H. Arndt	S. Car.	cotton seed	1938	C.H. Arndt
24	Fusarium vasinfectum Atk.	W.W. Ray	Okla.	cotton stem	1939	W.W. Ray
27	Fusarium vasinfectum Atk.	W.W. Ray	Okla.	cotton stem	1939	W.W. Ray
34	Fusarium semitectum Berk. et Rav.	K. Starr Chester	Okla.	cotton seed	1938	C.D. Sherbakoff
36	Fusarium solani Mart. v. suffuscum Sherb.	K. Starr Chester	Okla.	cotton seed	1938	C.D. Sherbakoff
38	Fusarium chlamydosporum Wr. et Rg.(?)	K. Starr Chester	Okla.	cotton seed	1938	C.D. Sherbakoff
42	Fusarium bullatum Sherb. v. minus Wr. et Rg.	K. Starr Chester	Okla.	cotton seed	1938	C.D. Sherbakoff
44A	Fusarium scirpi Lamb. et Fautr.	K. Starr Chester	Okla.	cotton seed	1938	C.D. Sherbakoff
D	Fusarium scirpi Lamb. et Fautr.	J. Harvey McLaughlin	Okla.	cotton seed	1940	J. Harvey McLaughlin
B	Fusarium scirpi Lamb. et Fautr. v. acuminatum (Ell. & Ev.) Wr.	J. Harvey McLaughlin	Okla.	cotton seed	1940	J. Harvey McLaughlin
20	Glomerella gossypii (South) Edg.	C.H. Arndt	S.Car.	cotton seed	1938	C.H. Arndt
R37	Sclerotium bataticola Taub.	W.W. Ray	Okla.	cotton root	1939	W.W. Ray
41	Sclerotium bataticola Taub.	G.Tennyson	Okla.	cotton root	1939	G. Tennyson
7	Rhizoctonia solani Kühn	J. Harvey McLaughlin	Okla.	potato tuber	1940	J. Harvey McLaughlin
R	Rhizoctonia solani Kühn	David Dunn	Okla.	cowpea stem	1940	David Dunn
61	Alternaria species	W.W. Ray	Okla.	cotton seed (external)	1939	W.W. Ray
P	Penicillium species	J. Harvey McLaughlin	Okla.	cotton seed (external)	1940	J. Harvey McLaughlin
A	Aspergillus niger v. Tieg.	J. Harvey McLaughlin	Okla.	cotton seed	1940	J. Harvey McLaughlin

EXPERIMENTAL RESULTS

Experiment I

One hundred sixty pots of soil were inoculated with the following organisms, as indicated:

Culture No.	Organism	No. Pots
18	Fusarium moniliforme	20
20	Glomerella gossypii	20
24	Fusarium vasinfectum	20
27	Fusarium vasinfectum	20
115	Fusarium moniliforme	20
R37	Sclerotium batatícola	20
C I	Uninoculated check	20
CII	Uninoculated check	20

Each pot was planted with 6 surface-sterilized Oklahoma Acala seed of the 1938 crop. After seedling emergence the plants were thinned to 3 plants per pot. Ten pots inoculated with each fungus were placed in the laboratory by a window with southern exposure. The remaining 10 pots of each series were placed in a greenhouse where the temperature was usually between 70° and 80°F. The plants were watered daily with boiled water. At the termination of 22 days each individual plant was removed from its respective pot and examined for evidence of fungal injury. Three degrees of injury were recognized: "S",--slight injury, no outstanding evidence of disease was observable yet the root systems or hypocotyls were visibly attacked; "H",--heavy infection with marked damage to the roots or hypocotyl, lesions very pronounced in most cases, very evident dark discolorations, growth of plant visibly affected and retarded, and "M",--moderate injury, roots or hypocotyl obviously attacked but apparently only slight injurious effects suffered by the plants. Plants which showed no injury or attack whatever were scored "N". These degrees of attack and injury are used throughout the remainder of this paper. The results of this experiment are summarized in Table III.

Table III. Tabulated results of experiment I showing percentage infection by each organism and severity of infection. Symbols are as indicated on page 8.

Organism	Total No. Pots	Total No. Plants	Total %age Infected	Total % age "H"	Total % age "M"	Total % age "S"	Total % age "N"
Laboratory tests							
No. 18 Fusarium moniliforme	10	29	24.1	0	10.3	13.7	75.8
No. 20 Glomerella gossypii	10	30	96.6	83.3	13.3	0	3.4
No. 24 Fusarium vasinfectum	10	29	37.9	0	6.9	31.	62.
No. 27 Fusarium vasinfectum	10	30	33.3	0	3.3	30.	66.6
No.115 Fusarium moniliforme	10	30	33.3	0	0	33.3	66.6
No.R37 Sclerotium bataticola	10	30	20.	0	0	20.	80.
No. CI Uninoculated check	10	30	0	0	0	0	100.
No.CII Uninoculated check	10	30	6.6	0	0	6.6	93.3
Greenhouse tests							
No. 18 Fusarium moniliforme	10	30	83.	0	20.	63.3	16.6
No. 20 Glomerella gossypii	10	30	100.	66.6	20.	13.3	0
No. 24 Fusarium vasinfectum	10	30	40.	0	20.	20.	60.
No. 27 Fusarium vasinfectum	10	30	53.3	6.6	3.3	43.3	46.6
No.115 Fusarium moniliforme	10	30	60.	10.	16.6	33.3	40.
No.R37 Sclerotium bataticola	10	30	40	0	0	40.	60.
No. CI Uninoculated check	10	30	13.3	0	0	13.3	86.6
No.CII Uninoculated check	10	30	23.3	0	3.3	20.	76.6

The root systems of the plants grown in the laboratory were very sparse with few lateral roots whereas the plants grown in the greenhouse were almost root-bound and with normal growth of lateral roots.

The above ground portions of the greenhouse plants were well developed. The results with the plants in the laboratory indicate that Glomerella gossypii is a severe pathogen on cotton seedlings and that the other organisms are only slightly pathogenic under the conditions of the experiment. The results with the greenhouse plants also show Glomerella gossypii to be a very severe pathogen. Fusarium moniliforme attacked quite generally in the greenhouse but with only slight severity.

Experiment II

Experiment II was set up to determine the pathogenicity of the organisms already tested, at cooler temperatures than usual for cotton. The arrangement was the same as in experiment I, with 3 additional organisms added to the list being tested, as may be noted in Table IV. One-half of each series was placed in the laboratory by a window with southern exposure and the remaining half of each series was placed in a cool portion of the greenhouse where the temperature varied very little and averaged about 65°F. The results are given in Table IV.

Table IV. Tabulated results of experiment II showing percentage infection by each organism and severity of infection
Symbols are as indicated on page 8.

Organism	Total No. Pots	Total No. Plants	Total %age Infected	Total % age "H"	Total % age "M"	Total % age "S"	Total % age "N"
Laboratory tests							
No. 18 Fusarium moniliforme	10	30	86.6	23.3	23.3	40.	13.3
No. 20 Glomerella gossypii	10	31	100.	77.4	22.6	0	0
No. 24 Fusarium vasinfectum	10	32	81.2	21.9	31.2	28.1	18.7
No. 27 Fusarium vasinfectum	10	29	89.6	20.7	27.5	41.3	10.3
No.115 Fusarium moniliforme	10	31	100.	16.1	41.9	41.9	0
No.R37 Sclerotium bataticola	10	31	93.3	13.3	26.6	53.3	6.6
No. A Aspergillus species	10	29	82.7	24.1	27.5	31.1	17.3
No. P Penicillium species	10	30	83.3	20.	20.	43.3	16.6
No. 7 Rhizoctonia solani	10	26	92.3	23.	26.9	42.3	7.7
No. C Uninoculated check	10	30	26.6	6.6	6.6	13.3	73.3
Greenhouse tests							
No. 18 Fusarium moniliforme	10	28	75.4	14.3	17.9	42.9	25.
No. 20 Glomerella gossypii	10	30	100.	93.3	3.3	3.3	0
No. 24 Fusarium vasinfectum	10	32	96.8	15.6	21.8	59.3	3.1
No. 27 Fusarium vasinfectum	10	30	96.6	6.6	43.3	46.6	3.3
No.115 Fusarium moniliforme	10	30	100.	16.6	40.	43.3	0
No.R37 Sclerotium bataticola	10	32	81.2	21.9	12.5	46.8	18.7
No. A Aspergillus species	10	30	83.3	3.3	13.3	66.6	16.6
No. P Penicillium species	10	30	93.3	6.6	16.6	70.	6.6
No. 7 Rhizoctonia solani	10	31	80.6	6.4	16.1	58.	19.3
No. C Uninoculated check	10	31	16.1	0	0	16.1	83.8

These plants were grown in the pots for a period of 28 days before examining. Examination revealed that practically all the plants both in the greenhouse and laboratory were becoming root-bound. The plants growing in soil infected with Glomerella gossypii were the only ones to show above-ground effects of disease. All other plants appeared externally healthy. These results again show that G. gossypii is a serious plant pathogen on cotton. It may be observed that all organisms used in the experiment showed general attack and infection, however, with the exception of G. gossypii, the major portion of the attacks were classed as slight. These plants although infected, showed as good growth as did the check plants.

Experiment III

Experiment III was arranged to determine the effect of constant light and temperature upon the pathogenicity of the organisms. The same group of organisms were used in this experiment as in experiment II. One-half of each series was placed in a warm room of the greenhouse. The other half of each series was placed in a windowless room with two doors which were kept closed. Two 300-watt electric lights were so placed as to throw continuous light upon the plants from a distance of approximately 3 feet. An overhead light was also kept burning continuously. Thus light was constant and continuous and the temperature varied between 73° and 78°F. The results are given in Table V.

Table V. Tabulated results of experiment III showing percentage infection by each organism and severity of infection. Plants in laboratory grown under continuous light. Symbols are as indicated on page 8.

Organism	Total No. Pots	Total No. Plants	Total % age Infected	Total % age "H"	Total % age "M"	Total % age "S"	Total % age "N"
Laboratory tests							
No. 18 Fusarium moniliforme	10	30	46.6	0	3.3	43.3	53.3
No. 20 Glomerella gossypii	10	30	100.	100.	0	0	0
No. 24 Fusarium vasinfectum	10	30	53.3	3.3	10.	40.	46.6
No. 27 Fusarium vasinfectum	10	30	46.6	0	3.3	43.3	53.3
No.115 Fusarium moniliforme	10	30	70.	3.3	10.	56.6	30.
No.R37 Sclerotium batatícola	10	30	70.	0	6.6	63.3	30.
No. A Aspergillus species	10	30	16.6	3.3	0	13.3	83.3
No. P Penicillium species	10	29	10.3	0	0	10.3	89.6
No. 7 Rhizoctonia solani	10	30	50.	3.3	13.3	33.3	50.
No. C Uninoculated check	10	30	6.6	3.3	0	3.3	93.3
Greenhouse tests							
No. 18 Fusarium moniliforme	10	30	93.3	0	20.	73.3	6.6
No. 20 Glomerella gossypii	10	29	100.	100.	0	0	0
No. 24 Fusarium vasinfectum	10	31	90.3	16.1	0	74.2	9.3
No.R37 Sclerotium batatícola	10	29	79.3	10.	10.	58.6	20.6
No. A Aspergillus species	10	30	40.	0	0	40.	60.
No. P Penicillium species	10	30	30.	3.3	3.3	23.3	70.
No. 7 Rhizoctonia solani	10	30	53.3	3.3	10.	40.	46.6
No. C Uninoculated check	10	30	0	0	0	0	100.

These plants were grown in the pots for a period of 14 days at which time they were examined. All plants growing in soil inoculated with Glomerella gossypii were severely injured. The root systems of all other plants were becoming root-bound, however all plants except those infected by G. gossypii were apparently healthy. The tops of the plants grown in continuous light were 6 to 7 inches in height, the other plants averaged 3 to 4 inches. The results of this experiment again show that G. gossypii is a severe, killing cotton seedling pathogen. The remaining organisms caused very little damage under continuous light; in no case causing more than slight injury. However, Fusarium moniliforme, No. 18 and F. vasinfectum, No. 24, caused approximately 75% slight infection respectively although the only apparent above-ground damage was a slight stunting of the growing plants.

Experiment IV

Experiment IV was arranged to test the pathogenicity of the following additional organisms:

Culture No.	Organism	No. Pots
34	Fusarium semitectum	20
36	F. solani v. suffuscum	20
38	F. chlamydosporum	20
42	F. bullatum v. minus	20
44A	F. scirpi	20
61	Alternaria species	20
B	F. scirpi v. acuminatum	20
D	F. scirpi	20
C	Uninoculated check	20

Observations in the previous experiments disclosed the fact that the root systems were growing out through the small opening in the bottom of the pot and were thus exposed to attack from all organisms in the neighborhood of the pot. To eliminate this factor each clay

pot of this and succeeding experiments was placed in a paper pot which had no opening in the bottom. Table II shows the origin of the above cultures, all of which were isolated from the internal structures of cotton seed except Alternaria sp. Ten pots of each of the series were placed under the constant light and controlled temperature conditions and the remaining ten pots of each series were placed in a cool portion of the greenhouse. The results were as indicated in Table V.I.

Table VI. Tabulated results of experiment IV showing percentage infection by each organism and severity of infection. Plants in laboratory grown under continuous light. Symbols are as indicated on page 8.

Organism	Total No. Pots	Total No. Plants	Total % age Infected	Total % age "H"	Total % age "M"	Total % age "S"	Total % age "N"
Laboratory tests							
No. 34 Fusarium semitectum	10	30	100.	23.3	43.3	33.3	0
No. 36 F. solani v. suffuscum	10	29	100.	51.7	31.	17.2	0
No. 38 F. chlamydosporum	10	29	100.	72.4	24.1	3.4	0
No. 42 F. bullatum v. minus	10	28	96.4	82.1	10.7	3.5	3.5
No. 44A F. scirpi	10	30	76.6	20.	23.3	33.3	23.3
No. 61 Alternaria species	10	31	74.1	12.8	9.6	51.6	25.8
No. B F. scirpi v. acuminatum	10	30	100.	90.	10.	0	0
No. D F. scirpi	10	30	100.	86.6	10.	3.3	0
No. C Uninoculated check	10	30	33.3	6.6	10.	16.6	66.6
Greenhouse tests							
No. 34 Fusarium semitectum	10	30	96.6	10.	26.6	63.3	3.3
No. 36 F. solani v. suffuscum	10	30	80.	0	20.	60.	20.
No. 38 F. chlamydosporum	10	31	90.3	45.1	12.9	32.2	9.6
No. 42 F. bullatum v. minus	10	28	64.2	21.4	14.2	28.5	35.7
No. 44A F. scirpi	10	31	100.	32.2	29.	38.7	0
No. 61 Alternaria species	10	30	86.6	20.	16.6	50.	13.3
No. B F. scirpi v. acuminatum	10	30	100.	56.6	40.	3.3	0
No. D F. scirpi	10	31	100.	67.7	29.	3.2	0
No. C. Uninoculated check	10	30	43.3	13.3	10.	20.	56.6

Examination of the plants grown under constant light brought out the following facts:

Plants growing in soil inoculated with Fusarium solani v. suffuscum, F. chlamydosporum, F. bullatum v. minus and F. scirpi (culture D) were stunted with severely infected roots.

Plants growing in soil inoculated with Fusarium scirpi,(culture 44A), F. semitectum and Alternaria species showed some evidence of slight stunting, however, the plants in general were nearly normal.

Plants growing in soil inoculated with Fusarium scirpi v. acuminatum were slightly stunted with good above-ground development. The roots, however, were severely affected just at and slightly above the ground line. This infection was apparently the most severe of the group tested although the plants gave little evidence of being harmed at the time of examination. It is believed that these plants would have died as soon as the supply of food in the cotyledonary leaves was exhausted.

The check plants were in an apparently healthy condition.

These results indicate that a number of Fusarium species found on and in cotton seed may cause serious losses to the cotton seedlings under favorable conditions.

The results obtained in this experiment with cultures 44A (Fusarium scirpi) and D (Fusarium scirpi) indicate considerable variation in virulence in this species of Fusarium. A similar situation is seen in the results obtained in experiments I, II, and III with cultures 18 and 115 (Fusarium moniliforme) and also 24 and 27 (Fusarium vasinfectum)

Experiment V

Experiment V was arranged to obtain further data on the organisms used in experiment IV. Ten pots of soil inoculated with each organism were placed in the laboratory by a window with a southern exposure, the remaining 10 pots of soil inoculated with the organisms were placed in a cool part of the greenhouse. The plants were removed and examined at the end of 20 days.

In this experiment a poor stand was obtained. An average of 11.6 per cent of the total number of seed planted germinated and grew with the roots extending upward; the tip of the roots apparently injured by a fungus in each instance (root tip brown and with a damping off appearance). The plants soon wilted, due to the lack of water and minerals from the soil.

Examination revealed that 50.8 per cent of the total number of seed planted failed to germinate and were decayed. Comparable results were obtained in germination tests of these seed on water agar in which case 63 per cent of the seed failed to germinate and were internally decayed although not yielding pathogenic fungi nor bacteria.

The results of this experiment are given in Table VII which show that 100 per cent infection was the general rule with the major portion of the infections classed as slight.

Table VII. Tabulated results of experiment V showing percentage infection by each organism and severity of infection. Symbols are as indicated on page 8.

Organism	Total No. Pots	Total No. Plants	Total % age Infected	Total % age "H"	Total % age "M"	Total %age "S"	Total % age "N"
Laboratory tests							
No. 34 Fusarium semitectum	10	24	100.	8.3	8.3	83.3	0
No. 36 F. solani v. suffuscum	10	7	100.	0	28.5	71.4	0
No. 38 F. chlamydosporum	10	8	100.	25.	12.5	62.5	0
No. 42 F. bullatum v. minus	10	4	100.	0	0	100.	0
No. 44A F. scirpi	10	25	100.	4.	32.	64.	0
No. 61 Alternaria species	10	28	100.	14.2	42.8	42.8	0
No. B F. scirpi v. acuminatum	10	15	100.	6.6	20.	73.3	0
No. D F. scirpi	10	23	100.	13.	34.3	52.1	0
No. C Uninoculated check	10	30	36.6	9.9	3.3	23.3	63.3
Greenhouse tests							
No. 34 Fusarium semitectum	10	24	100.	8.3	16.6	75.	0
No. 36 F. solani v. suffuscum	10	12	100.	33.3	33.3	33.3	0
No. 38 F. chlamydosporum	10	14	100.	21.4	0	78.5	0
No. 42 F. bullatum v. minus	10	7	85.7	28.5	14.3	42.8	14.3
No. 44A F. scirpi	10	18	100.	38.8	22.2	38.8	0
No. 61 Alternaria species	10	26	61.5	0	23.1	38.4	38.4
No. B F. scirpi v. acuminatum	10	19	89.4	21.1	31.5	38.8	10.6
No. D F.scirpi	10	22	100.	13.6	31.8	54.5	0
No. C Uninoculated check	10	26	30.7	0	0	30.7	69.2

Experiments VI to XI Inclusive

Examination of the references in the introduction indicate that a few organisms are found to predominate in cotton seedling troubles. The organisms most frequently mentioned in the literature are Glomerella gossypii, Fusarium moniliforme, Fusarium vasinfectum, Rhizoctonia solani and Sclerotium bataticola. Experiments VI to XI inclusive were arranged to test the pathogenicity of these organisms under various environmental conditions. Fusarium scirpi was added to the list being tested because of its virulence as seen in Experiment IV. It has been frequently isolated from cotton seed and diseased seedlings in Oklahoma and the majority of the investigators list "Fusarium species" as a group attacking cotton. It is believed that Fusarium scirpi occurs frequently under this designation. Each experiment was conducted under a slightly different environment.

Each experiment consisted of 60 pots of soil inoculated with the above organisms at the rate of 10 pots for each organism and 10 uninoculated check pots. The method of inoculation was the same as in previous experiments. All pots in the experiments except those in experiment IX were placed in the greenhouse for the duration of the experiment. Experiment VI was placed under standard conditions, that is, as nearly as possible the plants were cared for as were the preceding experiments. The plants of the remaining experiments of the group were subjected to a variety of environments as indicated below. The plants were grown in the pots for a period of 21 days with the exception of those in experiment IX which were examined after 18 days growth.

The temperatures in the greenhouse for the duration of these experiments were as follows: for the week of April 13 to April 20, the maximum was 104°F., the minimum 54°F. with an average of approximately 80°F.;

for the week of April 21 to April 27, the maximum was 96°F., the minimum 57°F with an approximate average of 77°F; for the week of April 28 to May 4, the maximum was 98°F., the minimum 55°F. with an approximate average of 78°F.

Experiment VI

The results of the group of inoculations under "standard" conditions are given in Table VIII. From the data tabulated in the table it is

Table VIII. Tabulated data of experiment VI showing infection results with organisms under standard conditions. Symbols are as indicated on page 8.

Organism	Total No. Pots	Total No. Plants	Total % age Infected	Total % age "H"	Total % age "M"	Total % age "S"	Total % age "N"
Glomerella gossypii	10	28	100.	60.7	7.1	32.1	0
Fusarium vasinfectum	10	29	86.2	0	0	86.2	13.7
Fusarium moniliforme	10	30	89.9	3.3	6.6	80.	10.
Sclerotium bataticola	10	30	89.9	0	6.6	83.3	10.
Fusarium scirpi	10	30	89.9	13.3	20.	56.6	10.
Rhizoctonia solani	10	30	86.6	30.	20.	36.6	13.3
Uninoculated check	10	28	7.6	0	0	7.6	92.3

observed that all of the organisms produced high percentages of infection. Glomerella gossypii gave its usual large amount of heavy infection, Rhizoctonia solani and Fusarium scirpi ranked second and third respectively in severity. These three organisms produced some stunting of growth whereas the remaining ones produced only slight attacks on the hypocotyls of the plants without stunting. The uninoculated check plants were relatively uninfected. Comparison with the data of the preceding experiments shows

that there were essentially the same degrees of infection for each organism in the respective experiments, under similar environmental conditions.

Experiment VII

Table IX reports the results of a group of inoculated plants under conditions of excessive watering. In the table it can be

Table IX. Tabulated data of experiment VII showing infection results of organisms under conditions of excess moisture. Symbols are as indicated on page 8.

Organism	Total No. Pots	Total No. Plants	Total % age Infected	Total % age "H"	Total % age "M"	Total % age "S"	Total % age "N"
Glomerella gossypii	10	30	96.6	73.3	0	23.3	3.4
Fusarium vasinfectum	10	29	55.1	0	10.3	44.8	44.8
Fusarium moniliforme	10	30	73.3	20.	13.3	40.	26.6
Sclerotium bataticola	10	30	93.3	10.	10.	73.3	6.6
Fusarium scirpi	10	28	92.8	25.	21.4	46.4	7.1
Rhizoctonia solani	10	28	96.4	39.3	17.8	39.3	3.6
Uninoculated check	10	30	40.	3.3	6.6	30.	60.

observed that Glomerella gossypii again proved its seedling-destroying power. Under conditions of excessive moisture, each organism in the series became more destructive than under dryer conditions, with the exception of Fusarium vasinfectum which decreased strikingly in its attack. Fusarium moniliforme became very pathogenic under these conditions. Glomerella gossypii, Fusarium moniliforme, Fusarium scirpi and Rhizoctonia solani each produced a few noticeably stunted plants. The uninoculated checks gave a high percentage of slight infection which may be attributed to the excessive watering and the accidental transfer

of pathogenic organisms in caring for the experiment.

Experiment VIII

The plants in the series were watered on the average once every two days. An attempt was made to water the plants just enough to prevent serious wilting due to lack of water. The plants did not grow well; they averaged about 2⅛ inches in height at the end of 21 days and were of lighter green color than the other plants. The cotyledonary leaves were small, thin, and somewhat leathery. The data tabulated in Table X shows that all the organisms except Glomerella gossypii, Rhizoc-

Table X. Tabulated data of experiment VIII showing relationship of infection to xerophytic conditions. Symbols are as indicated on page 8.

Organism	Total No. Pots	Total No. Plants	Total % age Infected	Total % age "H"	Total % age "M"	Total % age "S"	Total % age "N"
Glomerella gossypii	10	30	100.	86.6	3.3	10.	0
Fusarium vasinfectum	10	30	83.3	10.	3.3	70.	16.6
Fusarium moniliforme	10	30	90.	10.	6.6	73.3	10.
Sclerotium bataticola	10	30	93.3	13.3	26.6	53.3	6.6
Fusarium scirpi	10	30	93.3	23.3	30.	40.	6.6
Rhizoctonia solani	10	29	90.	68.9	6.9	17.2	6.9
Uninoculated check	10	30	33.3	0	3.3	30.	66.6

tonia solani and Fusarium scirpi decreased in their pathogenicity under xerophytic conditions. The most significant point was the increase of pathogenicity of Rhizoctonia solani under these conditions. It is commonly assumed that R. solani is most virulent under conditions of excessive moisture. Glomerella gossypii was observed to produce some stunting of growth in this series.

Experiment IX

Experiment IX was arranged to test the pathogenicity of the above organisms under conditions which varied from time to time. The pots were placed in the greenhouse for 5 days to obtain germination equal to that of the other experiments of this group. On the fifth day the pots were removed to a shaded area outside of the greenhouse where they were kept for 3 days. These pots were then replaced in the greenhouse in such a way as to receive normal sunlight and temperature. The pots were watered daily to prevent wilting and to promote proper growth activities. The last 3 days these pots were outside the greenhouse where it was windy with very cool nights. The plants were whipped about by the wind and a great many of them bent and broke in the region between the ground line and cotyledons. The plants were removed from the pots and examined for fungous injury after a period of 18 days. The results are tabulated in Table XI.

Table XI. Infection results of experiment IX, under variable conditions. Symbols are as indicated on page 8.

Organism	Total No. Pots	Total No. Plants	Total % age Infected	Total % age "H"	Total % age "M"	Total % age "S"	Total % age "N"
Glomerella gossypii	10	30	100.	83.3	16.6	0	0
Fusarium vasinfectum	10	30	96.6	13.3	40.	43.3	3.4
Fusarium moniliforme	10	30	100.	30.	36.6	33.3	0
Sclerotium bataticola	10	30	73.3	33.3	13.3	26.6	26.6
Fusarium scirpi	10	30	100.	30.	43.3	26.6	0
Rhizoctonia solani	10	30	100.	70.	13.3	16.6	0
Uninoculated check	10	30	23.4	10.	10.	3.4	76.6

From these results it is observed that infection was general, with Glomerella gossypii, and Rhizoctonia solani giving high percentages of heavy infection. Fusarium scirpi again gave indications of being a severe pathogen when under favorable conditions.

It as observed that in the pots containing soil inoculated with Glomerella gossypii, the plants were very much stunted with the majority being wilted and discolored at the groundline. Many of the plants were dead. In pots inoculated with Fusarium vasinfectum, F. moniliforme, and Sclerotium bataticola, the plants were normal with only a slight amount of stunting. Some evidence of hypocotyl attack was visible in a few instances. Plants growing in soil inoculated with Fusarium scirpi and Rhizoctonia solani showed general stunting of growth with slight hypocotyl attacks above ground. The uninoculated check plants were normal and relatively uninfected.

Experiment X

The soil in this series was mixed with a small amount of calcium carbonate. The pH at the time of inoculating and planting was approximately 9.6. At the time the plants were removed from the soil, the pH tested 8.2. The cotton plants in this soil grew very well; the average height was about 4½ inches. The hypocotyls were stout; the cotyledonary leaves were thick and dark green in color. There were no apparent above-ground lesions. Glomerella gossypii produced stunting in approximately 50 per cent of the plants growing in soil inoculated with the organism. Rhizoctonia solani, although very pathogenic caused no stunting. It is seen from the data in Table XII that although infection in general was very high, only Glomerella gossypii and Rhizoctonia solani produced notable percentages of heavy infection. Most of

the infections produced by the remaining organisms were very slight in nature. These results indicate that Glomerella gossypii and Rhizoctonia solani are severe pathogens in alkaline soil but the remaining 4 organisms were of little consequence under the conditions of the experiment.

Table XII. Tabulated results of experiment X showing infection results in an alkaline soil. Symbols are as indicated on page 8.

Organism	Total No. Poss	Total No. Plants	Total % age Infected	Total % age "H"	Total %age "M"	Total % age "S"	Total %age "N"
Glomerella gossypii	10	30	100.	96.7	0	3.3	0
Fusarium vasinfectum	10	30	66.6	10.	0	56.6	33.3
Fusarium moniliforme	10	30	86.5	6.6	16.6	63.3	13.3
Sclerotium bataticola	10	30	90.	3.3	10.	76.6	10.
Fusarium scirpi	10	30	96.6	0	3.3	93.3	3.3
Rhizoctonia solani	10	30	100.	40.	13.3	46.6	0
Uninoculated check	10	30	33.3	0	13.3	20.	66.6

Experiment XI

The soil in this series was acidified by the addition of hydrochloric acid. The acid (66°Baumé) was diluted 1 to 500 (approximately) and sprinkled on the soil, then the soil was thoroughly mixed and dried. The soil was then inoculated and planted as described on pages 5 and 6. The plants which developed were very spindly and stunted in appearance. Examination revealed that the roots were all swollen just below the ground line. The tips of the root systems were blackened and soft in all series including the uninoculated checks. Close examination of the hypocotyls, however, did reveal many lesions and indications of infection. Evidently the acid in this series caused the major part of the damage

and hence the data are not valid for comparison with the preceding data. The pH of this soil at the time of inoculation was 4.0. At the close of the experiment the pH was approximately 5.4.

Experiments XII to XVII Inclusive

Experiments XII to XVII inclusive were arranged as duplications of experiments VI to XI inclusive. The purpose of the duplications was to obtain more data on the pathogenicity of the organisms being used and thus make the information more valid. The plants were grown for a period of 18 days.

The temperatures in the greenhouse for the duration of the experiments were: for the week of April 28 to May 4, the maximum was 98°F., the minimum 55°F. with an approximate average of 78°F.; for the period of May 5 to May 8, the maximum was 103°F., the minimum was 75°F. with an approximate average of 90°F.; for the period of May 9 to May 11, the maximum was 98°F., the minimum 60°F. with an approximate average of 76°F.; for the period of May 12 to May 15, the maximum was 101°F., the minimum 64°F. with an approximate average of 80°F.

Experiment XII

The plants growing under "standard" conditions reacted as shown in Table XIII.

Table XIII. Tabulated data of experiment XII showing infection results with organisms under standard conditions. Symbols are as indicated on page 8.

Organism	Total No. Pots	Total No. Plants	Total % age Infected	Total % age "H"	Total % age "M"	Total % age "S"	Total % age "N"
Glomerella gossypii	10	30	100.	83.3	13.3	3.3	0
Fusarium vasinfectum	10	30	76.6	30.	6.6	40.	23.3
Fusarium moniliforme	10	30	76.6	6.6	10.	60.	23.3
Sclerotium bataticola	10	30	73.3	3.3	3.3	66.6	26.6
Fusarium scirpi	10	30	86.6	6.6	20.	60.	13.3
Rhizoctonia solani	10	30	89.9	40.	13.3	36.6	10.
Uninoculated check	10	30	23.3	6.6	3.3	13.3	76.6

These data are comparable to those of experiment VI with the exception of Fusarium vasinfectum which became more pathogenic in this experiment. The plants in this series were severely stunted by Glomerella gossypii and Rhizoctonia solani. Fusarium vasinfectum, F. moniliforme and F. scirpi, culture D, produced a slight amount of stunting. The plants inoculated with Sclerotium bataticola and the uninoculated checks were normal.

Experiment XIII

A poor stand of plants were obtained in the experiment in which they were subjected to excessive watering. Species of algae and saprophytic fungi developed on the surface of the soil in the pots. All of the plants except those in the uninoculated check pots were more or less stunted.

Examination of the data in Table XIV shows results which are comparable

to those of experiment VII with the exception of Rhizoctonia solani which became very virulent under the conditions of this experiment.

Table XIV. Tabulated data of experiment XIII showing infection results of organisms under conditions of excess moisture. Symbols are as indicated on page 8.

Organism	Total No. Pots	Total No. Plants	Total % age Infected	Total % age "H"	Total % age "M"	Total % age "S"	Total % age "N"
Glomerella gossypii	10	20	100.	60.	20.	20.	0
Fusarium vasinfectum	10	18	94.5	22.2	22.2	50.	5.5
Fusarium moniliforme	10	13	92.3	15.4	23.1	53.8	7.7
Sclerotium bataticola	10	21	76.2	9.5	14.3	52.4	23.8
Fusarium scirpi, D	10	24	95.9	12.5	16.7	66.7	4.1
Rhizoctonia solani	10	16	100.	81.2	18.7	0	0
Uninoculated check	10	26	23.1	3.8	7.7	11.5	76.9

Experiment XIV

The results of inoculations under conditions of deficient moisture are given in Table XV.

Table XV. Tabulated data of experiment XIV showing relationship of infection to xerophytic conditions. Symbols are as indicated on page 8.

Organism	Total No. Pots	Total No. Plants	Total % age Infected	Total % age "H"	Total % age "M"	Total % age "S"	Total % age "N"
Glomerella gossypii	10	30	100.	100.	0	0	0
Fusarium vasinfectum	10	26	100.	80.7	3.8	15.4	0
Fusarium moniliforme	10	30	100.	73.3	6.6	20.	0
Sclerotium bataticola	10	28	100.	75.	3.6	21.4	0
Fusarium scirpi, D	10	29	100.	75.8	10.3	13.8	0
Rhizoctonia solani	10	24	100.	83.3	8.3	8.3	0
Uninoculated check	10	28	71.5	28.5	14.3	28.5	28.5

The data in the table shows that all of the organisms in this experiment produced high percentages of heavy infection. The plants growing in soil inoculated with Glomerella gossypii and Rhizoctonia solani were severely stunted with 40 to 50 per cent of the plants killed. The plants growing in soil inoculated with the remaining organisms showed normal above-ground growth. The uninoculated check plants were normal in appearance.

Experiment XV

The plants in this experiment were grown under conditions which varied from time to time. The pots were placed in the greenhouse for 4 days, then removed from the greenhouse and covered for 3 days to allow the plants to harden to external conditions. The covers were then removed and the plants remained outside the greenhouse for the rest of the experiment.

Table XVI. Infection results of experiment XV, under variable conditions. Symbols are as indicated on page 8.

Organism	Total No. Pots	Total No. Plants	Total % age Infected	Total % age "H"	Total % age "M"	Total % age "S"	Total % age "N"
Glomerella gossypii	10	30	100.	73.3	16.6	10.	0
Fusarium vasinfectum	10	30	90.	3.3	13.3	73.3	10.
Fusarium moniliforme	10	27	77.8	14.8	7.4	55.5	22.2
Sclerotium bataticola	10	30	50.	0	0	50.	50.
Fusarium scirpi, D	10	30	83.3	16.6	23.3	43.3	16.6
Rhizoctonia solani	10	27	100.	63.	7.4	29.6	0
Uninoculated check	10	29	27.5	10.3	3.4	13.8	72.4

The data in Table XVI indicates that under conditions which approximate those to which field-grown cotton seedlings are exposed, only Glomerella

gossypii and Rhizoctonia solani proved to be seriously pathogenic.

Experiment XVI

The soil in this series was mixed with calcium carbonate. The pH at the time of inoculating and planting was approximately 8.3. It tested approximately 8.1 after the plants were removed. The plants grew rapidly, producing dark green, thick cotyledonary leaves. They averaged 6 to 7 inches in height. Rhizoctonia solani and Glomerella gossypii produced some stunting.

From Table XVII it is observed that Glomerella gossypii produced

Table XVII. Tabulated results of experiment XVI showing infection results in an alkaline soil. Symbols are as indicated on page 8.

Organism	Total No. Pots	Total No. Plants	Total % age Infected	Total % age "H"	Total % age "M"	Total % age "S"	Total % age "N"
Glomerella gossypii	10	30	100.	96.6	3.3	0	0
Fusarium vasinfectum	10	30	63.3	13.3	0	50.	36.6
Fusarium moniliforme	10	30	76.6	3.3	6.6	66.6	23.3
Sclerotium bataticola	10	28	75.	0	7.2	67.8	25.
Fusarium scirpi, D	10	30	80.	0	20.	60.	20.
Rhizoctonia solani	10	26	100.	38.4	15.4	46.1	0
Uninoculated check	10	30	6.6	3.3	0	3.3	93.3

very severe infection, 96.6 per cent infection being designated as heavy ("H"). Rhizoctonia solani produced 38.4 per cent heavy infection. The remaining organisms produced high percentages of slight infection but such infection is not believed to be a serious factor in killing seedlings. These results agree closely with the results in a comparable experiment (Exp. X).

Experiment XVII

The soil in this series was acidified by mixing with flowers of sulphur. The pH at the time of inoculating and planting was approximately 6.4 At the time the plants were removed it tested 6.0 to 6.1. The plants in this soil averaged 3 to 4 inches in height. The cotyledonary leaves were smaller than in the other experiments in the series.

Table XVIII. Tabulated results of experiment XVII showing infection results in an acid soil. Symbols are as indicated on page 8.

Organism	Total No. Pots	Total No. Plants	Total % age Infected	Total % age "H"	Total % age "M"	Total % age "S"	Total % age "N"
Glomerella gossypii	10	30	66.6	40.	3.3	23.3	33.3
Fusarium vasinfectum	10	29	62.1	13.8	3.4	44.8	37.9
Fusarium moniliforme	10	30	43.3	3.3	0	40.	56.6
Sclerotium bataticola	10	25	24.	8.	0	16.	76.
Fusarium scirpi, D	10	26	69.3	19.3	11.5	38.4	30.7
Rhizoctonia solani	10	30	93.3	56.6	10.	26.6	6.6
Uninoculated check	10	30	26.6	20.	0	6.6	73.3

The data of this experiment (Table XVIII) show Rhizoctonia solani to be the most severe pathogen under acid conditions with Glomerella gossypii ranking second. The uninoculated check plants showed 20 per cent heavy infection, possibly related to direct injury from the acid as seen in experiment XI.

Experiment XVIII

This experiment was arranged to test the protective qualities of ethyl mercury phosphate ("New Improved Ceresan") against heavy soil inoculations of Glomerella gossypii. Twenty pots of soil were inoculated

with G. gossypii according to the standard method outlined above. Ten of these pots were planted with acid-delinted, graded, "New Improved Ceresan"-treated seed and the remaining 10 pots with "New Improved Ceresan" treated fuzzy seed. Ten uninoculated check pots of acid delinted, graded, and "New Improved Ceresan"-treated seed and 10 of uninoculated "New Improved Ceresan"-treated fuzzy cotton seed were also planted. The results were as given in Table XIX.

Table XIX. Tabulated results of Experiment XVIII showing the relationship between "New Improved Ceresan" cotton seed treatment and infection with Glomerella gossypii from the soil. Symbols are as indicated on page 8.

Cotton seed	Total No. Pots	Total No. Plants	Total % age Infected	Total % age "H"	Total % age "M"	Total % age "S"	Total % age "N"
Delinted, treated[1] Inoculated soil	10	30	73.3	63.3	3.3	6.6	26.6
Delinted, treated[1] Uninoculated soil	10	30	43.3	3.3	3.3	36.6	56.6
Fuzzy, treated[1] Inoculated soil	10	29	100.	24.1	44.8	31.	0
Fuzzy, treated[1] Uninoculated soil:	10	27	85.1	3.7	22.2	59.2	14.8

1. treated with "New Improved Ceresan".

The D. and P.L. 11A seed used in this experiment had been found to be internally infested with G. gossypii. This will account for th infection occurring in the group of uninoculated check plants.

It may be concluded from these results that under a high infection potential of the soil "New Improved Ceresan" has protective power but it is limited, being more potent in fuzzy seed due to the greater amount of "New Improved Ceresan" adherent to the fuzz.

Experiment XIX

The arranging and conducting of experiments, such as the ones above, involves considerable work and many necessary precautions. Time and space are often at a premium in the laboratory or greenhouse. The possibility that test tube cultures might be used to simplify evaluating the index of

of infection of a particular organism or a group of organisms was considered. An experiment was arranged with this in mind. Two hundred and ten tubes of water agar were prepared. Into each tube was placed one acid-delinted, sinking, surface-sterilized cotton seed. As soon as the seed germinated the hypocotyls were inoculated with the organisms listed in Table II in series of 10 tubes per organism. The inoculating was performed by placing a bit of the fungal mycelium, mixed with spores in most instances, on the hypocotyl near the agar line. The tubes were kept plugged with cotton until the cotyledonary leaves came into contact with the cotton. The seedlings were grown for a period of 10 days after inoculating. The results are given in Table XX.

Table XX. The results of test tube cultures showing the percentages of injury and index of infection for each organism.

Organism		No. tubes cultured	Total % age Infected	Total % age "H"	Total % age "M"	Total % age "S"	Total % age "N"	Index of Infection
No. 18	Fusarium moniliforme	10	100.	0	30.	70.	0	130
No.115	Fusarium moniliforme	10	100.	40.	30.	30.	0	210
No. 24	Fusarium vasinfectum	10	80.	0	30.	50.	20.	110
No. 27	Fusarium vasinfectum	10	100.	0	10.	90.	0	110
No. 34	Fusarium semitectum	10	80.	0	0	80.	20.	80
No. 36	F. solani v. suffuscum	10	100.	20.	30.	50.	0	170
No. 38	F. chlamydosporum	10	100.	0	10.	90.	0	110
No. 42	F. bullatum v. minus	10	70.	0	20.	50.	30.	90
No. 44A	Fusarium scirpi	10	90.	10.	40.	40.	10.	150
No. D	Fusarium scirpi	10	100.	100.	0	0	0	300
No. B	F. scirpi v. acuminatum	10	100.	0	20.	80.	0	120
No. 20	Glomerella gossypii	10	100.	80.	20.	0	0	280
No.R37	Sclerotium bataticola	10	80.	10.	30.	40.	20.	130
No. 41	Sclerotium bataticola	10	100.	0	60.	40.	0	160
No. 7	Rhizoctonia solani	10	100.	20.	30.	50.	0	170
No. R	Rhizoctonia solani	10	100.	100.	0	0	0	300
No. 61	Alternaria species	10	90.	0	0	90.	10.	90
No. P	Penicillium species	10	50.	10.	10.	30.	50.	80
No. A	Aspergillus niger	10	60.	0	20.	40.	40.	80
No. C	Uninoculated check	10	10.	0	0	10.	90.	10
No. CII	Uninoculated check	10	0	0	0	0	100.	0

Reisolations of pathogens from representative samples of the plants in each experiment were made to determine that the organisms involved in the infections were identical with those which the soil had been originally inoculated. The samples were obtained by slicing thin portions of diseased tissue from the plants and, after surface sterilizing in 10 per cent calcium hypochlorite ("B-K" powder) solution for a period of 5 minutes, placing the pieces on potato-dextrose agar in culture tubes. The fungi which developed were identical with the original inoculum in practically every instance. Portions of hypocotyls of the plants growing in uninoculated soil were also cultured to determine whether or not the check plants were infected. Table XXI shows the percentage of recovery of the organisms in each experiment.

Table XXI. Data showing percentage recovery of original inoculum.

Experiment No.	Number of tubes cultured from		Percentage recovery of organisms	Percentage of uninfected check plants
	Inoculated Seedlings	Uninoculated Seedlings		
Experiment I	40	12	92.5	50.0
Experiment II	40	4	67.5	75.0
Experiment III	42	5	28.5	80.0
Experiment IV	33	4	66.0	75.0
Experiment V	37	10	0	100.0
Experiment VI	17	3	58.8	100.0
Experiment VII	17	3	64.7	100.0
Experiment VIII	17	3	76.4	66.6
Experiment IX	17	3	29.4	100.0
Experiment X	17	3	58.8	100.0
Experiment XI	17	3	94.1	33.3
Experiment XVIII	6	6	100.0	16.6

It is believed that the strength of the calcium hypochlorite powder varies and although the sterilizing solution was mixed at the ratio of 1 part powder to 10 parts water in every case, there is no assurance that the solution was of the same chemical strength in all

instances. A probable explanation of the low percentage of recovery of the organisms in some of the experiments is that the calcium hypochlorite solution was strong enough to kill the organisms in the tissues as well as the external surface.

DISCUSSION

The experiments recorded above do not permit direct comparison of the infectivities of the various organisms because of differences in moisture, temperature, light, soil reaction, and provenience in various experiments. It seemed desirable to arrange a classification of the 19 organisms used in this study according to their relative pathogenicity. In order to accomplish this some system of weighting the infection percentages was necessary. Arbitrarily, "H" (heavy infection) was given a value of 3 points, "M" (moderate infection) 2 points, and "S" (slight infection) 1 point. It was found that the study could be divided into 3 sets of experiments that were each under comparable conditions and with comparable organisms, viz. Exp. I, II, and III; Exp. IV, and V; and Exp. VI to XVII. An index of infection for each organism in each experiment was calculated by multiplying the percentage of heavy infection by 3, that of moderate infection by 2 and that of slight infection by 1, totaling and averaging. These values are shown in Tables XXII, XXIII & XXIV.

Table XXII. Index of infection of the organisms in Exp. I, II & III.

Culture No.	Organism	Index of Infection
18	Fusarium moniliforme	96
115	Fusarium moniliforme	113
24	Fusarium vasinfectum	98
27	Fusarium vasinfectum	94
20	Glomerella gossypii	283
R37	Sclerotium bataticola	86
7	Rhizoctonia solani	103
P	Penicillium species	79
A	Aspergillus niger	81
C	Uninoculated check	15

Table XXIII. Index of infection of the organisms in Exps. IV and V.

Culture No.	Organism	Index of Infection
34	Fusarium semitectum	154
36	F. solani v. suffuscum	165
38	F. chlamydosporum	186
42	F. bullatum v. minus	161
44A	F. scirpi	168
D	F. scirpi	213
B	F. scirpi v. acuminatum	209
61	Alternaria species	126
C	Uninoculated check	56

Table XXIV. Index of infection of the organisms in Exps. VI through XVII.

Culture No.	Organism	Index of Infection
115	Fusarium moniliforme	129
27	Fusarium vasinfectum	125
D	Fusarium scirpi	150
20	Glomerella gossypii	259
41	Sclerotium bataticola	113
R	Rhizoctonia solani	221
C	Uninoculated check	50

In every instance in which Glomerella gossypii was used it proved to be the most serious cotton seedling pathogen, and other investigators report similar results; therefore G. gossypii was given an arbitrary weighted infection index of 100. Referring to Table XXII, G. gossypii has an infection index of 283. In order to give G. gossypii the arbitrary weighted index of 100 the index, 283, was divided by 2.83, after which each of the other indices was divided by the same number. Table XXIV was weighted in a similar manner by dividing the index of infection of each organism by 2.59. Comparison of Table XXIII and Table XXIV shows Fusarium scirpi, culture D, to be the one organism common to both groupings. In Table XXIV F. scirpi, culture D has a weighted value of 58 in comparison to the 100 rating of Glomerella gossypii therefore the value of F. scirpi, culture D in Table XXIII was weighted to equal 58. Each

of the other organisms in Table XXIII was then rated on the same basis by dividing each index of infection by 3.67 (213 ÷ 3.67 = 58). Thus the infection indices of all of the 19 organisms used in this study were weighted on a comparable basis. These values are shown in Figure 1 on page 40. Figure 2 shows the weighted indices of infection of the culture tube tests which are described in experiment XIX.

Comparison of the two charts shows that the organisms are distributed in much the same order in relation to the other organisms in the charts. The weighted indices of the culture tube tests are uniformly higher than those of the soil inoculation tests, probably because the pathogens in pure culture are free from antagonism by other soil organisms. It is notable that Rhizoctonia solani and Fusarium scirpi, D are rated above Glomerella gossypii in pathogenicity in figure 2. G. gossypii and R. solani are rated as severe pathogens in both charts; the other species of Fusarium are grouped more or less together with indices between 30 and 60; and the species of Aspergillus, Alternaria and Penicillium occur at the lower end of the group.

Information on the distribution of the organisms attacking cotton seedlings is not very extensive. Some knowledge concerning their distribution and prevalence may be gained from the following tables.

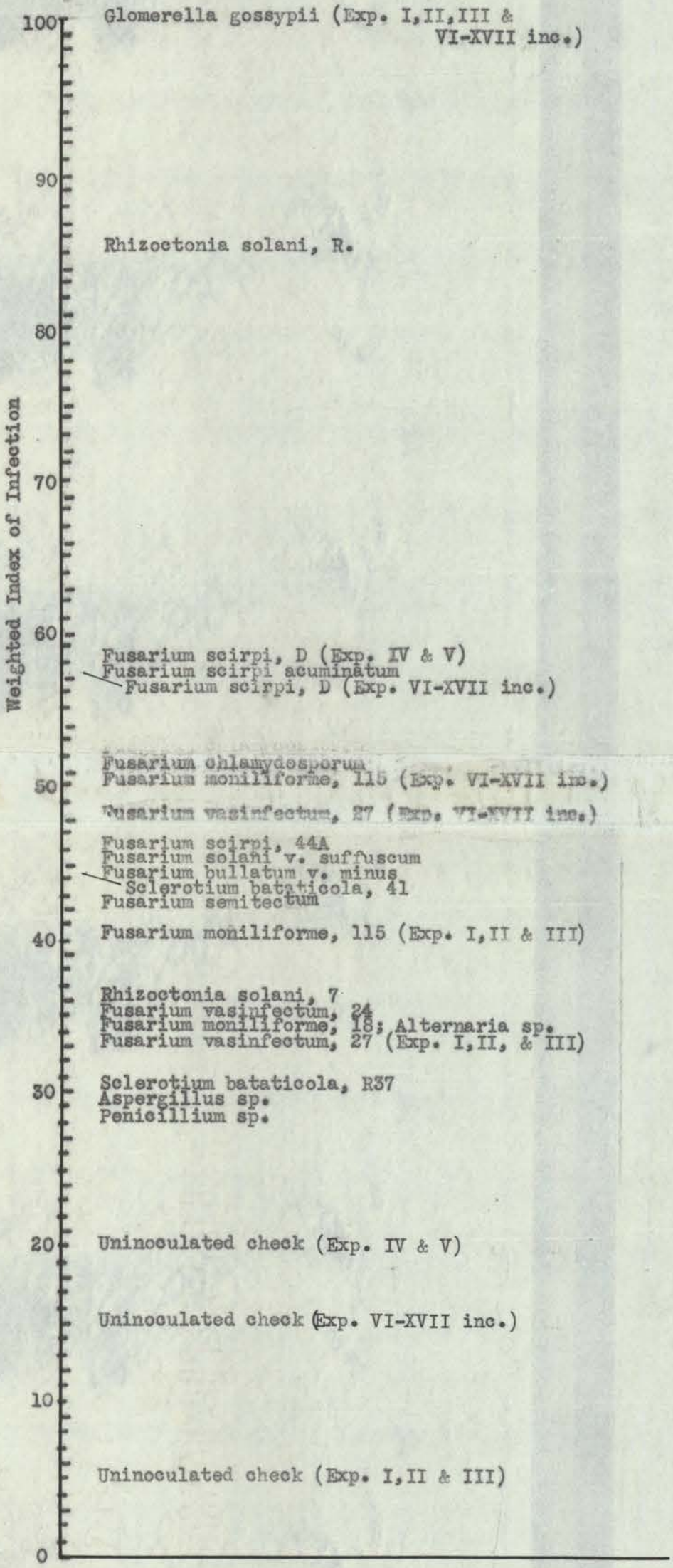

Fig. 1. Chart showing weighted index of infection of 19 organisms used in soil inoculation experiments.

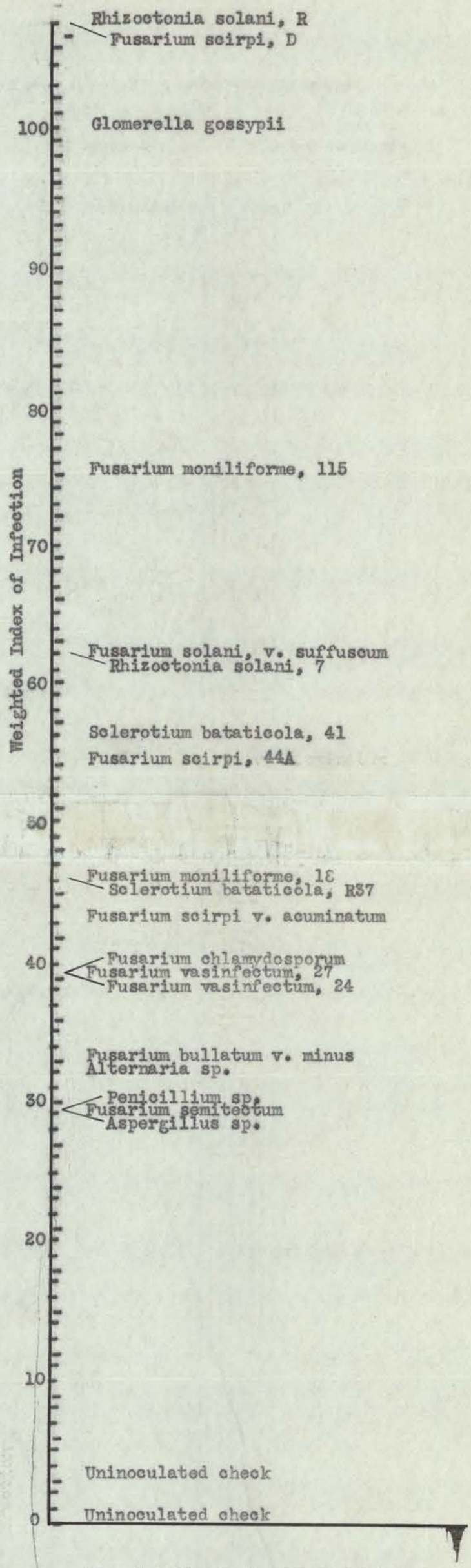

Fig. 2. Chart showing weighted index of infection of 19 organisms used in culture tube experiments.

Table XXV. Number of samples of seedlings from each state found infected by the various fungi indicated. From Miller and Weindling[13].

State	No. of Samples	G.g.	F.m.	Rh.s.	F.s.	D.g.	Rh.b.	Other fungi
Alabama	49	49	47	8	2	0	0	12
Arkansas	34	33	33	7	21	1	1	18
Georgia	84	79	80	10	18	1	4	46
Kansas	5	4	4	0	1	0	0	5
Louisiana	60	60	55	17	39	0	1	33
Mississippi	48	47	38	6	30	0	5	23
Missouri	5	5	5	0	2	0	0	1
North Carolina	20	16	13	1	13	3	5	15
Oklahoma	17	0	6	0	5	1	2	16
South Carolina	104	90	96	12	40	4	2	67
Tennessee	7	7	6	0	6	0	0	3
Texas	4	0	0	0	4	0	0	3
Virginia	25	23	24	0	10	0	1	14
Total	462	413	407	61	191	10	21	256
Percentage found with fungi		89.4	88.1	13.2	41.3	2.2	4.5	55.4

Abbreviations: G.g.: Glomerella gossypii; F.m.: Fusarium moniliforme; Rh.s.: Rhizoctonia solani; F.s.: Fusarium sp.; D.g.: Diplodia gossypina; Rh.b.: Rhizoctonia (Sclerotium) bataticola. "Other fungi" includes: Alternaria sp., Penicillium sp., Aspergillus sp. and unidentified species.

Table XXV indicates the presence of Glomerella gossypii and Fusarium moniliforme throughout the cotton belt with the exception of Texas. F. moniliforme is probably grouped in the "Fusarium species" by the Texas investigators. The small amount of Rhizoctonia solani is of interest since it is such a virulent pathogen on cotton seedlings. This may be explained, to some extent, by the fact that R. solani is a soil-borne organism for the most part, attacking only the young seedlings, whereas G. gossypii is seed-borne. Seed-borne organisms are usually more evenly distributed in fields than are soil-borne organisms and consequently spot sampling may not give comparable results with the two types of pathogens.

It should also be pointed out that the results of isolations vary according to the techniques used, and these techniques vary from one

investigator to another.

Table XXVI. Results of isolations from wilted and diseased cotton seedlings collected in southern Oklahoma in May, 1939.

Name of Organism	No. times isolated	Percent of total fungi isolated	Percent of parasitic fungi isolated
Fusarium moniliforme	61	30.	40.
F. scirpi	8	2.8	3.9
F. vasinfectum	5	1.5	2.4
F. scirpi v. acuminatum	2	.7	.98
F. solani	21	7.5	10.3
F. solani martii	20	7.2	9.9
F. bullatum v. minus	1	.35	.49
Rhizoctonia solani	40	15.	19.8
Sclerotium bataticola	24	8.6	11.8

(Unpublished data of Dr. W. W. Ray and cited with his permission.)

The data of Table XXVI reveal F. moniliforme to be present in 40 per cent of the diseased cotton seedlings examined. Figure 1 shows Fusarium moniliforme to vary in its weighted index of infection, from 34 to 50. This would indicate that, except under very favorable conditions, Fusarium moniliforme is probably a minor factor in Oklahoma cotton seedling disease problems.

Rhizoctonia solani was isolated from approximately 20 per cent of the seedlings examined and because of its severe pathogenic rating under many variable conditions it is probably the most damaging of cotton seedling pathogens occurring in Oklahoma.

Sclerotium bataticola was isolated from approximately 12 per cent of the seedlings, however, in view of its low rating in pathogenicity, it is believed to be of slight consequence as a primary pathogen in Oklahoma seedling disease.

The many species of Fusarium isolated occurred in low percentages of the total number of parasitic fungi isolated and since the majority of the Fusarium species appear to be pathogens of only moderate virulence, it is believed that these organisms are of slight consequence to the cotton grower.

The occurrence of Aspergillus sp., Penicillium sp., and Alternaria sp. in isolations from seedlings may be almost disregarded because of their low ratings in pathogenicity. Fusarium vasinfectum, (the causal organism of cotton wilt) because of its low pathogenic rating and infrequent occurrence in Oklahoma, may be classed with this group, although, it will prove more pathogenic occasionally under favorable conditions of host and fungus.

The experiments have indicated Fusarium scirpi to rank third in severity in the soil inoculations under the many various environmental conditions. This organism was isolated from a small number of seedlings in 1939, however, if conditions were to prevail favoring growth of the organism it might possibly prove to be a very destructive field pathogen.

Glomerella gossypii is recognized as probably the most severe and important cotton seedling pathogen east of Oklahoma and Texas. Experimental results throughout this study have indicated that the organism is a severe killing pathogen under all conditions tested. Diseased cotton seedlings from Oklahoma and Texas have failed repeatedly to yield this organism in isolation cultures. In view of this, it is suggested that Glomerella gossypii is not a cotton seedling pathogen of importance to Oklahoma or Texas cotton growers under ordinary circumstances.

There is some question as to whether the results obtained in the soil inoculation experiments are faithful as indicators of field infections from these organisms. There are factors involved which necessitate revision of these results before using them to indicate field infections. In all probability, the presence of the organisms in the concentrations used in these experiments would seldom be en-

countered in soils. Organisms in soils are never found in pure culture. There are always large numbers of parasitic and saprophytic fungi and bacteria found in samples of soil. The phenomenon of competition in the soil flora is known to be a tempering factor in raising and lowering the abundance and virulence of organisms in the soil. Therefore, field results would probably indicate lower "weighted indices of infection" for these organisms. Comparison shows that the"weighted indices of infection"of the organisms in figure 2 are uniformly higher than those in figure 1. It is probable that there are as great or even greater differences to be found between the soil inoculation experiments as conducted and acutal field experiments with these organisms. On the other hand, the plants used in these experiments were favored in most cases by adequate moisture, favorable temperatures, and freedom from wind, insects, and other field hazards. To plants under stress of the rigorous conditions of life in the open field, even pathogens of moderate to low indices might prove very harmful.

It is known that serious losses occur annually from cotton seedling diseases and that in most instances one or more of the organisms studied in this paper are responsible. These losses are usually the result of all of these organisms, present in the soil, working together to produce wounds and lesions which will permit the secondary organisms in the soil to gain entrance and work their destruction. The entire group of 19 organisms listed in Table II were found to be capable of producing various degrees of attack under at least some of the conditions to which they were exposed. When these organisms are not pathogenic enough to cause serious losses by working alone, they may be thought of as the fungal pioneers that open the way for the numerous other secondary fungi

which make up the soil flora and which, in all probability, often are more severe agents of destruction to the young seedlings than the original pathogen which opened the plant to attack.

SUMMARY

Nineteen cultures of fungi, isolated from diseased cotton seed and seedlings, were used in a series of greenhouse and laboratory infection experiments to determine the degrees of pathogenicity of the various organisms under a variety of environmental conditions.

Glomerella gossypii proved to be a serious cotton seedling pathogen under all experimental conditions to which it was subjected, however, it is not believed to be a serious problem in Oklahoma because of its infrequency in seedling isolations.

Rhizoctonia solani was found to be a serious cotton seedling pathogen under all of the experimental conditions used and, because of its frequent occurrence in isolations from diseased seedlings, is believed to be the most serious cotton seedling pathogen under Oklahoma conditions.

Fusarium scirpi was sufficiently virulent to be considered an important seedling pathogen when present in abundance and when suitable growth conditions for the fungus and host prevail.

A number of other species of Fusarium were found to rank in a group of moderate pathogenicity and probably are of slight consequence in Oklahoma except under very suitable conditions for infection or when acting in combination with one another or with other organisms.

Alternaria sp., Penicillium sp., and Aspergillus sp. were ranked as slightly pathogenic with probably no importance in causing serious losses. These species may even serve to inhibit the growth of some of the more severe pathogens.

Charts (figures 1 and 2) are presented showing the "weighted indices of infection" of the 19 organisms used in the experiments and the correlation between soil inoculation and culture tube experiments.

Extensive experiments were conducted with 6 of the most common organisms in the group studied (Exp. VI to XVII inc.) Glomerella gossypii was found to be the most strongly virulent in the experiments conducted under standard, xerophytic, and variable conditions, also in the alkaline soil experiments. Rhizoctonia solani ranked highest in virulence in the acidified soil with Glomerella gossypii second and Fusarium scirpi third. Under conditions of excessive moisture both Glomerella gossypii and Rhizoctonia solani proved to be strongly virulent with the remaining organisms showing stronger virulence than under previous conditions. It is commonly assumed that Rhizoctonia solani is most virulent under conditions of excessive moisture, however evidence is presented which indicates that it may be just as severe under xerophytic conditions.

The conclusion was reached from the results of experiment XVIII that under a high infection potential of the soil "New Improved Ceresan" has a limited protective power, being more potent on fuzzy seed than on delinted seed, due to the greater amount of the dust adherent to the fuzz.

Plate I. Cotton seedlings of Exp. VI growing under standard conditions. Rows of plants inoculated from left to right with: uninoculated checks; Rhizoctonia solani; Fusarium scirpi; Sclerotium bataticola; Fusarium moniliforme; Fusarium vasinfectum; and Glomerella gossypii.

Plate II. Cotton seedlings of Exp. VII growing under conditions of excessive watering. Rows of plants inoculated from left to right with: uninoculated checks; Rhizoctonia solani; Fusarium scirpi; Sclerotium bataticola; Fusarium moniliforme; Fusarium vasinfectum; and Glomerella gossypii.

Plate III. Cotton seedlings of Exp. VIII growing under deficient moisture conditions. Rows of plants inoculated from left to right with: uninoculated checks; Rhizoctonia solani; Fusarium scirpi; Sclerotium bataticola; Fusarium moniliforme; Fusarium vasinfectum; and Glomerella gossypii.

Plate IV. Cotton seedlings of Exp. XVIII produced from seed which had been dusted with "New Improved Ceresan". Left to right: fuzzy seed in uninoculated soil; fuzzy seed in Glomerella gossypii infested soil; delinted seed in uninoculated soil; and delinted seed in Glomerella gossypii infested soil.

Plate V. Cotton seedlings of Exp. IX growing under conditions which were varied from time to time. These plants were severely injured by wind and cool night temperatures.

Plate VI. Cotton seedlings growing in soil which had been alkalinized by the addition of calcium carbonate (Exp. X). Rows of plants inoculated from left to right with: uninoculated checks; Rhizoctonia solani; Fusarium scirpi; Sclerotium bataticola; Fusarium moniliforme; Fusarium vasinfectum; and Glomerella gossypii.

Plate VII. Cotton seedlings growing in soil which had been acidified by the addition of a weak solution of hydrochloric acid (Exp. XI). Rows of plants inoculated from left to right with: uninoculated checks; Rhizoctonia solani; Fusarium scirpi; Sclerotium bataticola; Fusarium moniliforme; Fusarium vasinfectum; and Glomerella gossypii.

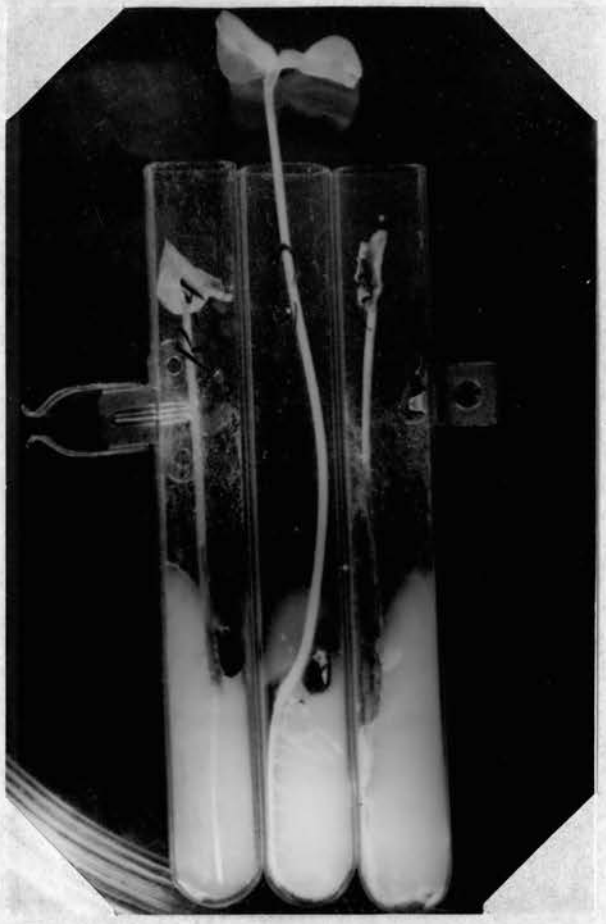

Plate VIII. Cotton seedlings growing in culture tubes of water agar. Left to right: inoculated with Rhizoctonia solani; uninoculated check; inoculated with Rhizoctonia solani.

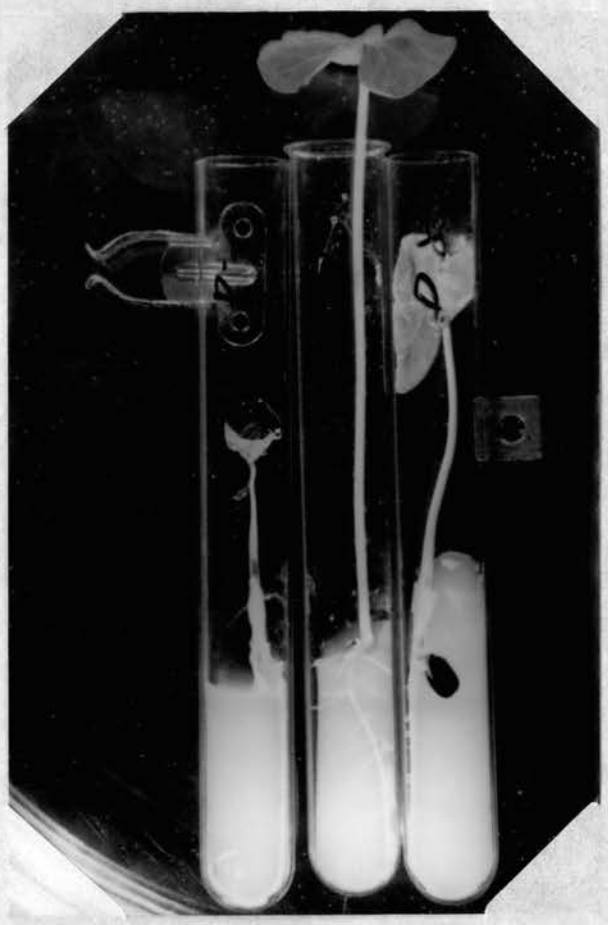

Plate IX. Cotton seedlings growing in culture tubes of water agar. Left to right: inoculated with Fusarium scirpi,D; uninoculated check; inoculated with Fusarium scirpi,D.

BIBLIOGRAPHY

1. Archibald, R. G. 1927. Sulfuric acid treatment of cotton seed. Soil Science 23:1-3.

2. Arndt, C. H. 1935. The etiology of damping off of cotton seedlings. Phytopath. 25:968-969.

3. Arndt, C. H. 1937. The comparative role of certain nematodes and fungi in the etiology of damping off or soreshin of cotton. Phytopath. 27:569-572.

4. Arndt, C. H. 1935. Cotton seedling diseases. S. C. Agr. Exp. Sta. Ann. Rep. 48:38-43.

5. Brown, J. G. 1939. Permeability of testa of normal and treated cotton seeds. Phytopath. 29:753.

6. Camp, A. F. et al. 1928. Plant diseases. Fla. Agr. Exp. Sta. Rep. 38-39 and 47.

7. Chester, K. Starr. 1938. Gravity grading, a method for reducing seed-borne diseases in cotton. Phytopath. 28:745-749.

8. Chester, K. Starr. and G. Tennyson. 1940. The effect of a diseased cotton seed on an adjacent healthy seed. Phytopath. 30:4.

9. Gordon, Thomas B. 1937. Report of cotton anthracnose survey in Oklahoma. Pl. Dis. Rep. 21:385-386.

10. Lehman, S. G. 1938. Seed infestation with Glomerella and Fusarium in the 1936 cotton crop of North Carolina. Pl. Dis. Rep. 22: 4-6.

11. Miller, Paul R. 1936. Observations on cotton disease in the Mississippi delta. Pl. Dis. Rep. 20:272-273.

12. Miller, Paul R. 1938. A survey of cotton seedling diseases and the fungi associated with them. Pl. Dis. Rep. 22:260-263.

13. Miller, Paul R., and R. Weindling. 1939. A survey of cotton seedling diseases in 1939 and the fungi associated with them. Pl. Dis. Rep. 23:210-214.

14. Miller, Paul R. 1940. Microorganisms associated with cotton boll rot in 1939. Proc. 5th Ann. Meeting of Cotton Disease Council. Feb. 6-9. Birmingham, Ala. (mimeographed).

15. Shaw, Luther. 1936. Cotton diseases in North Carolina during the season of 1936. Pl. Dis. Rep. 20:347-348.

16. Smith, A.L., and H.W. Rankin. 1936. A cotton wilt survey in the Georgia coastal plain area in 1936. Pl. Dis. Rep. 20: 342-346.

17. Taubenhaus, J.J., and W.N. Ezekiel. 1932. Seed transmission of cotton wilt (Fusarium vasinfectum). Science 76: 61-62.

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