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To the Graduate Council:

I am submitting herewith a thesis written by Albert Young Chambers entitled "Evaluation of Fungicidal Soil Treatments in the Control of Seedling Diseases of Cotton." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agronomy.

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EVALUATION OF FUNGICIDAL SOIL TREATMENTS IN THE CONTROL OF SEEDLING DISEASES OF COTTON

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements of the Degree

Master of Science

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by

Albert Young Chambers

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CHAPTER I

INTRODUCTION

Seedling diseases of cotton, incited by seed- and soil-borne microorganisms, are widespread throughout the world where they pose a constant threat to cotton production. Seedling disease losses sustained by cotton growers in the United States amount to an estimated 73,000,000 dollars annually. Cotton farmers of Tennessee share over 1,000,000 dollars of these losses.

Reduction in yield due to poor stand is the most apparent result of seedling diseases, but the time and expense involved in replanting when a stand is lost constitute possibly an even greater loss. Another loss from seedling diseases, many times unrecognized, is the decreased vigor and yield in plants which survive but whose root systems are seriously damaged.

Attacks on cotton by seedling disease organisms are composed of two distinct types, each made up of a number of phases. In pre-emergence attacks, the cotton seed is rotted, or the young seedling is killed before emergence from the soil. In post-emergence injury, the emerging seedling is attacked slightly below the soil surface. The root system is damaged, and the taproot is completely rotted in severe cases. Lesions are produced on the stem of the seedling which may encircle it and cause the young plant to break over and die. Some of the common names for the post-emergence phases include "black root", "seedling blight", "damping-off", and "soreshin".

Chemicals used in seed treatments are effective in controlling those organisms infesting the seed but offer little protection from soil-borne pathogens. Attempts have been made in recent years to control the soil-borne organisms by treating the soil around the planted cotton seed with fungicides. This is commonly called "in-furrow" fungicide treatment. Results in most in-furrow fungicide experiments have not been consistent and have been discouraging.

Seedling diseases of cotton continue to be important in Tennessee. An important aspect of cotton production is the obtaining and maintenance of adequate, healthy stands from early plantings or in other periods of cool, damp weather when all conditions for disease development are optimum. The present study, therefore, was made in an effort to obtain consistent and usable data on in-furrow fungicide treatments in the control of seedling disease organisms. The objectives of the study were (1) to record the soil-borne organisms causing seedling diseases of cotton in Tennessee, (2) to study the effects of certain environmental factors on the growth and pathogenicity of the causal organisms and on disease development, and (3) to evaluate fungicides applied directly to the soil by in-furrow treatment in the control of seedling diseases of cotton.

CHAPTER II

REVIEW OF LITERATURE

As early as 1892, Atkinson (4) described the soreshin symptoms of cotton and reported that this post-emergence phase of seedling diseases was causing important losses in Alabama.

Rhizoctonia solani Kuehn was considered the causal organism of post-emergence injury for several years. From the work of Shapovalov (38) in 1925 and 1926 in which Fusarium spp. were also isolated from diseased cotton seedlings, the concept has arisen that the post-emergence seedling disease problem is caused by a disease complex in which any one or a combination of several fungi may cause typical disease symptoms. Weindling et al. (49) found F. moniliforme Sheld. and several other unidentified Fusarium spp. associated with R. solani on diseased cotton seedlings. Blank et al. (7) and Staffeldt (45) reported Thielaviopsis basicola (Berk. & Br.) Ferr., a tobacco root rot organism, to be a part of the cotton seedling disease complex. Brinkerhoff et al. (10) repeatedly isolated Pythium spp. from diseased cotton seedlings collected from field plots during periods of cold, damp weather when a pathogen other than R. solani was suspected. Ranney and Bird (26, 29) reported R. solani, Fusarium spp. and Pythium spp. to be the predominant organisms involved in the seedling disease complex. Other workers (6, 9, 13, 14, 15, 18, 39, 51) have found seedling diseases to be incited by several soil-borne fungi but reported R. solani to be the principal causal organism.

Considerable work has been done in studying the effects of environmental factors on seedling disease development and on the pathogenicity of

the causal organisms. Walker (48) reported an optimum temperature range for the death of cotton seedlings due to soreshin of 17 to 23° C. No minimum temperature for seedling mortality was established since the cotton seed used failed to germinate at temperatures below 15° C., but a maximum temperature of 34° C. was recorded. Ray and McLaughlin (33) found Glomerella gossypii (South.) Edg., R. solani, F. scirpi Lamb & Fautr., and F. moniliforme to be highly pathogenic at 80° F. on cotton seedlings in greenhouse tests. At 65° F. only G. gossypii and R. solani caused serious injury to plants. Blank et al. (7) observed that cool temperatures, around 69° F., were the most favorable for disease expression of T. basicola in cotton seedlings. Arndt (3) reported the temperature ranges at which 10 pathogens were able to attack cotton seedlings. Fulton and Bollenbacher (14) found R. solani and a Pythium sp. to be the most pathogenic of 22 fungi tested individually on cotton seedlings in the laboratory and greenhouse at a temperature of 21° C. Fusarium spp. were only moderately to mildly pathogenic in this test. In general, all of the fungi tested were more pathogenic when incubated at 21° C. than when incubated for 1 week at 27° C. and then changed to 16° C. for 2 weeks.

Walker (48) reported that any soil moisture level at which cotton would grow was sufficient for penetration of cotton seedlings by pathogenic fungi if the soil temperature were favorable. Kernkamp et al. (19) pointed out that soil moisture had less effect than soil temperature on the pathogenicity of R. solani on cotton seedlings. Ray and Mclaughlin (33) found that watering cotton seedlings heavily several times a day in the greenhouse generally increased the pathogenicity of seedling disease organisms while

watering just enough to keep the plants growing slightly decreased pathogenicity.

Kadow and Anderson (17) observed that R. solani was able to grow in culture media having a minimum pH of 2.4 up to a maximum pH of 10.4 with an optimum of near neutral or slightly acid. A Pythium sp. grew on media ranging in pH from 3.0 to 8.5 with optimum cultural growth occurring between pH 4.5 to 6.5. Ray and McLaughlin (33) reported that R. solani and G. gossypii were pathogenic to cotton seedlings over a wide range of soil pH levels.

Kadow and Anderson (17) found little difference in seedling disease development due to soil type, but light sandy soils were somewhat less favorable for damping-off.

Kernkamp et al. (19) observed that the pathogenicity of R. solani on cotton seedlings was enhanced by the presence of other fungi.

Neal and Newsom (25) reported that cotton seedlings weakened by thrip damage were more susceptible to soreshin. Control of these insects helped to control soreshin to some extent.

Reynolds and Hanson (34) found that the incidence of cotton seedling diseases caused by R. solani increased when root-knot nematodes were present in the soil. They also found that other factors which affect seedling vigor, such as mechanical injury, contribute to the amount of damping-off.

Wiles (53, 54) reported that a deficiency of calcium in the upper one inch of soil due to leaching or other factors, even in a highly alkaline soil, may increase the incidence of seedling diseases of cotton.

Reginned seed rolled in gypsum produced vigorous plants which were less affected by seedling diseases. He suggested that the beneficial effects of many fungicides were derived from the calcium diluents used in the materials rather than from the active fungicidal chemicals.

Bollenbacher and Fulton (8) stated that seedlings from artificially deteriorated cotton seed were more susceptible to pathogenic organisms than seedlings from undeteriorated seed.

A number of workers (1, 9, 10, 12, 20, 21, 22, 32, 33, 42) have tested many fungicides for treating cotton seed in an effort to control seedling diseases. Some of the materials tested gave effective control of seed rot and pre-emergence seedling mortality, but none gave adequate protection from post-emergence seedling damage.

The in-furrow fungicide method of application of chemicals in the control of seedling diseases of cotton stemmed from a laboratory testing technique described by Arndt (2). He mixed fungicides with soil used to cover cotton seed during planting and obtained significant protection from R. solani with treatments of Dowicide F, Seedox, and Mathieson 275-D during a 12-day culture period.[1] Godfrey (16) reported that in-furrow treatments with Mathieson 275 and Dithane Z-78 in both dust and spray application gave very outstanding increases in stand over untreated plots in a field in which cotton was severely damaged by seedling diseases in the two previous seasons. Significant increases in stand were also obtained with Stauffer N-521, Dithane D-14, Arasan, and Vancide 51-ZW.

1/ The chemical composition of fungicides is given in Appendix A.

Brinkerhoff et al. (9) found that Arasan and Thiram 50 were fairly consistent in controlling R. solani injury to cotton seedlings in the greenhouse in artificially inoculated soil which had not been sterilized. Mathieson 275 and Dithane Z-78 gave comparable results in later tests. Arasan, Thiram 50, and Mathieson 275 were the least injurious to cotton seedlings of the fungicides tested. Brinkerhoff et al. (10) obtained excellent control of R. solani with Mathieson 275 dust applied in the seed row in artificially inoculated field plots, but the same chemical was ineffective in naturally infested field plots from which Pythium spp. were repeatedly isolated. Dithane Z-78 and Thiram 50 gave reasonably good protection in the same naturally infested plots while Orthocide 50 was variable. Cooper (11) reported highly significant cotton stand increases, almost two-fold, from in-furrow applications of Captan 50W and Arasan, but Stauffer N-521, Dithane Z-78, and Mathieson 275 were not effective.

Whitehead and Brown (51) tested 10 fungicides separately and in certain combinations applied in the seed furrow in inoculated and non-inoculated plots as possible protectants against cotton seedling diseases. Five pathogenic species of fungi were used for inoculation. Treatments with Captan 50W, Dithane D-14, Dithane D-14 plus Terraclor, and Dithane D-14 plus zinc sulfate gave significantly higher plant populations. Two mercury seed treating compounds were toxic to seedlings when applied in the furrow at rates comparable to the rates at which the other fungicides were used.

Sinclair (39) screened 17 fungicides, alone and in combination, in the laboratory and greenhouse and found that Terraclor, Captan 50W, Dithane D-14, and combinations of these materials were the most promising in

controlling R. solani when applied to the soil in the seed furrow. In later greenhouse tests, he (40) screened 42 fungicides and antibiotics for the control of R. solani. Terraclor, Captan 50W, Dithane D-14, and Phygon XL gave the best control when used alone while combinations of these materials were even better. Sinclair et al. (43) obtained significantly better stands of cotton in field tests by in-furrow applications using Terraclor plus Captan 50W plus Dithane Z-78, Captan 50W plus Dithane Z-78, Captan 50W plus Terraclor, Terraclor plus Phygon XL, Terraclor plus Dithane D-14, and Dithane D-14 plus calcium chloride. Erwin et al. (13) reported that field plots treated in the seed furrow at planting time with Terraclor, Terraclor plus Captan 75W, Terraclor plus Dithane Z-78, and Dithane Z-78 had significantly better emergence and survival of cotton seedlings than those plots receiving no treatment.

Bird et al. (6) tested Terraclor, Captan 50W, Dithane D-14, Dithane Z-78, Arasan, and Vancide 51 alone and in combination by in-furrow application to the seed furrow at different locations and in different soil types. No single fungicide or mixture gave good results in all locations or soil types. Wiles (52) reported the results of the in-furrow experiments for 4 years. Dithane D-14, Captan 50W, Terraclor, and combinations involving Terraclor plus other fungicides gave uniform stands with significantly higher plant populations in some seasons but not in others.

Sinclair (41) found that Captan 50W plus Terraclor and Dithane D-14 plus Terraclor gave highly significant increases in percentages of healthy cotton seedlings in field plots inoculated with 4 isolates of R. solani. Kennedy and Brinkerhoff (18) obtained good protection for cotton seedlings

against R. solani with in-furrow treatments of Dithane Z-78, Terraclor, Tersan 75, and Orthocide 50. Orthocide 50, Tersan 75, and Dithane Z-78 were effective against P. debaryanum, but Terraclor was ineffective. None of the fungicides was effective when the environment favored severe disease development.

Bird and Ranney (5) and Ranney and Bird (27, 28) reported that effective control of seedling diseases of cotton was obtained with in-furrow application of a combination of three fungicides, Captan 50W, Dithane Z-78, and Terraclor at 1 1/2, 2, and 1 1/2 pounds respectively per acre. This combination of materials gave consistent results over a wide range of locations with different soil types, temperatures, and pH levels. Another fungicide, Dithane D-14, was found to be effective but only on soils with a pH less than 7.3.

Some work has been done in an attempt to evaluate the effects of a number of environmental factors on the effectiveness of in-furrow fungicide treatments in controlling seedling diseases of cotton. Rushdi and Jeffers (36, 37) tested the effects of 7 soil factors on the effectiveness of 4 fungicides, Puratized Agricultural Spray, Vancide 51-ZW, Dithane D-10, and Acti-dione, in controlling R. solani. Increasing amounts of sand in a soil increased the effectiveness of the fungicides. Increases in pH from 4.6 to 7.1 decreased the effectiveness of Acti-dione but increased the effectiveness of the others. Moisture level or soil temperature did not affect fungicidal efficiency. The addition of 1% of starch or 1% of hay or wheat straw to the soil greatly reduced the effectiveness of Dithane D-10 and Puratized Agricultural Spray. The addition of 1% of casein had no effect, but adding of 5% clover hay reduced the effectiveness of all the

fungicides.

Ranney and Bird (26) found that, in controlled-temperature experiments, Dithane Z-78, Captan 50W, Terraclor, and other fungicides did not give the same seedling disease control at 70° F. and at 80° F. The variable results in naturally infested soil were attributed to changes in the soil microflora due to temperature. Isolations from diseased cotton seedlings indicated that Fusarium spp. were predominant at 80° F., but Fusarium spp., Phythium spp., and R. solani were all active at 70° F., Fulton et al. (15) found that the date of planting of cotton influenced the effectiveness of in-furrow treatments with Terraclor. They suggested that variations in soil temperature at different planting dates affected the soil microflora as to which organisms were predominant. They added further that many fungicides are specific in that they will control only one or a few fungi.

Ranney and Hillis (30) reported that the pattern of placement and dispersion of the fungicide obtained by in-furrow application affected the control of seedling diseases of cotton in a study using a fluorescent indicator to show the placement pattern and degree of dispersion. Brinkerhoff et al. (9) stated that thorough mixing of fungicides with the soil of the seed furrow increased the protection afforded cotton seedlings from soil pathogens.

Munnecke (23) studied the persistence in the soil of 4 fungicides, Captan 50W, Semesan, Dithane D-14, and Fermate. Captan 50W was stable up to 150 days, but Dithane D-14, Semesan, and Fermate were rapidly inactivated. Erwin et al. (13) also found that Dithane D-14 retained its

effectiveness in the soil for only a short time.

Seedling diseases of cotton may continue to cause losses beyond the seedling stage. Neal (24) noted that seedling disease organisms attacked cotton in the flowering stage after cold, wet weather in late spring. Many of the plants were almost devoid of root systems and were growing prostrate. Some of the stems were nearly severed by deep-seated cankers. Stewart and Whitehead (46, 47) described a mature plant phase of seedling diseases in cotton called "nub-root". This condition results from the rotting of the taproot and the proliferation of lateral roots if sufficient moisture prevails in the seedling stage. They suggested that nub-root causes greater yield reduction than loss in stand due to damping-off.

CHAPTER III

METHODS AND PROCEDURE

Experimental work for the present study was carried out in the laboratory, greenhouse, and field plots of the Plant Pathology Department at the West Tennessee Agricultural Experiment Station at Jackson and in field plots on the Davis Brothers' Farm in Tipton County. The purposes of the study were to isolate and record the soil-borne organisms causing seedling diseases of cotton in Tennessee, to study the effects of certain environmental factors on the growth and pathogenicity of the causal organisms and on disease development, and to test various fungicides applied as in-furrow fungicide treatments in the control of seedling diseases.

Isolation of Seedling Disease Organisms

Soil samples collected and planted to cotton

Thirty bulk soil samples were obtained by sampling three cotton fields in each of Crockett, Dyer, Fayette, Gibson, Haywood, Lake, Lauderdale, Madison, Shelby, and Tipton Counties in 1958. Each sample was used to fill pots in the greenhouse. The pots were planted with cotton seed of the Tennessee 19 experimental variety that had been acid-delinted, gravity separated, dried, and treated with Panogen 15 at 1 ounce per bushel to eliminate seed-borne organisms. A low greenhouse temperature, kept as closely to 65° F. as possible, and a high moisture level in the pots were maintained to induce a high incidence of seedling diseases.

Isolation of organisms

Ten diseased seedlings were selected from the pots of each bulk samples and washed in running water for 48 hours. Stem sections of diseased tissue approximately 1/2 inch in length were cut from the 10 seedlings, surface sterilized by dipping momentarily in 70% isopropyl alcohol, immersed in a 1% solution of sodium hypochlorite for 2 minutes, and plated in petri dishes containing potato-dextrose agar (PDA). One drop of 20% lactic acid was added to each 15 ml. of medium just before pouring to control bacterial contamination and allow the growth of such organisms as Pythium spp. The culture dishes were incubated at 20° C., and transfers of the different organisms growing from the stem sections were made to test tube slants of PDA. Transfers of Penicillium spp. and Rhizopus spp. were not maintained since these fungi are usually non-pathogenic. Hyphal tip transfers were made later from each culture. The culture medium used throughout this study was PDA prepared as described by Riker and Riker (35).

Tests for pathogenicity of isolates

Each isolate obtained was tested for pathogenicity according to Koch's postulates. Equal quantities of inoculum of each isolate were mixed with sterilized sandy loam soil in 4 pots. The soil in the pots was kept moist by adding sterile water as needed. The inoculum was incubated for 2 weeks in the greenhouse, and 5 cotton seed of the lot described earlier were sown in each pot. After 10 days, the cotton seedlings were lifted from the pots and examined for symptoms of seedling diseases. Three seedlings were selected from the pots of each isolate which produced

disease symptoms, and the isolation methods described above were used to obtain the disease organism in pure culture. Each organism re-isolated was compared with the original isolate and identified by macroscopic and microscopic examination.

Tests for virulence of pathogenic isolates

The degree of pathogenicity of each of the pathogenic isolates obtained in the previous tests was measured on cotton seedlings in two separate tests. In the first test, a petri dish method was used in which 3 apparently healthy cotton seed of the lot described earlier were placed on moistened blotter paper in a sterile petri dish around each of 3 squares (approximately 6 mm. by 6 mm.) of inoculum on PDA of the isolate being tested. Sterile water was added periodically to the blotter paper to insure good germination and seedling growth. Three inoculated dishes and one uninoculated control dish were used to test the virulence of each isolate. All dishes were incubated for a period of 10 days at a temperature of 20° C.

In the second test, 4 equal quantities of inoculum of each pathogenic isolate were mixed with sterilized sandy loam soil in 4 pots. A control pot which contained sterilized soil but no inoculum was included for each isolate. The soil in the pots was kept moist by adding sterile water as needed. After the inoculum had incubated for 2 weeks in the greenhouse, 5 cotton seed of the lot used in the first test were planted in each pot. The pots remained in the greenhouse for 10 days.

The seedlings in the inoculated petri dishes and pots were examined and compared with the controls. Each pathogenic isolate was rated for

virulence according to the disease severity symptoms manifested on the cotton seedlings. A disease index rating scale is given in table 1.

One of the more virulent isolates of each of the 13 pathogenic fungi identified was selected, and subcultures of these representative isolates were used in obtaining additional inoculum for use in succeeding tests.

Testing of the Effects of Environmental Factors on the Growth and Pathogenicity of Causal Organisms

Effects of temperature on fungous growth

Comparative growth rates at 8 temperatures were recorded in the following way for the 9 pathogenic fungi isolated most frequently. A petri dish containing 15 mm. of PDA was inoculated in the center with a 6-mm. disk of inoculum cut from the outer edge of a vigorously growing culture. Six subcultures of each fungus were incubated at 5, 10, 15, 20, 25, 30, 35, and 40° C. over a 48 hour period. The growth of a culture was then obtained by averaging 4 measurements taken across the colony and subtracting the diameter of the inoculum disk. The mean growth for each fungus at each temperature was obtained by averaging the colony growth in 6 dishes.

Effects of soil pH

The effects of soil pH on seedling emergence, pathogenicity of the inciting organisms, disease development, and effectiveness of a fungicidal treatment were measured in 3 separate tests. Soil from a Lintonia silt loam was placed in greenhouse flats and autoclaved for 1 hour at 15 pounds

Table 1.--Disease index rating scale used to indicate the degree of pathogenicity of fungal isolates tested by the petri dish-blotter paper and inoculated pot methods

Pathogenicity rating	Description of symptoms and damage	
	Petri dish	Inoculated pot
1 - slightly	Only a small amount of fungal growth and damage on roots and stems of seedlings.	No evidence of injury observable above the soil line, yet the roots visibly attacked.
2 - moderately	Considerable fungal growth and damage on roots and stems of seedlings.	Slight wilting of seedlings and roots obviously attacked.
3 - severely	Serious damage with seedlings killed soon after germination.	Wilting of seedlings with roots seriously rotted and resulting seedling mortality.
4 - very severely	Very serious damage with seedlings killed before complete germination.	All seedlings killed quickly and few emerge from the soil.

pressure. The autoclaved soil had a pH of 5.5. Each flat was divided into 3 sections, one of which was adjusted to pH 6.5 and another to 7.5 by adding hydrated lime. The remaining section was retained at pH 5.5. In each test 3 flats were used, each of which constituted a replicate.

In the first test, the effects of different soil pH levels on seedling emergence were studied. Twenty-five reginned cotton seed, Empire variety treated with Ceresan 100, were planted in each section of the three flats. After the flats were maintained in the greenhouse for 2 weeks, stand counts were taken. The results were analyzed by an analysis of variance to find if there were significant differences in seedling emergence due to soil pH.

In a second test, the effects of different soil pH levels on pathogenicity and disease development as measured by seedling survival were studied. The pH values of the soil in the sections of the flats were adjusted as previously described. Grain sorghum inoculum of each of the 13 pathogenic fungi was prepared by using the techniques of growing inoculum as described by Whitehead (50). The grain sorghum inoculum of each of the 13 fungi was combined into a composite mixture in approximately the same weight proportion as the isolates of each fungus were of the total pathogenic isolates obtained. For example, R. solani made up approximately 24% of the pathogenic isolates; therefore 24 g. of R. solani sorghum inoculum was used in each 100 g. of the composite mixture of the 13 fungi. Five g. of the composite inoculum was used for inoculating each of the three sections of a flat. The inoculum was mixed with the soil in the flats 1 week previous to planting with cotton seed as in the first

test. Stand counts were made after 2 weeks and analyzed as described previously. Statistical methods described by Snedecor (44) were used throughout the present study.

In the last test, the effects of different soil pH levels on fungicidal effectiveness as measured by the survival of healthy seedlings were tested. The pH adjustments and flat inoculations were carried out as described previously. During the planting process the soil covering the seed in each flat section was mixed with a fungicidal combination including Orthocide 50, Dithane Z-78, and Terraclor at 1 1/2, 2, and 1 1/2 pounds per acre respectively. Stand count data were taken after 2 weeks and analyzed as in the preceding tests.

Effects of different soils

The effects of different soils on seedling emergence, pathogenicity of the disease-causing organisms, disease development, and effectiveness of a fungicidal treatment as measured by the survival of healthy seedlings were studied in 3 tests similar to those described for soil pH. Soil was obtained from a Robinsonville sandy loam, a Lintonia silt loam, and a Dexter silty clay loam. The sections of each greenhouse flat were filled with a different soil. Each test was made up of 3 flats or replicates, and the soils were randomized in the flats. Sterilization of the soil, inoculation, planting, fungicidal treatment, and greenhouse conditions were identical to the pH test. Taking of stand count data and analysis of results were also identical.

Testing of Fungicides

Laboratory tests

Six fungicides were tested at four rates each in two laboratory experiments for the control of the three predominant genera of the seedling disease organisms isolated. R. solani, F. oxysporum f. vasinfectum (Atk.) Snyd. & Hans., and P. ultimum Trow were used in this test. Orthocide 50, Phygon XL, Terraclor, Arasan 75, and Dithane Z-78 were tested at rates of 3, 4, 5, and 6 pounds per acre. Dithane D-14 was tested at 1/2, 3/4, 1, and 1 1/4 gallons per acre.

In the first test, distilled water solutions or suspensions of the fungicides were added to PDA just before pouring into petri dishes. The wettable powders were added to the medium at 240, 200, 160, and 120 ppm. The liquid fungicide Dithane D-14 was added at 450, 360, 270, and 180 ppm. These rates were calculated to be equal to the pounds per acre and gallons per acre rates. The petri dishes were inoculated with 6-mm. disks cut from the edge of vigorously growing cultures of the 3 fungi. Three dishes of each dilution of a fungicide were used to test for inhibition of growth of each fungus. Three untreated control dishes were included with the dishes of each fungus. The inoculated dishes were incubated at the optimum growth temperature of each fungus as recorded in the temperature test, and colony growth measurements were made of R. solani and P. ultimum after 48 hours and of F. oxysporum f. vasinfectum after 96 hours. The results were analyzed by an analysis of variance to find if certain treatments gave significantly better inhibition of each fungus than no treatment.

The same fungicidal treatments were evaluated in a second test in which the methods used were a modification of the testing techniques described by Zentmyer (55). Twenty-gram aliquots of autoclaved sandy loam soil were placed in test tubes above a small cotton wad inserted in the tube to provide drainage and prevent saturation of the soil. An inoculum disk of 1 of the 3 fungi was placed 1/2 inch below the surface of the soil in each tube. Five milliliters of fungicidal solution or suspension were added to the soil in each test tube. Each rate of each fungicide was repeated 3 times for each fungus. The fungicidal solutions or suspensions added for each rate contained the fungicides in amounts necessary to bring the soil plus the solution rate of treatment to the ppm. rates used in the first test. Three control test tubes, treated only with distilled water, were included for each fungus. After incubation for 48 hours, the soil in the tubes was placed on aluminum screen wire, and the soil was washed through with a fine stream of water. The inoculum disks were picked up with sterile forceps and placed on PDA in petri dishes. Incubation conditions, measurements made, and statistical analysis were similar to the first laboratory fungicide test.

Greenhouse tests

In the first of 2 greenhouse tests, the 6 fungicides used in the laboratory experiments were evaluated at the same rates in an additional experiment in the greenhouse. Clay pots 4 inches in diameter were filled with autoclaved sandy loam soil. The soil in each pot was inoculated by mixing 1/2 of a petri dish subculture of 1 of the 3 disease inciting fungi into the upper inch of soil. The inoculum was incubated for 2 weeks in

the greenhouse, and 10 acid-delinted seed of the Tennessee 19 lot described previously were planted in each pot. The soil in the pots used to cover the seed was treated by spraying with the respective fungicides during the planting operation. Four pots of each fungus were included for each rate of each fungicide, and four untreated pots inoculated with each fungus were included as checks. All pots were maintained in the greenhouse for 1 month, after which stand counts were made. Effectiveness of the different treatments was ascertained by making an analysis of variance of the stand counts.

In a second greenhouse test, 14 treatments, made with fungicides showing promise in earlier tests applied alone and in various combinations, were evaluated for control of all 13 pathogenic fungi in greenhouse flats planted to cotton. Soil consisting of 1/2 compost and 1/2 washed river sand was sterilized with methyl bromide and placed in flats partitioned into 3 sections. The soil in each section was inoculated with 5 g. of a composite grain sorghum inoculum prepared as described previously. The inoculum was incubated in the flats for 2 weeks in the greenhouse, and 25 reginned cotton seed (Deltapine 15 variety) treated with Panogen 15 were planted in each section. The fungicides tested were sprayed at 5 pounds of wettable powder or 1 gallon of liquid per acre on the open seed furrow and on the soil used to cover the seed furrow as the soil was closed over the planted seed. A randomized block design with 3 replications was used. One untreated section was included in each replication. One month after planting stand counts were made, and an analysis of variance was made to find if stands were improved

significantly by the various fungicidal treatments.

Field tests

Eight fungicides that gave the highest stand counts in the greenhouse tests were evaluated in both inoculated and uninoculated field plots at Jackson in 1959. Three-row plots that were 9 by 20 feet in size were used. A randomized block design with 4 replications was used for both the uninoculated and inoculated tests, and the same randomization was used for both tests. An untreated check plot was included in each replication.

In the inoculated test, the plots were inoculated with 240 g. of composite grain sorghum inoculum per plot or at the rate of 4 g. of inoculum per foot of row. The inoculum was applied to the center of each row with a Planet Jr. garden planter and placed directly under where the cotton seed would be planted later. The plots were planted 2 weeks after inoculation. The fungicides were applied at 5 pounds of wettable powder or 1 gallon of liquid in 10 gallons of water per acre during the planting operation with a planter that had been adapted for spray in-furrow application (see figure 1). A low pressure sprayer was mounted on the tractor, and one spray nozzle was placed directly behind the planter sword to treat the open seed furrow, the seed, and soil pushed into the seed furrow by the packer wheel. The other nozzle was attached behind the packer wheel and sprayed a narrow band on the filled seed furrow. The front nozzle sprayed at twice the rate of the rear nozzle. A pressure of 40 psi. was used for spraying. The applicator used was a modification of the equipment described by Ranney and Hillis (31).



Figure 1.--A planter adapted to apply in-furrow fungicide treatment during the planting operation.

Reginned seed (Empire variety) that had been treated with Ceresan 100 were planted at 1 bushel per acre on May 11. An extra man rode the tractor during planting to observe the dropping of the seed by the planter to insure that the number of seed planted was as uniform as possible with the planting equipment used. Stand counts were made of all of the cotton seedlings surviving in the center row of each plot after 1 month, and the data were analyzed by an analysis of variance to ascertain the effectiveness of the treatments.

In the uninoculated test, the methods of planting, application of fungicides, taking of data, and statistical analysis were identical to those used in the inoculated test.

In a third field test, the three fungicidal treatments that gave the highest stand counts in the greenhouse tests were included in an experiment on the Davis Brothers' Farm in Tipton County. A Latin square design with four replications was used, and a check plot was included in each replicate. Plot size was the same as that in the two previous tests. The same tractor and planter were used for planting and fungicide application as were used in the previous tests. Reginned seed (Empire variety) treated with Ceresan M were planted at 1 1/2 bushels per acre on April 30. An extra rider observed the seed flow as described previously. Stand counts were taken as described earlier, and the data were analyzed to find if the treated plots had significantly improved stands over the untreated plots.

CHAPTER IV

RESULTS

Recording of Seedling Disease Organisms

Organisms isolated

Cotton seedlings were grown in 30 soil samples collected from 10 West Tennessee counties, and a total of 465 isolates of fungi were obtained from the diseased plants produced. The isolates were tested for pathogenicity on cotton seedlings, and the pathogenic isolates reisolated according to Koch's postulates. The different species and forms identified after reisolation and the number of times that each was isolated originally are listed in table 2. The percentage that the isolates of each fungus were of the total is also shown. Approximately 95% of the isolates were pathogenic on cotton seedlings with R. solani, F. oxysporum f. vasinfectum, and F. moniliforme making up 64% of the total. The number of nonpathogenic fungi isolated was small since Penicillium spp. and Rhizopus spp., easily recognized and usually considered to be nonpathogenic, were eliminated during isolation.

Pathogenicity ratings of isolates

Each of the pathogenic isolates was tested for degree of pathogenicity on cotton seedlings in petri dishes as previously described. For illustration a petri dish with cotton seedlings was very severely damaged by an isolate of R. solani and a control dish with normal seedlings are shown in figure 2. Each isolate of the 13 pathogenic fungi

Table 2.--Number of isolates of different fungi obtained from diseased cotton seedlings grown in soil samples from 10 counties in West Tennessee, 1958

Name of organism	No. of isolates	Percent of total
Fusarium decemcellulare Brick.	26	5.6
F. moniliforme Sheld.	92	19.8
F. oxysporum f. conglutinans (Wr.) Snyd. & Hans.	6	1.3
F. oxysporum f. vasinfectum (Atk.) Snyd. & Hans.	100	21.5
F. semitectum Berk. & Rav.	11	2.4
F. solani (Mart.) App. & Wr.	40	8.6
Helicobasidium purpureum (Tul.) Pat.	8	1.7
Pellicularia rolfsii (Curzi) West	1	0.2
Pythium debaryanum Hesse	6	1.3
P. ultimum Trow	39	8.4
Rhizoctonia solani Kuehn	106	22.8
Sclerotium bataticola Taub.	6	1.3
Thielaviopsis basicola (Berk. & Br.) Ferr.	1	0.2
Nonpathogenic Fungi	23	4.9

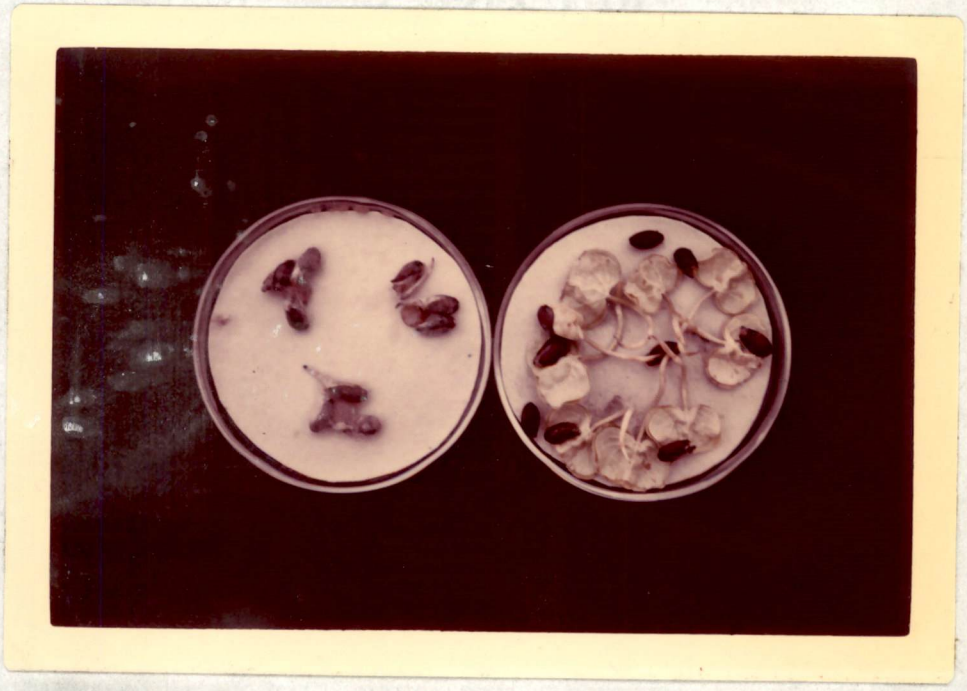

Figure 2.--The petri dish on the left contains cotton seedlings with very severe damage by an isolate of Rhizoctonia solani; the other dish contains normal seedlings.

was given a pathogenicity rating, and these are listed in table 3. The results of a further test for relative pathogenicity in which cotton seedlings were grown in pots inoculated with the pathogenic isolates are given in table 4. The pathogenicity ratings were similar in both tests for all of the fungi with the exception of the Fusarium spp. which were less pathogenic in soil than in petri dishes. The Fusarium spp. were generally less pathogenic in both tests than were the other fungi. A greater proportion of the isolates of R. solani and P. ultimum had higher pathogenicity ratings than the other fungi isolated frequently.

Environmental Factors Affecting Growth and Pathogenicity of Pathogens

Effects of temperature

Growth rates on PDA of a representative isolate of each of the 9 fungi obtained most frequently were measured at 8 temperatures. The mean growth rates per 48 hours are shown in table 5. R. solani and H. purpureum (Tul.) Pat. (a Rhizoctonia-type fungus) made their most rapid growth at 20° C. P. ultimum and the Fusarium spp. grew faster at higher temperatures. All the fungi tested grew over a fairly wide range of temperatures, from 15 to 35° C. with the optimum for most being 25° C.

Effects of soil pH

The results of a test to study the effects of different soil pH levels on seedling emergence, pathogenicity of the causal organisms, disease development, and fungicidal effectiveness are summarized in

Table 3.--Number of isolates of species and forms of pathogenic fungi at each pathogenicity level when tested on cotton seedlings by the petri dish-blotter paper method

Name of organism	Pathogenicity rating			
	1[a]	2	3	4
Fusarium decemcellulare	1		25	
F. moniliforme	6	26	60	
F. oxysporum f. conglutinans		2	4	
F. oxysporum f. vasinfectum		26	74	
F. semitectum		4	7	
F. solani		9	31	
Helicobasidium purpureum		2		6
Pellicularia rolfsii			1	
Pythium debaryanum			3	3
P. ultimum		2	3	34
Rhizoctonia solani		5	8	93
Sclerotium bataticola				6
Thielaviopsis basicola			1	

a/Ratings were made as follows: 1 - slightly pathogenic, 2 - moderately pathogenic, 3 - severely pathogenic, 4 - very severely pathogenic.

Table 4.--Number of isolates of species and forms of pathogenic fungi at each pathogenicity level when tested on cotton seedlings by the inoculated pot method

Name of organism	Pathogenicity rating			
	1[a]	2	3	4
Fusarium decemcellulare	1	25		
F. moniliforme	8	83	1	
F. oxysporum f. conglutinans		6		
F. oxysporum f. vasinfectum	16	84		
F. semitectum	3	8		
F. solani	1	39		
Helicobasidium purpureum			2	6
Pellicularia rolfsii			1	
Pythium debaryanum			3	3
P. ultimum		2	1	36
Rhizoctonia solani		8	8	90
Sclerotium bataticola				6
Thielaviopsis basicola		1		

a/Ratings were made as follows: 1 - slightly pathogenic, 2 - moderately pathogenic, 3 - severely pathogenic, 4 - very severely pathogenic.

Table 5.--Growth rates in millimeters per 48 hours of 9 fungi causing seedling diseases of cotton when grown at various temperatures on agar medium

Name of organism	Temperature in °C.							
	5	10	15	20	25	30	35	40
Fusarium decemcellulare	0[a]	0	7	12	24	20	13	0
F. moniliforme	0	0	6	19	26	21	13	0
F. oxysporum f. conglutinans	0	0	8	16	24	26	8	0
F. oxysporum f. vasinfectum	0	2	5	17	31	27	14	0
F. semitectum	0	0	6	12	20	21	12	0
F. solani	0	0	6	14	21	13	2	0
Helicobasidium purpureum	1	5	13	58	40	14	13	0
Phythium ultimum	0	1	37	55	82	76	54	0
Rhizoctonia solani	0	0	15	69	68	65	32	0

a/Each figure represents an average of 24 measurements: 4 per petri dish and 6 petri dishes.

Table 6. Seedling emergence was not measurably affected by different soil pH levels. A very high germination of seed and seedling stand were obtained at all three pH levels. The soil pH also had no measurable effect on pathogenicity and disease development. All of the cotton seedlings were killed quickly at the three pH values. The effectiveness of the fungicide, a mixture of Orthocide 50, Dithane Z-78, and Terraclor, was not significantly affected by pH level as measured by stand count differences. Excellent control of seedling diseases was obtained at all three pH levels.

Effects of different soils

The pathogenicity of 13 organisms on cotton and the effectiveness of a fungicide combination in controlling these pathogenic organisms were tested in 3 different soils in 3 separate tests. The results of the tests are summarized in table 7. No measurable difference was found in seed germination and seedling emergence in the three soils. The three soils had little or no effect on virulence of the disease-inciting organisms as measured by stand count differences. All of the plants were killed in the soil from the Robinsonville sandy loam, but there were no significant differences in stand even though a few plants survived in the other two soils. Fungicidal effectiveness was not measurably affected by the different soils. Excellent stands of seedlings were obtained in all three soils by treatment with the Orthocide 50, Dithane Z-78, and Terraclor combination.

Table 6.--Average stand counts of cotton seedlings after 2 weeks in greenhouse flats at 3 soil pH levels having 3 different treatments

Soil pH	Sterile[a] soil	Inoculated[b] soil	Treated[c] soil
5.5	24.3[d]	0.0	17.3
6.5	23.7	0.0	17.3
7.5	21.0	0.0	20.7
L. S. D. (.05)	N.S.	N.S.	N.S.

a/The soil used in the first test was sterilized by autoclaving for 1 hour at 15 psi.

b/Sterile soil was inoculated with a composite grain sorghum inoculum of the 13 pathogenic fungi isolated for the second test.

c/In the last test, the soil was sterilized, inoculated with the disease fungi, and treated with a fungicide mixture including Orthocide 50, Dithane Z-78, and Terraclor as the seed were being covered during planting.

d/Each figure represents an average of the number of seedlings in the 3 flat sections of each treatment from 25 seed planted per section.

Table 7.--Average stand counts of cotton seedlings after 2 weeks in greenhouse flats containing 3 different soils having 3 different treatments

Soil	Sterile[a] soil	Inoculated[b] soil	Treated[c] soil
Robinsonville sandy loam	24.3[d]	0.0	21.3
Lintonia silt loam	23.7	5.0	23.7
Dexter Silty clay loam	22.7	3.7	24.0
L. S. D. (.05)	N.S.	N.S.	N.S.

a/The soil used in the first test was sterilized by autoclaving for 1 hour at 15 psi.

b/Sterile soil was inoculated with a composite grain sorghum inoculum of the 13 pathogenic fungi isolated for the second test.

c/In the last test, the soil was sterilized, inoculated with the disease fungi, and treated with a fungicide mixture including Orthocide 50, Dithane Z-78, and Terraclor as the seed were being covered during planting.

d/Each figure represents an average of the number of seedlings in the 3 flat sections of each treatment from 25 seed planted per section.

Results of Fungicidal Testing

Laboratory tests

In the first laboratory test, the amount of growth of F. oxysporum f. vasinfectum, P. ultimum, and R. solani on PDA to which 6 fungicides each at 4 rates had been added was measured. The results of this test are presented in table 8. All treatments, with the exception of Dithane Z-78 at 3 pounds per acre, gave very highly significant inhibition of the growth of F. oxysporum f. vasinfectum. Only Dithane D-14 at 1 1/4, 1, and 3/4 gallons per acre rates completely inhibited growth of this fungus. Growth was slight on the medium containing Arasan 75. Orthocide 50, Dithane D-14, Phygon XL, and Arasan 75 were very effective in inhibiting the growth of P. ultimum. Terraclor and Dithane Z-78, with the exception of the 4-pound rate which was significant, did not inhibit growth of P. ultimum. Dithane D-14 at all rates and Phygon XL at 6 pounds per acre completely stopped the growth of this fungus. All treatments gave very highly significant reduction of the growth of R. solani. The three highest rates of Dithane D-14 completely inhibited growth.

Results of the second laboratory test in which the same treatments were evaluated by drenching soil which contained an inoculum disk of F. oxysporum f. vasinfectum, P. ultimum, or R. solani are presented in table 9. Treatments with Dithane D-14, Terraclor, Arasan 75, Dithane Z-78 at 5 and 6 pounds per acre, and Orthocide 50 at 4 pounds per acre gave very highly significant control of F. oxysporum f. vasinfectum. Dithane Z-78 at 3 and 4 pounds and Phygon XL at 4 pounds significantly

Table 8.--Growth in millimeters of F. oxysporum f. vasinfectum, P. ultimum, and R. solani on agar medium in petri dishes mixed with 6 fungicides at 4 rates each

Treatment	Rate per acre[a]	Test organism		
		Fusarium	Pythium	Rhizoctonia
Untreated		22.3[b]	71.7	62.0
Orthocide 50	6 lb.	8.7**	8.7**	6.0**
	5 lb.	10.3**	13.7**	6.7**
	4 lb.	8.0**	15.3**	8.7**
	3 lb.	11.3**	9.7**	9.7**
Dithane D-14	1 1/4 gal.	0.0**	0.0**	0.0**
	1 gal.	0.0**	0.0**	0.0**
	3/4 gal.	0.0**	0.0**	0.0**
	1/2 gal.	1.3**	0.0**	0.7**
Phygon XL	6 lb.	8.3**	0.0**	9.7**
	5 lb.	9.7**	10.0**	8.0**
	4 lb.	12.0**	25.0**	11.0**
	3 lb.	9.0**	36.0**	13.3**
Terraclor	6 lb.	9.7**	73.3	17.7**
	5 lb.	9.7**	73.0	18.0**
	4 lb.	9.0**	72.3	24.7**
	3 lb.	9.3**	73.0	21.7**
Arasan 75	6 lb.	1.0**	4.3**	5.0**
	5 lb.	2.0**	4.3**	4.7**
	4 lb.	1.3**	5.7**	5.7**
	3 lb.	1.7**	9.0**	5.7**
Dithane Z-78	6 lb.	15.7**	69.0	23.3**
	5 lb.	18.0**	68.3	24.3**
	4 lb.	18.0**	63.0*	25.7**
	3 lb.	20.0	73.7	28.7**
L. S. D. (.01)		3.8	11.2	2.8
(.05)		2.8	8.4	2.1

a/Fungicides were added to the medium at ppm. rates comparable to the rates per acre listed.

b/Each figure represents an average of 12 colony measurements: 4 per petri dish and 3 petri dishes.

Table 9.--Growth in millimeters of F. oxysporum f. vasinfectum, P. ultimum, and R. solani on agar medium after treatment of inoculum disks with 6 fungicides at 4 rates each by drenching soil in test tubes

Treatment	Rate per acre[a]	Test organism		
		Fusarium	Pythium	Rhizoctonia
Untreated		43.0[b]	72.7	54.0
Orthocide 50	6 lb.	35.0	44.0	17.3**
	5 lb.	36.0	47.0	16.7**
	4 lb.	28.7**	70.0	13.7**
	3 lb.	35.0	70.7	27.7**
Dithane D-14	1 1/4 gal.	12.3**	0.0**	0.0**
	1 gal.	7.0**	0.0**	0.0**
	3/4 gal.	8.3**	0.0**	0.0**
	1/2 gal.	9.0**	0.0**	0.0**
Phygon XL	6 lb.	39.3	25.0*	25.7**
	5 lb.	39.0	24.7*	28.3**
	4 lb.	32.0*	46.3	21.7**
	3 lb.	37.7	72.3	29.7**
Terraclor	6 lb.	27.0**	68.3	27.0**
	5 lb.	26.3**	68.0	28.3**
	4 lb.	26.0**	42.3	22.3**
	3 lb.	23.3**	68.7	26.0**
Arasan 75	6 lb.	30.7**	44.3	23.3**
	5 lb.	27.7**	66.7	24.0**
	4 lb.	25.3**	71.7	23.0**
	3 lb.	27.3**	68.3	20.3**
Dithane Z-78	6 lb.	26.0**	43.3	15.7**
	5 lb.	28.7**	70.7	18.3**
	4 lb.	32.7*	65.7	22.7**
	3 lb.	32.0*	63.0	16.0**
L. S. D. (.01)		12.0	48.6	8.4
(.05)		9.0	36.4	6.3

a/Fungicides were added to the soil at ppm. rates comparable to the rates per acre listed.

b/Each figure represents an average of 12 colony measurements: 4 per petri dish and 3 petri dishes.

controlled fungous growth. The remaining rates of Phygon XL and Orthocide 50 did not inhibit growth significantly. Only Dithane D-14 gave very highly significant inhibition of the growth of P. ultimum. Phygon XL at 5 and 6 pounds significantly inhibited growth. Only Dithane D-14 completely stopped growth. All other treatments were not significant. All treatments gave very highly significant control of R. solani. Dithane D-14 completely inhibited fungous growth.

Greenhouse tests

The same treatments tested in the laboratory were evaluated in a greenhouse experiment in which the fungicides were applied to the soil used to cover cotton seed during the planting of pots inoculated with F. oxysporum f. vasinfectum, P. ultimum, and R. solani. The average stand counts of seedlings surviving in each treatment after one month are listed in Table 10. The number of surviving cotton seedlings in the treated pots which were inoculated with F. oxysporum f. vasinfectum did not differ significantly from the untreated, inoculated pots. That the incidence of disease was low is evidenced by the number of seedlings in the untreated pots. Dithane D-14 at 1 and 1 1/4 gallons per acre, Terraclor at 4 and 5 pounds, Arasan 75 at 4 and 5 pounds gave highly improved stands in pots inoculated with P. ultimum. Treatments of Phygon XL at 6 pounds, Terraclor at 3 pounds, Arasan 75 at 3 pounds, and Dithane Z-78 at 6 pounds significantly improved seedling numbers. No other treatment was significantly different from stands obtained in the untreated pots. Treatments with Terraclor at all rates, Arasan 75

Table 10.--Average number of cotton seedlings surviving after 1 month from 10 seed planted in pots in soil inoculated with F. oxysporum f. vasinfectum, P. ultimum, and R. solani and treated with 6 fungicides at 4 rates each by in-furrow application

Treatment	Rate per acre	Test oganism		
		Fusarium	Pythium	Rhizoctonia
Untreated		6.0[a]	0.0	0.0
Orthocide 50	6 lb.	7.5	0.0	1.0
	5 lb.	7.5	1.0	0.0
	4 lb.	8.0	0.0	0.0
	3 lb.	7.5	1.0	0.0
Dithane D-14	1 1/4 gal.	7.0	3.5**	4.5**
	1 gal.	5.5	3.5**	0.5
	3/4 gal.	7.0	0.0	0.0
	1/2 gal.	6.5	0.0	0.0
Phygon XL	6 lb.	6.5	2.0*	4.0**
	5 lb.	8.5	1.5	0.0
	4 lb.	6.0	0.0	1.0
	3 lb.	7.5	1.5	0.5
Terraclor	6 lb.	7.0	1.0	6.0**
	5 lb.	7.5	3.0**	7.5**
	4 lb.	7.0	3.0**	7.0**
	3 lb.	7.5	2.0*	5.0**
Arasan 75	6 lb.	6.5	1.5	5.5**
	5 lb.	6.0	6.5**	3.5**
	4 lb.	6.0	3.5**	3.0*
	3 lb.	5.5	2.0*	3.5**
Dithane Z-78	6 lb.	9.0	2.0	0.0
	5 lb.	7.5	0.5	0.0
	4 lb.	7.0	0.0	0.0
	3 lb.	4.5	0.0	0.0
L. S. D. (.01)		N.S.	2.4	3.1
(.05)		N.S.	1.8	2.4

a/Each figure represents an average of the number of seedlings in the four pots of each treatment.

at 3, 5, and 6 pounds, Dithane D-14 at 1 1/4 gallons, and Phygon XL at 6 pounds gave improved stands that were very highly significant in pots inoculated with R. solani. Arasan 75 at 4 pounds significantly improved seedling numbers. All other treatments were not significant.

In a second greenhouse test, 14 fungicidal treatments, made with the fungicides showing promise in the 3 previous experiments applied alone and in various combinations, were evaluated in flats inoculated with the composite inoculum of all 13 pathogenic fungi. The average stand counts obtained in the flat sections of each treatment are presented in table 11. All treatments tested gave very highly significant increases in stand over the untreated flats except Dithane D-14 and Orthocide 50 plus Dithane D-14. Orthocide 50 plus Dithane D-14 was significant at the 95% level of probability.

Field tests

Eight fungicidal treatments which gave the highest stand counts in the greenhouse tests were evaluated in both inoculated and uninoculated field plots. The 13 pathogenic fungi were used for inoculation. The stand count results of these two tests are summarized in table 12. In the inoculated test, 6 of the 8 treatments gave very highly significant control of seedling diseases as measured by stand counts over the untreated plots. Arasan 75 and Dithane D-14 plus Arasan 75 did not differ significantly. A plot treated with Dithane Z-78 plus Terraclor with an excellent stand of cotton and a plot treated with Arasan 75 with a very poor stand are shown in figure 3. Weather conditions which prevailed during the test period are presented in Appendix B.

Table 11.--Average number of cotton seedlings surviving after 1 month from 25 seed planted in greenhouse flats in soil inoculated with seedling disease fungi and treated with 14 fungicides and fungicide mixtures by in-furrow application

Treatment	Rate per acre	No. surviving seedlings
Untreated		2.0[a]
Orthocide 50	5 lb.	16.7**
Dithane D-14	1 gal.	8.7
Terraclor	5 lb.	18.0**
Arasan 75	5 lb.	18.3**
Orthocide 50 + Dithane D-14	2 1/2 lb., 1/2 gal.	11.3*
Orthocide 50 + Terraclor	2 1/2 lb., 2 1/2 lb.	21.0**
Orthocide 50 + Dithane Z-78 + Terraclor	1 1/2 lb. 2 lb., 1 1/2 lb.	22.7**
Orthocide 50 + Arasan 75	2 1/2 lb., 2 1/2 lb.	15.7**
Dithane D-14 + Terraclor	1/2 gal., 2 1/2 lb.	20.0**
Dithane D-14 + Arasan 75	1/2 gal., 2 1/2 lb.	18.0**
Terraclor + Dithane Z-78	2 1/2 lb., 2 1/2 lb.	19.3**
Terraclor + Arasan 75	2 1/2 lb., 2 1/2 lb.	17.7**
Phygon XL	5 lb.	16.7**
Phygon XL + Terraclor	2 1/2 lb., 2 1/2 lb.	21.3**
L. S. D. (.01)		9.9
(.05)		7.3

a/Each figure represents an average of the number of seedlings in the three flats of each treatment.

Table 12.--Average number of cotton seedlings surviving after 1 month in inoculated and uninoculated plots treated with 8 fungicides and fungicide mixtures by in-furrow application, Jackson, Tennessee, 1959

Treatment	Rate per acre	Inoculated plots	Uninoculated plots
Untreated		0.8[a]	73.8
Orthocide 50 + Dithane Z-78 + Terraclor	1 1/2 lb., 2 lb., 1 1/2 lb.	73.8**	106.0
Phygon XL + Terraclor	2 1/2 lb., 2 1/2 lb.	64.8**	95.8
Orthocide 50 + Terraclor	2 1/2 lb., 2 1/2 lb.	88.2**	94.0
Dithane D-14 + Terraclor	1/2 gal. 2 1/2 lb.	43.2**	87.5
Dithane Z-78 + Terraclor	2 1/2 lb., 2 1/2 lb.	61.8**	97.2
Arasan 75	5 lb.	22.5	93.2
Dithane D-14 + Arasan 75	1/2 gal. 2 1/2 lb.	13.0	96.5
Terraclor	5 lb.	81.2**	87.5
L. S. D. (.01)		33.3	N.S.
(.05)		24.6	N.S.

a/Each figure represents an average of the number of seedlings in the center rows of the four plots of each treatment.



Figure 3.--The three-row plot on the left was treated with Dithane Z-78 plus Terraclor by in-furrow fungicide application; the plot on the right was treated with Arasan 75.

In the uninoculated test, none of the treatments gave stands which differed significantly from the untreated plots at the 95% level of probability. Significantly improved stands were obtained, however, at the 80% level.

In a third field test, three fungicidal treatments were tested for control of seedling diseases of cotton on a farm in Tipton County. All three treatments used gave significantly improved stands over those obtained in the untreated at the 95% level of probability. The stand count results of this test are given in table 13.

Table 13.--Average number of cotton seedlings surviving after 1 month in plots treated with 3 combinations of fungicides by in-furrow application, Tipton County, Tennessee, 1959

Treatment	Rate per acre	No. surviving seedlings
Untreated		107.5[a]
Orthocide 50 + Dithane Z-78 + Terraclor	1 1/2 lb., 2 lbs., 1 1/2 lb.	140.5*
Phygon XL + Terraclor	2 1/2 lb., 2 1/2 lb.	129.8*
Orthocide 50 + Terraclor	2 1/2 lb., 2 1/2 lb.	126.5*
L. S. D. (.01)		N.S.
(.05)		17.8

a/Each figure represents an average of the number of seedlings in the center rows of the four plots of each treatment.

CHAPTER V

DISCUSSION

Information has been provided by the data obtained in the present study as to the soil-borne organisms causing seedling diseases of cotton in Tennessee. Although all of the organisms reported to be a part of the seedling disease complex by workers in other states were not isolated, only a few of the less frequently occurring species were not found. Care was taken during collection of the 30 soil samples used in isolating the disease organisms to obtain the samples from widely scattered locations and different soil types within each county.

The large number of isolates of Fusarium spp. obtained, over 50% of the isolations, indicates that species of that genus may be more important in the seedling disease complex than previously thought. Even though the Fusarium spp. accounted for over one-half of the isolations, they were generally less pathogenic than the other fungi in the degree of pathogenicity tests conducted. They were also less virulent in soil in the inoculated pots than in the petri dish test. When pots were inoculated with F. oxysporum f. vasinfectum for greenhouse testing of fungicides, little seedling mortality was observed in the untreated, inoculated pots, but stem lesions and some root damage were noted. Ray and McLaughlin (33) reported similar observations of the pathogenicity of Fusarium spp. and concluded that they were important in causing injury to cotton seedlings due to their frequent occurrence although they are usually only moderately pathogenic.

Work of Ranney and Bird (26) and Fulton et al. (15) help to give further explanation, in addition to frequent occurrence, for the large number of isolates of Fusarium spp. obtained. They reported that Fusarium spp. were the predominant organisms isolated from diseased cotton seedlings grown at temperatures of 80° F. or higher while R. solani, Pythium spp., and Fusarium spp. were predominant when seedlings were grown at 65 and 70° F. Seedlings for the present study were grown in the greenhouse during March and April with the temperature regulated as closely as possible to 65° F., but there were great fluctuations especially on sunny days when temperatures of 90 to 95° F. were common. This fluctuation of temperature with extremely high temperatures on some days undoubtedly had considerable influence on the microflora of the soil samples.

The results of the degree of pathogenicity tests indicate that R. solani and P. ultimum are the most pathogenic of the fungi frequently isolated in the present study. In culture both of these fungi grew more rapidly than the other pathogens obtained. R. solani made its most rapid growth at 20° C. while P. ultimum and the Fusarium spp. grew better at higher temperatures. The number of isolations of Pythium spp. was small although the greenhouse conditions which favored the large number of isolations of Fusarium spp. should have favored the isolations of Pythium spp. to some extent. These results all help to explain why R. solani is the organism most commonly isolated from diseased cotton seedlings during periods of cool, damp weather when seedling diseases are the most prevalent.

Data obtained from the tests to study the effects of different soils and soil pH levels on disease development suggest that these factors have little effect on seedling disease development. These results are generally in agreement with the results reported by Ray and McLaughlin (33) and Kadow and Anderson (17). More work is still needed on the effects of these and other environmental factors on disease development. Lower soil pH values should be tested especially.

The fungicidal combination consisting of Orthocide 50, Dithane Z-78, and Terraclor gave excellent control of seedling diseases over the range of soil pH levels and soil types used in the present study. These results support the findings reported by Ranney and Bird (27, 28) and Bird and Ranney (5). This mixture of fungicides should be tested over a wider range of conditions along with others.

The excellent control of R. solani by all fungicides at all rates in the laboratory and lack of control in the greenhouse except by Terraclor, Arasan 75, and the highest rates of Dithane D-14 and Phygon XL are difficult to explain. One possible explanation is that the mixing of the fungicides with the soil in the inoculated pots was not as thorough as the mixing of fungicides with the agar medium or the soil in the test tubes.

The apparent discrepancy between the control obtained with treatments of Dithane D-14 in the laboratory and in the greenhouse indicate that the effectiveness of this material may not be consistent under different conditions. Dithane D-14 was outstanding in inhibiting the growth of the disease fungi in the laboratory but failed to give control under the greenhouse conditions in which it was tested. The

lack of control by Dithane D-14 is better understood in view of the work of Rushdi and Jeffers (36, 37) who found that a high percentage of organic matter in the soil decreased the effectiveness of the active ingredient of the fungicide and of Mannecke (23) who found Dithane D-14 to be inactivated rapidly in the soil. The soil used to fill pots in the greenhouse had a fairly high content of organic matter, and the soil used to fill the greenhouse flats was composed of one-half compost. Also, the stand counts were made one month after planting while two weeks after planting stand counts were higher in the Dithane D-14 treatments than in any other treatment. Dithane D-14 was not tested in the field in the present study since the fungicides that gave poor results in the greenhouse were not included in the field tests. Further greenhouse and field testing would be desirable since Dithane D-14 is the least expensive of all the fungicides tested. Tennessee soils are generally low in organic matter, and two weeks of control of seedling diseases would be sufficient in most seasons.

Results in the laboratory and greenhouse tests of fungicides suggest that some of the fungicides gave excellent control of one fungus but little control of another. Thus, combinations of fungicides are likely to be effective against a wider range of pathogens.

Conclusions made from data obtained in the inoculated field tests for evaluation of fungicides indicate the degree of disease control that can be expected by in-furrow treatments. Only a few plants, an average of two plants in the center rows, survived in the untreated check plots, and these were damaged. Even with the extremely high incidence of disease, 6 of the 8 fungicidal treatments tested gave very good control

of seedling diseases. Seedling stands in these were highly significant over those obtained in the untreated plots.

Lack of disease expression in the plots of the uninoculated fungicide test at Jackson suggest that the seedling disease organism infestation of the land on which the plots were located was fairly low. One explanation for the lack of disease in these plots is that the land had not been in cotton for 15 years or more prior to this test. The possibilities of crop rotation are indicated and should be investigated.

The results obtained in the field test in Tipton County point out the importance of wet weather in the occurrence of seedling diseases of cotton. This test was planted during a dry period which continued for about 2 weeks after planting. Although the field in which the plots were located had a history of severe seedling disease losses in the two seasons prior to 1959, there was a fairly low incidence of disease in the check plots. The stands of seedlings in the treated plots were significantly better than the stands in the untreated plots at the 95% level, but seedling disease symptoms were just beginning to appear in the check plots when the stand counts were made 1 month after planting.

Data obtained in the present study indicate the possibilities of in-furrow fungicide treatments in the control of seedling diseases of cotton. Treatments of Orthocide 50 plus Dithane Z-78 plus Terraclor gave consistently good control of seedling diseases in greenhouse and field tests. The orthocide 50, Dithane Z-78, and Terraclor combination gave good control ovar a range of soil pH levels and soil types. Some of the other fungicides appear equally promising, but there are less data

available on them since they were not included in all of the field tests.

CHAPTER VI

SUMMARY AND CONCLUSIONS

In order to isolate and record the soil-borne organisms causing seedling diseases of cotton in Tennessee, soil samples were collected from 3 cotton fields in 10 counties in West Tennessee in 1958. Cotton seedlings were grown in the 30 samples, and 465 isolates of fungi were obtained from diseased seedlings. The isolates were tested for pathogenicity, and the pathogenic isolates were reisolated. The degree of pathogenicity of pathogenic isolates was measured in two tests. The pathogenic isolates were identified, and thirteen species and forms of fungi were found.

Of the 13 pathogens, R. solani and P. ultimum were the most pathogenic on cotton seedlings of the fungi that were isolated most frequently. The Fusarium spp. comprised over one-half of the isolations made, but they were generally less pathogenic than the other fungi tested.

The effects of three environmental factors on the growth of the causal organisms, disease development, and effectiveness of fungicidal treatment were measured in three tests. The effects of 8 temperatures on the growth of 9 of the 13 pathogens were studied, and it was found that R. solani and H. purpureum made their most rapid growth at lower temperatures than P. ultimum and the Fusarium spp. Most of the fungi were able to grow over a fairly wide range of temperatures. The effects of different soil pH levels and different soils on the pathogenicity of

the causal organisms and on disease development were tested, and no significant differences were found as measured by differences in stand. The fungicidal mixture, Orthocide 50 plus Dithane Z-78 plus Terraclor, gave excellent control of seedling diseases over the range of soil pH levels and soil types tested.

Six fungicides at four rates each were tested for the control of representative isolates of three specific organisms, R. solani, P. ultimum, and F. oxysporum f. vasinfectum. The fungicides were evaluated in two laboratory tests and one greenhouse test. Those found to have the greatest promise in controlling the individual organisms were tested in combination for controlling all of the pathogenic fungi isolated in the present study. The fungicidal mixtures that gave the best control in the greenhouse were tested in the field in three experiments. The fungicidal mixtures, Orthocide 50 plus Dithane Z-78 plus Terraclor, Phygon XL plus Terraclor, and Orthocide 50 plus Terraclor indicated the most promise in controlling the soil-borne organisms causing seedling diseases of cotton. Some of the other fungicides appeared equally promising, but there are less data available on them since they were not included in all of the field tests.

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APPENDIXES

APPENDIX A

Chemical composition of fungicides

1. Acti-dione; 90% cycloheximide
2. Arasan; 50% tetramethylthiuram disulfide
3. Arasan 75; 75% tetramethylthiuram disulfide
4. Captan 50W; 50% N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide
5. Captan 75W; 75% N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide
6. Ceresan M; 7.7% ethyl mercury p-toluene sulfonanilide
7. Ceresan 100; 3.1% ethyl mercury 2,3-dihydroxy propyl mercaptide plus 0.67% ethyl mercury acetate
8. Dithane D-10; 96.7% disodium ethylene bisdithiocarbamate
9. Dithane D-14; 19% disodium ethylene bisdithiocarbamate
10. Dithane Z-78; 65% zinc ethylene bisdithiocarbamate
11. Dowicide F; 80% sodium 2,3,4,6-tetrachlorophenoxide
12. Fermate; 76% ferric dimethyldithiocarbamate
13. Mathieson 275; 50% pentachloronitrobenzene
14. Mathieson 275-D; 20% pentachloronitrobenzene
15. Orthocide 50; 50% N-trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide
16. Panogen 15; 2.2% methyl mercury dicyan diamide
17. Phygon XL; 50% 2,3-dichloro-1,4-naphthoquinone
18. Puratized Agricultural Spray; 7.5% phenyl mercury triethanol ammonium lactate
19. Seedox; 50% 2,4,5-trichlorophenyl acetate

APPENDIX A (continued)

20. Semesan; 30% 2-chloro-4-(hydroxymercuri) phenol

21. Stauffer N-521; 25% tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione

22. Terraclor; 75% pentachloronitrobenzene

23. Tersan 75; 75% tetramethylthiuram disulfide

24. Thiram 50; 50% tetramethylthiuram disulfide

25. Vancide 51; 30% sodium dimethyldithiocarbamate

26. Vancide 51-ZW; 30% zinc dimethyldithiocarbamate

APPENDIX B

Record of climatological observations, West Tennessee Agricultural Experiment Station, Jackson, Tennessee, April-June, 1959

Date	April Temp. °F. high	April Temp. °F. low	April Rain inches	May Temp. °F. high	May Temp. °F. low	May Rain inches	June Temp. °F. high	June Temp. °F. low	June Rain inches
1	76	59		90	63		86	66	
2	70	41	0.82	89	61		80	65	0.56
3	68	59		88	65		80	56	
4	68	34		89	64		80	53	
5	78	44		93	61		81	60	
6	80	52		86	64		83	61	
7	84	57		81	62		86	61	
8	82	62		80	51		87	66	
9	77	45	0.42	85	59		81	68	0.77
10	58	45	0.03	86	63	0.04	81	67	0.98
11	57	41		81	63	0.44	77	69	0.27
12	55	43	0.46	72	66	0.41	87	66	0.27
13	55	39		81	60	0.24	86	69	
14	63	29		72	51	0.07	80	56	
15	69	35		67	41		86	52	
16	75	49		72	39		91	59	
17	80	57		75	57		91	62	
18	74	60	0.26	87	61	0.03	87	59	
19	79	56	0.44	84	66		80	62	
20	77	61	0.07	79	66	0.66	86	53	
21	64	46	0.08	85	66		87	58	
22	60	39		82	68	0.01	90	63	
23	67	33		86	59		87	69	
24	75	40		80	63		79	70	
25	81	57		89	65	0.01	89	69	1.31
26	79	69		85	67	0.03	89	73	
27	74	64		87	65	0.45	90	73	
28	72	64	0.10	88	65	0.36	92	72	
29	80	48		85	67		94	70	
30	85	51		81	67		94	72	
31				87	65				
Total rainfall			2.68			2.75			4.16