SOIL APPLICATION OF CHEMICALS FOR CONTROL OF TWO COTTON SEEDLING DISEASE PATHOGENS

By

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Bachelor of Science

Southeastern State College

Durant, Oklahoma

1951

Submitted to the Faculty of the Graduate School of
the Oklahoma Agricultural and Mechanical College
in Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE
August, 1955

SOIL APPLICATION OF CHEMICALS FOR CONTROL

OF TWO COTTON SEEDLING DISEASE PATHOGENS

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ACKNOWLEDGMENTS

Grateful acknowledgment is hereby extended to Professor L. A. Brinkerhoff for many suggestions and constructive criticisms offered during the course of this study. The author is also indebted to Dr. J. E. Thomas and Dr. W. W. Hansen for their guidance in preparing the final copies of this thesis. Further, the author takes pleasure in thanking Dr. F. D. Struble, Dr. R. H. Converse, and Dr. R. Reed for advice on certain aspects of this study.

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INTRODUCTION

The economic importance of seedling disease injury can hardly be over-emphasized. Many of the fungi associated with this type of plant malady are soil inhabitants, and are widely disseminated in agricultural soils. As a rule, the incidence of seedling disease injury fluctuates according to the existing environmental conditions. Departure from optimum conditions for the plant may operate so as to favor the parasite and like departure in the case of the parasite may enable the host to escape harm.

Seed rot and seedling injury of cotton is attributed to various seed or soil borne fungi, notably of the genera Glomerella, Rhizoctonia, Pythium, and Ascochyta. In addition to these, it is not an infrequent occurrence to find species of Fusarium and Sclerotium associated either singly or together with the above in the form of a complex (5, 24, 26, 35).

The hypocotyl of cotton seedlings may be slightly or severely damaged at or near the surface of the soil, or, the embryo may be completely decayed as the cotton seed is germinating. In the first case, the seedling may be injured and recover from the attack, while frequently in the latter instance the embryo is destroyed or damaged to such a degree that emergence from the soil is never accomplished.

Most cultivated upland cotton is very susceptible to attack by seedling disease organisms. At present a screening program is underway at the Oklahoma Agricultural Experiment Station in search of resistance or tolerance which might prove valuable for breeding

purposes. However, to date seed treatment and cultural practices are the principal control methods employed.

Seed treatment has not given complete control of cotton seedling diseases. Fungicides applied to the drill row as the seed are planted have been used with some success on an experimental basis for the control of both seed decay and post-emergence injury. Results obtained in the field have been variable and indicate a need for information on the specificity of fungicides to given pathogens, and also the length of time the different fungicides remain effective in the soil.

The purpose of this investigation was three fold: 1) to identify a species of Pythium isolated from infected seedlings, 2) to establish a method of infesting soil with Pythium to be used in greenhouse tests, and 3) to determine the fungicidal and residual effectiveness of the four chemicals (zineb, thiram, captan, and pentachloronitrobenzene) against the cotton seedling pathogens, Pythium debaryanum Hesse and Rhizoctonia solani Kuhn.

LITERATURE REVIEW

Literature Pertaining to Rhizoctonia

R. solani is worldwide in its distribution and its role in the seedling disease complex of many plants has been studied extensively. An excellent review was compiled by workers at the University of Minnesota and published in 1952 (21). Literature relative to its association with cotton has been adequately reviewed by Young (37) and Kortsen (22) and will not be further treated here.

Literature Pertaining to Pythium

Arndt (3) first reported *Pythium ultimum* Trow as a cotton seedling disease pathogen in the United States in 1934. Accompanying its presence he found reduced germination of seeds and impoverished stands. Miller in 1938 (24) while taking random samples of diseased seedlings from nine southern states, isolated *Pythium* 15 times out of 344 samples of diseased cotton seedlings. According to frequency of isolation it ranked fifth, preceeded by *R. solani*, *Fusarium* spp., *Fusarium moniliforme* (Sheld.), and *Glomerella gossypii* (Edg.) in that order. Five other species of fungi were isolated less frequently. Arndt (5) suggests that *P. ultimum* is inhibited by fungicides used for surface sterilizing of cotton seedlings and that this may be why more frequent recovery of this fungus from diseased seedlings is not obtained.

Weindling, Miller, and Ullstrup (35) list *Pythium* sp. along with eleven other organisms associated with diseases of cotton seedlings. Davis and Lund (11) found *Pythium* sp. predominating in certain soils

of the Mississippi Delta and proceeded with tests which established its pathogenicity to cotton. R. solani, due to its high degree of virulence under various environmental conditions, and because of its frequency in Oklahoma soils, was considered by Ray and McLaughlin (26) to be the most important of some fourteen fungi involved in diseases of cotton seedlings in the state. Pythium was not isolated during the course of this study.

Taxonomy of the Organism. P. ultimum was first described in 1901 (33). Being unable to inoculate healthy cress plants with the isolate obtained from a rotted-off seedling, Trow's conclusion was that the fungus must be a saprophyte. All attempts to induce the organism to produce zoospores, so common to other species in the genus, proved negative. Due to his belief that this species exhibited greater adaptation to a terrestrial existence as evidenced by the fact that it alone had lost all power of producing zoospores, the specific name was chosen to call attention to its position in the genus.

Some contention eventually arose in connection with the validity of new classification, due primarily to the fact that close similarity existed between the organism and P. debaryanum described earlier. P. debaryanum is said to be distinguished from P. ultimum by its typical plurality of antheridia which originate some distance from the oogonium (23). Drechsler (12) reported similar observations of the two species. Van Luijk (34) believed the antheridial character insufficient for specific segregation, preferring to consider P. ultimum synonymous with P. debaryanum.

Later, Drechsler (13) in 1946 and Ark (2) in 1949 reported ways of inducing P. ultimum to produce zoospores. In 1942 Reinking (27)

discovered the existence of races in P. ultimum which varied in their ability to decay pea seed in moist soils. Campbell and Sleeth (9) reported in 1946 that three types of cultures were found on diseased guayule plants. One type produced only oospores, while another produced only sporangia, while the third produced both.

P. debaryanum and P. ultimum grow abundantly on many types of media at wide variations in temperature. Ordinarily, isolates tend to grow best at temperatures between 25° and 30° C, yet the minimum and maximum may reach as low as 1° C and as high as 40° C respectively. Middleton (23) points out that the two fungi are very common members of the genus in the United States and are frequently reported throughout the world.

Temperature and Moisture Relations. It has been a general observation by several mycologists that species of the genus Pythium exhibit preference to a cool humid type of existence. Severe pre-emergence injury to cotton plants has been observed to occur during cool wet weather (5, 7). Beach (6) reported that the severity of attack on tomato increases as the soil moisture increases and is most severe at the point of saturation.

In 1934, Arndt (3) reported experiments which indicate that P. ultimum is principally a pre-emergence rather than a post-emergence pathogen of cotton. At a constant temperature of 30° C, all seedlings in his tests emerged but 6 per cent were finally killed. Fourty-five, 70, and 100 per cent of the seedlings were killed at temperatures of 27°, 24°, and 21° C respectively. Growing cotton seedlings at 30° C for 6 or 13 days and then lowering the temperature to 21° C resulted in some injury, but this was much less severe than in the first experiment,

thus indicating that the critical period for cotton seedlings is during the first week of germination.

In 1943 the same author published data substantiating the above findings and made an additional series of tests at 18° C that showed only 3 per cent emergence (5).

Alexander, Young, and Kiger (1) had earlier reported a similar temperature relationship while working with the same organism on tomato seedlings. In this case, high soil moisture was also considered favorable to damping-off.

The optimum for growth and optimum for pathogenicity are very dissimilar. Harter (14) found that 12° and 15° C temperatures were highly favorable to the sweet potato decay caused by P. ultimum, while laboratory cultures of the organism grown on corn meal agar were most luxuriant at 32° C. While using a peat soil known to be infested with P. ultimum and P. debaryanum, Hoppe (15) found that untreated corn seed planted and incubated at either a temperature of 4° C or 11° C for ten or fifteen days was attacked and the resulting disease was practically 100 per cent fatal. Leach found that when watermelons or beets are retarded by low temperature during germination and emergence, susceptibility to P. ultimum and R. solani is increased.

An interesting report by Jacobs (18), to the converse of any of the foregoing data, states that emergence of alfalfa seedlings growing in soil infested with P. ultimum and P. debaryanum was not correlated with temperature variations over a range of 7° to 22° C.

Control. Emphasis on control has been directed primarily toward seed or soil treatments. Such control has seldom been 100 per cent effective.

A formaldehyde drench applied so as to thoroughly wet the soil was reported in 1931 (1) as a satisfactory control for damping-off of tomato seedlings where species of Pythium were the chief inciting agents. Soaking the seeds in different solutions of formaldehyde and mercuric chloride did not control the disease. Soil disinfection by use of copper sulphate was unsatisfactory because phytotoxicity occurred when effective quantities were used. However, Horsefall (16) in the year following, found copper oxide to be effective as a seed treatment against damping-off of several vegetables grown in the greenhouse.

Sulphuric acid has been reported to control P. ultimum in an alkaline soil by reducing the pH (29). However, in Oklahoma, damping-off of Siberian Elm was observed on soils in which pH values ranged from 5.8 to 6.4 (36). Jackson (17), while working with spruce and pine in other regions found P. ultimum flourished at pH values similar to the above when grown on artificial media in the laboratory, yet damping-off was most severe at pH 8 and negligable at pH 5 or less. Soft rot of sweet potatoes incited by P. ultimum was most severe at pH 7 to 8.2 according to Jones (19).

If the occasion permits, steaming of the soil is as effective as any of the previously considered control measures (1, 16). However, tests have proved that susceptible plants seeded in steamed soil inoculated with Pythium are much more severely affected than those in similar soil where steaming has been omitted (1, 10, 36).

Some of the relatively newer organic fungicides have been found to be of considerable value. Literature on this phase of the subject is abundant. Depending upon the situation, the protective value of one chemical may be in direct proportion to the dosage applied (32), while

another may be relatively effective at several rates (30).

Large scale cultivation of the guayule plant in this country initiated further studies of chemical seed protectants where infested soils were used. Mersolite 19 (pheryl mercuric salicylate) and Mersolite 8 (pheryl mercuric acetate) when applied to the seed at rates of .1 to .5 per cent chemical per unit weight of seed were very effective (30). Arasan and U.S.R. #604 (2, 3-dichloro-1, 4-naphthquinone) were also considered promising.

Brinkerhoff et al. (7, 8) reported field studies in which good control of R. solani on cotton was obtained with Mathieson 275 (pentachloronitrobenzene) when applied in the drill row as a dust or spray at the time of seeding. Zineb and thiram applied in a similar manner appeared outstanding in their effectiveness against pre-emergence injury which occurred at low temperatures and was attributed to Pythium sp. Pythium sp. was obtained from decaying seed and seedlings during cool weather, and in both laboratory and field tests caused severe pre-emergence injury.

SOURCE AND IDENTIFICATION OF PATHOGENS

The culture of R. solani used in the following tests was originally isolated from an infected cotton seedling grown at Chickasha, Oklahoma in 1951. Since that time cultures of the fungus have been maintained on steamed grain sorghum seed, transfers each time being made only from those cultures which produced an abundance of sclerotia. This same isolate has been used in artificial inoculations since 1952.

The culture of Pythium used in these studies was isolated from decaying cotton seed during cool weather in April, 1954. Steamed sorghum seed also proved suitable as a substrate for culturing this fungus, and field tests were carried out during 1954 employing this isolate as the test pathogen (8).

The present investigation started with an attempt to identify the species of Pythium under consideration. In so far as observed by the writer, morphological characters of the fungus fruiting on water agar appeared to conform to those of P. ultimum (Figure 1). Plurality of antheridia were seen but seldom, and in no case was more than two found attached to any one oogonium.

However, distinguishing characteristics such as the number of antheridia and their point of attachment were not easily observed. As cultures sent to Dr. J. T. Middleton, University of California, Riverside, were identified as P. debaryanum, the isolate will be tentatively designated accordingly in this paper.

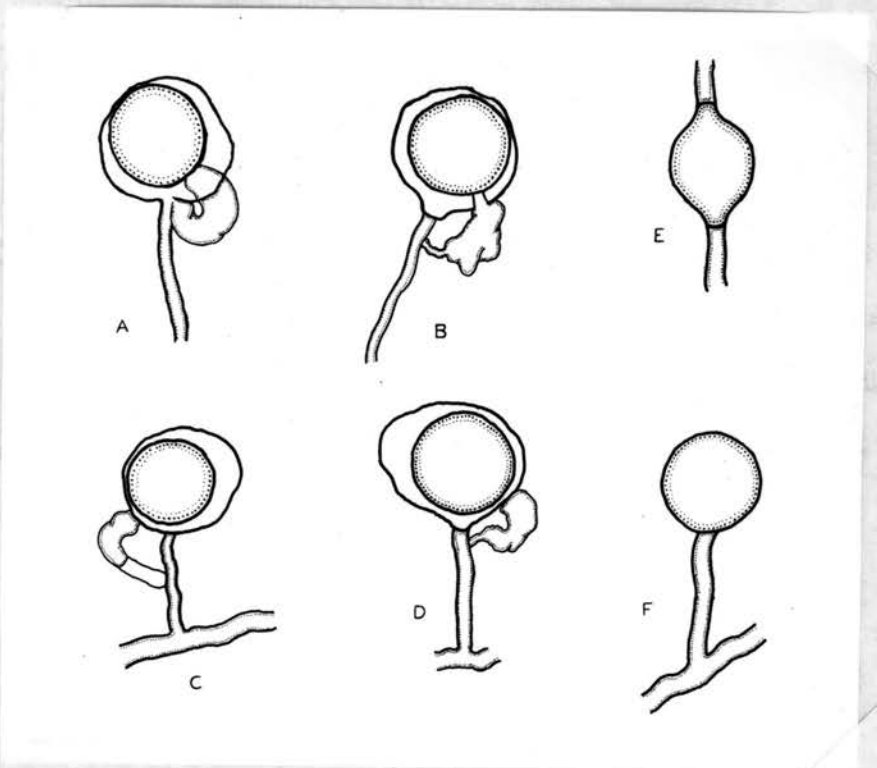

Figure 1. Oogonia and antheridia (A--D) and sporangial habit (E--F) of Pythium observed as it fruited on water agar.[1]

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[1]These drawings resemble P. ultimum. However, transfers of the original culture were identified as P. debaryanum by Dr. J. T. Middleton of the University of California. It is possible that the original culture was mixed, or that the media affected the number of antheridia that were produced.

MATERIALS AND METHODS

Methods of Inoculating. Several methods of infesting soil with *R. solani* have been developed for both field and greenhouse studies at the Oklahoma Agricultural Experiment Station (7, 22). Most of the methods employ the use of steamed grain sorghum seed as a culture medium and either whole grain or chopped hyphal suspensions to infest the soil. It was found in greenhouse tests that a direct correlation existed between the per cent of seedlings which emerged and the amount of hyphal suspension applied to the soil.

The whole grain method had proven effective for infesting soil in the field with *P. debaryanum* (8), but tests had not been made to determine what methods might be effective in the greenhouse.

Eight ounce prescription bottles were filled with the desired amount of rinsed sorghum seed and sufficient water was added to cover the seed, after which the bottles were plugged with cotton, capped and autoclaved for one hour on each of two successive days. After being inoculated with the fungus, each bottle was incubated at 70° F.

By the end of two weeks, mycelia of the organism had penetrated the entire mass of seed. Inoculum was prepared by chopping the cultures in a food blender with water and then straining the macerate through two layers of cheesecloth so that the resulting suspension could be applied with an ordinary sprinkling can. The hyphal suspension was then standardized by diluting each 125 ml of inoculum with sufficient water to make 1 liter.

When flats were used, the desired amount of stock suspension of

inoculum was added to sufficient water to make 400 ml total and then sprinkled evenly over the entire surface of a flat at the seed level. Unless otherwise specified, tests in 6 inch pots were inoculated similar to flats. Twenty-five ml of the diluted inoculum were applied per pot.

Soil. Initial preparation of soil consisted first of the addition of moisture (if needed) to soil in the storage bin. Subsequently it was screened and, in cases where partial steam sterilization was desired, placed in a steam chamber for a two hour period before using.

Seed. A one year old lot of acid delinted seed of the variety Deltapine 15 were used for all the following experiments. The light fraction was not removed when delinted, but all noticeably defective seeds were removed individually by hand. The remaining portion germinated between 70 and 100 per cent in laboratory and greenhouse tests, the germination being higher at high temperatures.

INOCULUM DOSAGE TESTS WITH PYTHIUM

Test 1. In test 1, wooden flats (4 x 14 x 16 inches) were filled 3/4 full with moist, steamed soil. Three different soils were used (see Table 1). Soil number 1 was collected from Perkins Farm and 2 and 3 from the Stillwater West Agronomy Farm. The upper one inch layer of soil in the flat was removed and the remaining portion leveled and packed lightly with a board. Five evenly spaced rows were marked across the flat. Twenty cotton seeds were planted along the length of each row. Seeds were then pressed lightly into the soil to help them maintain their position when liquid inoculum was applied.

Five flats were inoculated, receiving 0, 10, 30, 90, and 270 ml of stock P. debaryanum inoculum respectively. Enough water was added to the inoculum in each instance to make a total volume of 400 ml. The surface of each flat was then covered with one inch of loose, steamed soil and placed in a refrigerator held at 63° F. After four days, all flats were removed to the greenhouse. Seedling emergence counts were made four days later.

Temperatures were exceptionally high as exceedingly hot, dry weather prevailed during the summer when this test was made. Unfortunately, no facilities were available for controlling greenhouse temperatures. Because of rapid drying and crusting under these conditions, the soil surface was loosened to facilitate emergence of seedlings.

Table 1. Data pertaining to the three soils used in inoculum dosage tests.

Number	Soil type	pH	Phosphorus content	Potassium content	Organic matter (%)
1.	Norge sandy loam	6.4	Very high	Very high	2.9
2.	Port sandy loam	5.4	Very high	High	0.9
3.	Port silty clay loam	6.0	High	Very high	2.2

Results. The statistical analyses for test 1 are presented in tables 2, 3, and 4. Highly significant differences were calculated for inoculum rates in the case of each soil type. In the combined analysis found in table 5, highly significant differences were found to exist for soil types, inoculum rates, and soil x inoculum interaction. An interesting observation was that in both sandy loam soils (number 1 and 2), additional inoculum above a certain level did not result in additional decrease in emergence (Figure 2). Clay, on the other hand, required comparatively high inoculum dosages to seriously reduce emergence.

Table 2. Cotton seedling test showing the effect of different inoculum rates of P. debaryanum applied to Morge sandy loam soil (#1 from Perkins Farm).

Inoculum rate (in ml per flat)	Total surviving seedlings Replicates 1	2	3	4	Treatment mean
10	47	54	92	81	68.5
30	34	33	38	55	40.0
90	10	6	5	5	6.5
270	14	14	20	10	14.5
Check /a	91	86	98	97	93.0

Analysis of variance of Table 2.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	15	11,171.75	--	
Replications	3	554.75	--	
Treatments	3	9,408.75	3,136.25	23.36**
Error	9	1,208.25	134.23	

/a Checks were not included in the analysis.

Table 3. Cotton seedling test showing the effect of different inoculum rates of P. debaryanum applied to Port sandy loam soil (#2 from the Agronomy Farm, Stillwater).

Inoculum rate (in ml per flat)	Total surviving seedlings Replicates 1	2	3	4	Treatment mean
10	23	17	56	51	36.8
30	7	5	11	5	7.0
90	14	29	12	3	14.5
270	32	27	13	17	22.3
Check /a	97	89	94	89	92.3

Analysis of variance of Table 3.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	15	3,695.75	--	
Replications	3	45.00	--	
Treatments	3	1,939.25	646.41	7.17**
Error	9	1,711.40	190.16	

/a Checks were not figured in the analysis

Table 4. Cotton seedling test showing the effect of different inoculum rates of P. debaryanum applied to Port clay soil (#3 from the Agronomy Farm, Stillwater).

Inoculum rate (in ml per flat)	Total surviving seedlings Replicates 1	2	3	4	Treatment mean
10	84	83	77	90	83.5
30	70	65	75	72	70.5
90	29	70	30	52	45.3
270	21	11	23	5	15.0
Check /a	92	81	93	82	87.0

Analysis of variance of Table 4.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	15	12,465.94	--	
Replications	3	107.69	--	
Treatments	3	10,947.19	3,652.39	23.46**
Error	9	1,401.06	155.67	

/a Checks were not included in the analysis.

Table 5. Combined analysis of all three soils.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	47	36,490.98	--	
Rep. in soil	9	697.44	77.49	.484
Kind of soil	9	9,157.54	1,017.50	6.358**
Inoculum rates	3	15,337.23	5,112.41	31.947**
Soil x Inoculum	6	6,967.96	1,161.33	7.257**
Error	27	4,320.81	160.03	

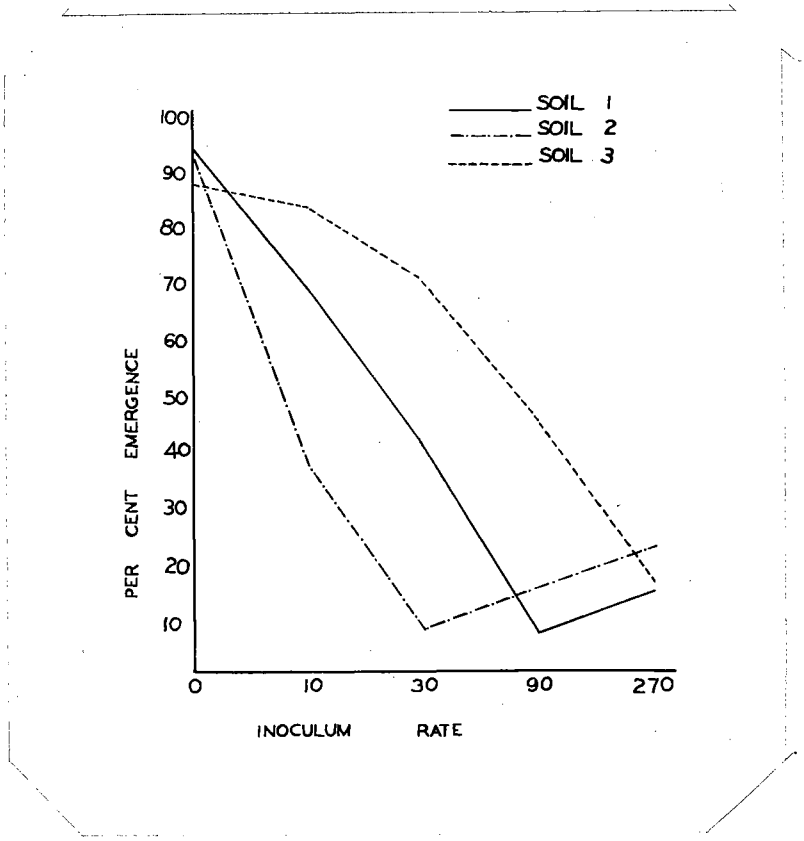

Figure 2. Curves representing emergence of cotton seedlings at different inoculum dosage rates of P. debaryanum (inoculum expressed in ml per flat). Soil 1 is Norge sandy loam; 2 is Port sandy loam; 3 is Port clay loam.

Figure 3. Flats of Port sandy loam soil inoculated with P. debaryanum showing check (lower left), 10 ml (lower right), 30 ml (upper right), 90 ml (center), and 270 ml of standard hyphal suspension applied at the seed level. Note that adding inoculum above 30 ml resulted in an increase rather than a decrease in total emergence.

Many surviving seedlings observed in inoculated flats of test 1 appeared to be unharmed. Examination of their root systems, however, revealed that the taproot of some plants had apparently been destroyed and adventitious roots had developed. Others appeared to have normal root systems but not infrequently exhibited soreshin or distorted cotyledons (see figure 4). Very few plants escaped injury. Pathogenic cultures of the fungus were isolated from injured tissues of either the cotyledonary leaves, the stems, or the roots. Decaying seeds and young plants unable to emerge were found to contain an abundance of oospores.

Figure 4. Ten day old cotton seedling infected with P. debaryanum showing characteristic symptoms in steamed Port sandy loam soil. Temperature for first 4 days after planting was 64° F and 80° to 110° F for the following 6 days.

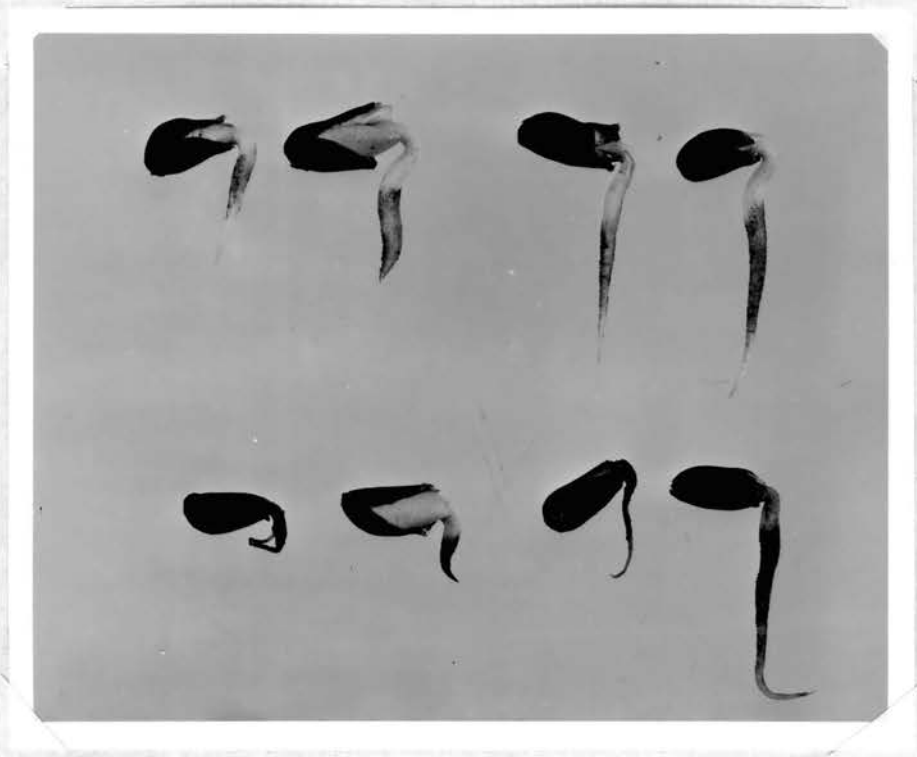

Figure 5. Typical pre-emergence injury of germinating cotton seeds incited by P. debaryanum (bottom) as compaired to healthy seeds (top).

Test 2. The objective of this test was to determine why additional inoculum above a certain level did not result in decreased emergence in the sandy loam soils of test 1. It was thought that something in the culture medium itself, possibly starch, was responsible for these differences.

In test 2, the same procedure was followed as for test 1 except for the following modifications: 1) Two checks accompanied each series of dosage rates. In one, 400 ml of water was substituted for inoculum; and in the other, 400 ml of a sterile grain-culture suspension was substituted which was prepaired in the same manner as the inoculum. 2) Sufficient grain suspension was added to each inoculum dosage to make the grain content comparable. 3) Only Port sandy loam soil was used. 4) Greenhouse temperatures averaged approximately 70° to 75° F compared to 80° to 115° F for the previous test which was made in the summer.

Results. All inoculum rates of P. debaryanum effected 100 per cent fatality of seedlings, thus thwarting the original objective. This was undoubtedly due to environmental conditions in the greenhouse which favored disease development. However, both tests were exposed to the same initial 4 day incubation at 63° F before being placed in the greenhouse. A fact noteworthy of mention is that there were 16 per cent more seedlings which emerged in the check flats receiving sterile grain suspension than in those receiving water; emergence averaged 79 and 63 per cent respectively.

Test 3. This test was designed to determine the feasibility of using pots rather than flats and to observe the action of the host-pathogen relationship in non-steamed soil when inoculum rates were

varied. Inoculum dosages were 2, 4, 8, and 16 ml of stock suspension per 6 inch pot. Inoculum was prepaired as in test 2.

For planting, the upper 1 inch layer of soil was removed from each pot and the remaining portion was shaken down by lightly striking the pot on the floor several times. Ten seeds were distributed over the surface of the soil. Inoculum was then applied and each pot was covered with the previously removed soil. After an initial 6 day incubation period at 63° F, all four replications were removed to the greenhouse and maintained at an average temperature of approximately 80° F.

Results. Only 2 plants emerged in the 16 inoculated pots. Hence, P. debaryanum appeared to be as severe in non-steamed soil as in steamed soil, and no differences were obtained with different amounts of inoculum. Stands ranged from 0 to 90 per cent in the 8 checks indicating that the test was conducted under conditions extremely favorable for disease development. Apparently disease development was the same regardless of whether pots or flats were used.

DETERMINATION OF FUNGICIDAL AND RESIDUAL EFFECTIVENESS OF FUNGICIDES TESTED AGAINST R. SOLANI AND P. DEBARYANUM

Identification of Chemicals

The 4 fungicides selected for these studies have shown promise as seedling protectants when used as sprays or dusts in the drill row in field tests (8). Information is needed in connection with their loss of potency as a function of time after soil application. The determination of minimum effective rates and the specificity of each product are incorporated in these studies.

According to their respective manufacturers, captan has an extremely short residual life in the soil while thiram is gradually broken down and its effectiveness is usually 4 to 8 weeks. Thiram has been found to persist for 2 months in sandy soil but disappeared from compost soil within 1 week (28). No comparable information was found for zineb or pentachloronitrobenzene.

Table 6. Data pertaining to the chemicals used in the following studies.

Chemical /a	Active principle	% Active principle	Manufacturer
1) Zineb	Zinc ethylene bisdithiocarbamate	65.0	Rohm and Haas
2) Thiram	Tetramethylthiuramdisulfide	75.0	DuPont
3) Captan	N-trichloromethylthio tetrahydrophthalimide	50.0	Calif. Spray Chem. Corp.
4) Mathieson 275	Pentachloronitrobenzene	75.0	Mathieson

/a The zineb was Dithane Z-78. Thiram was Tersan 75. Captan was Orthocide 50.

General Procedures

It will be observed that the following materials and methods apply to the entire series of tests with fungicides: 1) Chemicals were applied by mixing them with the soil; 2) only Port sandy loam soil was used; and 3) each test, with one exception, received an initial 6 day incubation period at 63° to 67° F immediately after planting. Conditioning of the soil, methods of inoculation, inoculum rates and chemical rates varied and will be described in the appropriate place under each experiment.

Tests in Flats

Soil taken from the field was very dry and required the addition of water to the storage bin to raise the moisture level to approximately 25 to 30 per cent of its waterholding capacity. By averaging the weights of 48 flats of moist screened soil, a standard weight was established and then converted to a volume standard. The volume standard thus obtained was used for all subsequent tests in flats.

The chemicals were sprinkled over the surface of the soil which had been measured and poured into a large galvanized pan. A thorough mixture with the upper inch or so of surface soil was accomplished by hand, afterward a hoe was used to facilitate mixing of the entire contents.

Test 1. A series of soil samples were treated with varying quantities of each commercial product in an attempt to determine the phytotoxicity of each chemical. Moist, non-steamed soil was placed in flats and mixed with each of the 4 fungicides at the rate of 1:20,000, 1:10,000, 1:5,000, and 1:2,000. Immediately after

planting, all flats were watered and placed in the greenhouse.

Results. The per cent emergence did not appear to be greatly lowered by any of the chemicals used. Thiram supressed seedling emergence. Plants were stunted at all rates (Figure 6). Stunting was especially severe in the two higher ones where few plants survived for 36 days at which time the experiment was terminated. Although unlike thiram in its toxic effects, captan produced noticeable damage at each rate, marginal leaf burning being the typical symptom (Figure 7). Increasing injury was noted as the rate was increased until approximately 1/2 the leaf area was destroyed at the highest rate. Necrotic areas were light brown in color and distinctly separated from healthy tissues. No plant injury was evident in any flats containing zineb or pentachloronitrobenzene. There was evidence that captan accelerated seedling emergence at all rates while thiram delayed emergence at high rates.

Figure 6. Six day old seedlings grown in soil treated at the following rates of thiram: A, 1:20,000; B, 1:10,000; C, 1:5,000; D, 1:2,000.

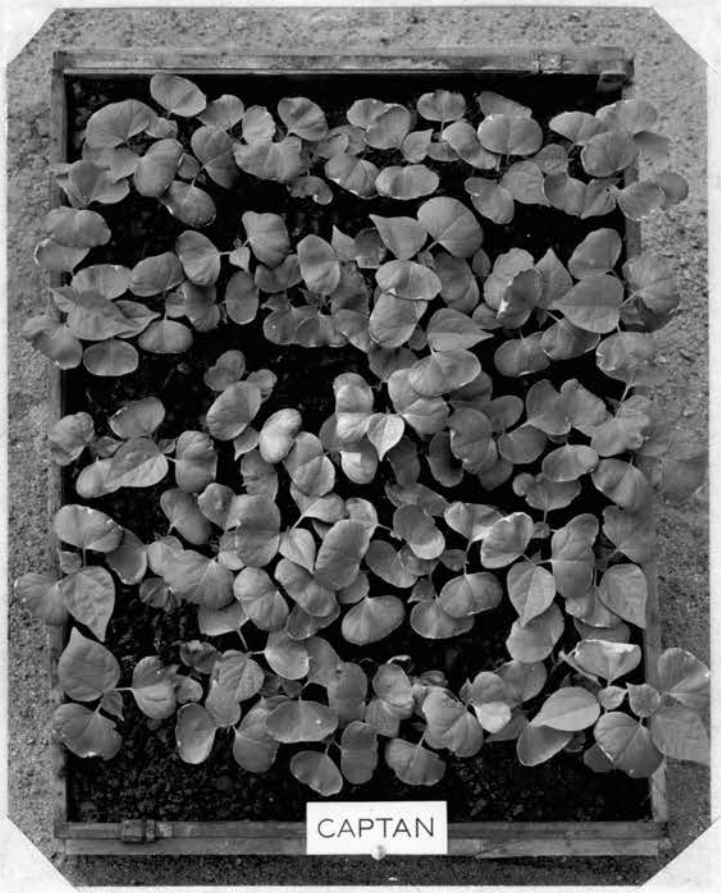

Figure 7. Three week old cotton seedlings depicting captan injury when used at the rate of 1:20,000.

Test 2. This test consisted of 3 trials or replicates. One part commercial chemical to 20,000 parts of moist steamed soil was prepaired and 4 flats were mixed at a time. In the end, there were 16 flats containing 4 lots of soil treated with each fungicide.

Identical series were planted after 0, 1, 2, and 4 weeks. Both an inoculated (90 ml of standard hyphal suspension per flat) and an uninoculated check accompanied each series. The entire experiment was conducted during early fall at a time when temperatures were unseasonably high (80° to 105° F). Counts were made when seedlings were 12 and 18 days old, but only the 18 day count was analyzed statistically.

Inoculation was attempted by 2 methods: 1) The upper inch of soil was removed, after which seeds were planted and the inoculum applied. The soil which had been removed was then replaced. 2) The soil was not removed, but a thin board was used to make a 1 inch deep furrow. Inoculum was applied to the entire soil surface of each flat.

Results. The first attempt was a failure as no infection occurred with either organism. The inoculation technique (method 2) was apparently at fault.

In the second trial using method 1, infection was excellent at the "0" planting; however, R. solani appeared to vary in its pathogenicity as the inoculated checks showed little infection in the 1 and 2 week series, yet was severe in the 4 week planting.

In the third trial, fluctuation in pathogenicity of R. solani was again evident while P. debaryanum remained uniformly pathogenic. Only the data obtained with P. debaryanum in trials 2 and 3 are presented (Table 7).

Table 7. Cotton seedling test showing the effect of chemicals mixed with moist, steamed soil and tested against P. debaryanum after a lapse of the indicated time.

Treatment	Rate /a	Surviving seedlings Mean of 2 replications (%) time before soil was inoculated (in weeks) 0	1	2	4	Mean
Inoculated						
Zineb	1:20,000	5.0	8.5	1.5	8.0	5.8
Thiram	"	87.0	78.5	60.5	45.5	67.9
Captan	"	76.5	83.0	81.5	72.5	78.4
Pentachloronitro-benzene /b	"	31.0	30.5	7.0	16.0	21.1
No chemical	—	3.0	8.0	3.0	5.5	4.9
Non-inoculated						
No chemical	--	88.5	87.5	77.0	90.5	85.9

Analysis of variance of table 7.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	39	56,095.9	--	
Times	3	646.5	215.5	1.89
Blocks Wn. Times	4	1,233.5	--	
Treats. Wn. Times	16	53,039.5	--	
Treatments	4	51,163.9	12,790.98	112.27**
Treats. x Times	12	1,875.6	156.3	1.37
Error Within	16	1,822.9	113.93	

/a All chemicals are reported on a commercial rather than an active ingredient basis.

/b Since data on pentachloronitrobenzene was variable, it was omitted from the analysis. Replicate 1 averaged 32.2 per cent surviving seedlings while replicate 2 averaged 1.6 per cent.

Figure 8. Twelve day old seedlings grown in soil infested with P. debaryanum and treated with the indicated chemicals immediately proir to planting. ("Ck + F" represents check + fungus.) Pentachloronitrobenzene failed to control the disease in the second replication.

Test 3. Non-steamed soil was used in this test and only P. debaryanum was used to infest the soil. Inoculum was applied according to the first method. Greenhouse temperatures were about 70° F for the 0, 1, and 2 week planting, but were raised so that the average was about 80° F for the 4 week planting. Otherwise this test conformed to test 2.

Results. Table 8 lists pertinent data on test 3. Captan alone showed promise of insuring seedling protection in the initial or 0 week planting and the 1 week planting. The 2 week planting revealed captan still in the lead, zineb second, and pentachloronitrobenzene and thiram about equal for third place as ranked by total surviving seedlings. Captan and pentachloronitrobenzene appeared equally effective in insuring seedling emergence for the 4 week planting when temperatures were more favorable for germination and growth of cotton.

Interpretation of results obtained in this test is difficult. No combined analysis was figured since true relationships would not have been pointed out due to greenhouse conditions not remaining constant throughout the entire experiment. Poor emergence of checks could probably be attributed to a departure from optimum growing conditions for the host in the presence of seedling disease pathogens which occurred naturally in the soil.

Table 8. Cotton seedling disease test showing the effect of chemicals mixed with non-steamed soil and tested against P. debaryanum after indicated time periods.

Treatment	Rate /a	Surviving seedlings Mean of 2 replications (%) time before soil was inoculated (in weeks) 0	1	2	4	Mean
Inoculated						
Zineb	1:20,000	1.0	3.0	21.0	30.5	13.8
Thiram	"	0.5	3.0	8.5	27.5	9.9
Captan	"	51.5	28.0	34.5	51.5	42.4
Pentachloronitrobenzene /b	"	0.5	5.5	10.0	48.5	16.1
No chemical	--	0.5	1.0	0.5	0.0	0.5
Non-inoculated						
No chemical	--	30.0	21.0	27.0	64.0	35.5

Analysis of variance of table 8.

Source of variation	Time in weeks 0	1	2	4
Total				
D.F.	11	11	11	11
S.S.	5,774	1,171	1,177	5,788
Blocks				
D.F.	1	1	1	1
S.S.	8	4	14	0
Treatment				
D.F.	5	5	5	5
S.S.	5,716	1,153	1,631	5,146
M.S.	1,143	231	326	1,029
Error				
D.F.	5	5	5	5
S.S.	50	15	32	642
M.S.	10	3	6	128
F value for treatments	114.3**	77.0**	54.3**	8.0**

/a Chemicals are reported on a commercial rather than an active ingredient basis.

Test 4. This test, using steamed soil, was set up contemporary with the 0, 1, and 2 week planting of test 3 when greenhouse temperatures were approximately 70° F. It was designed to compare the effectiveness of chemicals tested at varying rates. Ninety ml standard hyphal suspension of P. debaryanum was used to inoculate each flat. Checks in this case consisted of chemical rates used for residual tests 2 and 3, ie. 1:20,000. Due to their phytotoxicity in test 1, concentrations of thiram and captan were lowered. On the other hand, the concentration of pentachloronitrobenzene and zineb were increased. Counts were made when seedlings were 19 days old.

Results. Table 9 lists stand counts and chemical rates used in test 4. Captan, even when used at relatively low rates, insured seedling protection under the conditions of this experiment. Almost no control was shown for the other chemicals. One exception was zineb at 1:3,000 which had 10 serviving seedlings.

Table 9. Cotton seedling test comparing the effectiveness of four chemicals at varying rates against *P. debaryanum* applied to steamed soil at the seed level.

Treatment	Rate /a	Surviving seedlings (%) (after 19 days)
Thiram	1:20,000	0
"	1:25,000	0
"	1:30,000	0
"	1:40,000	1
Pentachloro-nitrobenzene	1:20,000	0
"	1:15,000	0
"	1:10,000	0
"	1: 7,000	1
Captan	1:20,000	59
"	1:30,000	65
"	1:40,000	59
"	1:45,000	46
Zineb	1:20,000	0
"	1:15,000	0
"	1: 7,000	0
"	1: 3,000	10
Check + Fungus		0

/a All chemicals are reported on a commercial rather than an active ingredient basis.

Tests in Pots

A switch from flats to pots was considered because of limited greenhouse space. By making such a change, more replicates could be run; also, soil samples could be measured more accurately. All chemical-soil mixtures were prepaired according to a weight basis. Each 6 inch pot received 1500 g of soil before any fungicide was added. In every case, chemicals were figured on an active ingredient basis and were applied at the following rates: Captan 1:60,000; thiram 1:20,000; zineb 1:5,000; and pentachloronitrobenzene 1:5,000. Chemicals were added after first mixing them with sand that passed through a #40 sieve. Sand-fungicide mixtures used as stock contained 1 per cent active fungicide and all subsequent dilutions were made from there.

According to preliminary experiments, each product was used at a rate whereby, 1) it was not seriously phytotoxic; and 2) its fungitoxic effectiveness had been indicated at least in one or more tests for the test pathogen. A thorough mixture of soil and fungicide was obtained by placing each together and rolling them in a bucket fitted with a tight lid; several large bolts were included to aid in the mixing process.

Both R. solani and P. debaryanum were used as test organisms. Caps were removed from plugged prescription bottles in which cultures were grown, as it had been observed that both fungi grew better; also R. solani failed to produce sclerotia in capped bottles and when used for inoculum proved not to be pathogenic in some cases.

Ten seeds were planted in each pot after first removing the upper 1 inch of soil. Stand counts were made after 12, 18, and 22

days. Surviving seedlings were examined and a count made of plants free of soreshin.

Test 1. This was an attempt to substantiate the previous tests on how long the chemicals remained effective in the soil. In order to minimize variations in the chemical-soil ratios, the soil was air dried at room temperature. This was easily accomplished by pouring soil into a large container and stirring it several times each day with a hoe. Usually, the soil was dry within 4 to 7 days.

A sufficient quantity of soil was treated with each chemical so that 3 complete series were available. One was designated to be inoculated with R. solani, one with P. debaryanum, and the other used as an uninoculated check. All pots were then placed in the greenhouse and watered daily. Three and 6 weeks thereafter, identical series were prepared and placed in the greenhouse. When ready to be inoculated, 5 ml of the standard hyphal suspension were used for each inoculated pot.

At the end of 6 weeks all 3 lots with P. debaryanum were planted and removed to the refrigerator for 6 days of incubation. Due to mechanical difficulties, temperatures in the box for the first 24 hours were 54° to 59° F. A fan supplied with a heating unit was then used to raise the temperature to 63° F after which the R. solani and check series were planted. There was a similar incident which occurred in the greenhouse 5 days after the P. debaryanum series was placed there; soil temperatures dropped to 59° F for a period of about 6 hours. Otherwise, temperatures in the greenhouse ranged around 85° to 90° F.

At the rates used, all chemicals showed some promise of

controlling R. solani in inoculated pots (Table 10). Pentachloronitrobenzene gave the best control against both pre-emergence and post-emergence injury. Zineb at 1:5,000 and thiram at 1:20,000 also showed promise, whereas captan at 1:60,000 gave as good emergence but did not control post-emergence injury.

In the P. debaryanum series, no chemicals showed marked promise of controlling the organism under conditions prevailing for this test.

Of especial interest is the disease picture shown by the check (uninoculated) series. Treatment differences were significant at the 1 per cent level (Table 11). It was noted that zineb was very effective in preventing seedling injury to plants subjected to any organisms which might have prevailed in this soil. Thiram likewise offered a high degree of protection, but showed some tendency to lose its residual effectiveness. Captan and pentachloronitrobenzene, while rendering a great deal more protection than the untreated check, were still substantially lower in value than zineb and thiram.

Table 10. Cotton seedling disease test to determine residual effectiveness among four fungicides using P. debaryanum and R. solani as test organisms in non-steamed soil.

Treatment	Rate	Time (in weeks)	Total of 3 replications (%) Emerged (12 days)	R. solani Survived (18 days)	R. solani Free of lesions (22 days)
Zineb	1: 5,000	0	30.0	26.6	16.6
Thiram	1:20,000	"	40.0	33.3	16.6
Captan	1:60,000	"	26.6	13.3	0.0
Pentachloro-nitrobenzene	1: 5,000	"	23.3	23.3	13.3
Check	None	"	3.3	3.3	0.0
Zineb	(as above)	3	40.0	26.6	10.0
Thiram	"	"	16.6	13.3	6.6
Captan	"	"	40.0	26.6	13.3
Pentachloro-nitrobenzene	"	"	53.3	50.0	43.3
Check	"	"	3.3	3.3	3.3
Zineb	(as above)	6	50.0	30.0	20.0
Thiram	"	"	13.3	12.0	0.0
Captan	"	"	16.6	13.3	10.0
Pentachloro-nitrobenzene	"	"	46.6	46.6	36.6
Check	"	"	6.6	6.6	6.6
			P. debaryanum		
Zineb	(as above)	0	23.3	23.3	3.3
Thiram	"	"	10.0	6.6	0.0
Captan	"	"	6.6	6.6	0.0
Pentachloro-nitrobenzene	"	"	3.3	3.3	0.0
Check	"	"	0.0	0.0	0.0
Zineb	(as above)	3	6.6	3.3	0.0
Thiram	"	"	0.0	0.0	0.0
Captan	"	"	6.6	3.3	3.3
Pentachloro-nitrobenzene	"	"	10.0	3.3	0.0
Check	"	"	26.6	26.6	20.0
Zineb	(as above)	6	20.0	16.6	0.0
Thiram	"	"	10.0	10.0	6.6
Captan	"	"	16.6	16.6	3.3
Pentachloro-nitrobenzene	"	"	13.3	16.6	6.6
Check	"	"	0.0	0.0	0.0

Table 10 (continued).

Treatment	Rate	Time (in weeks)	Total of 3 replications (%) Uninoculated Emerged (12 days)	Survived (18 days)	Free of lesions (22 days)
Zineb	1: 5,000	0	93.3	96.6	96.6
Thiram	1:20,000	"	93.3	90.0	76.6
Captan	1:60,000	"	46.6	43.3	23.3
Pentachloro-nitrobenzene	1: 5,000	"	56.6	60.0	56.6
Check	None	"	53.3	16.6	16.6
Zineb	(as above)	3	100.0	100.0	96.6
Thiram	"	"	96.6	93.3	93.3
Captan	"	"	93.3	83.3	83.3
Pentachloro-nitrobenzene	"	"	80.0	66.6	56.6
Check	"	"	50.3	46.6	30.0
Zineb	(as above)	6	100.0	100.0	100.0
Thiram	"	"	83.3	83.3	66.6
Captan	"	"	93.3	93.3	90.0
Pentachloro-nitrobenzene	"	"	83.3	83.3	76.6
Check	"	"	63.3	60.0	56.6

Combined data showing totals for the R. solani, P. debaryanum, and uninoculated series.

Treatment	Surviving seedlings (%) R. solani	P. debaryanum	Uninoculated
Zineb	27.7	14.4	98.8
Thiram	18.8	5.5	91.1
Captan	17.7	8.8	76.6
Pentachloro-nitrobenzene	40.0	7.7	70.0
Check	4.4	8.8	41.1

Table 10 (continued). Combined analysis of both inoculated and non-inoculated soils.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Rhizoctonia				
Total	44	200,186		
Treatments	4	63,371	15,842	3.815*
Time	2	2,036	1,018	.245
Treatment x Time	8	10,205	1,276	.307
Error	30	124,574	4,152	
Pythium				
Total	44	90,082		
Treatments	4	2,531	632	.368
Time	2	2,296	1,148	.668
Treatment x Time	8	33,694	4,212	2.457*
Error	30	51,561	1,718	
Check (uninoculated)				
Total	44	207,563		
Treatments	4	88,609	22,152	8.800**
Time	2	21,231	10,615	4.217*
Treatment x Time	8	22,215	2,777	1.103
Error	30	75,508	2,517	

Test 2. The object of this experiment was to see what modifications in chemical protection would be encountered if test organisms were allowed to become established before fungicides were added. Each 1500 gram lot of soil for this test was weighed while moist, then inoculated with its respective pathogen by mixing 10 infested sorghum seeds throughout the soil sample. All pots were then placed in the greenhouse and watered daily for 20 days. Chemicals were then mixed with the soil as described in test 1, each pot was planted immediately, incubated 6 days at 63° to 66° F and again returned to the greenhouse.

Results. Captan at 1:60,000 showed a very marked tendency toward allowing seedlings to become parasitized after emergence. Thiram at 1:20,000 also showed the same tendency with P. debaryanum but not with R. solani. There was no indication that pentachloronitrobenzene at 1:5,000 inhibited P. debaryanum; however, its value against R. solani was clearly evident. Pre-emergence and post-emergence injury caused by either pathogen was greatly reduced with zineb.

Table 11. Cotton seedling disease test to determine effectiveness among four fungicides after R. solani had been allowed to become established in steamed soil.

Treatment	Rate	Cotton seedlings Total of 3 replications (%) Emerged (12 days)	Survived (18 days)	Free of lesions (22 days)
Inoculated				
Zineb	1: 5,000	76.6	76.6	60.0
Thiram	1:20,000	90.0	86.6	76.6
Captan	1:60,000	90.0	20.0	0.0
Pentachloronitro-benzene	1: 5,000	73.3	73.3	70.0
No chemical	--	20.0	3.3	0.0
Non-inoculated				
No chemical	--	80.0	80.0	53.3 /a

/a One uninoculated pot containing 8 plants was obviously contaminated thus leaving no plants free of injury.

Data for seedlings surviving after 18 days (%).

Treatment	Replicates 1	2	3	Mean
Inoculated				
Zineb	100	80	50	76.6
Thiram	100	90	70	86.6
Captan	0	20	40	20.0
Pentachloronitro-benzene	70	70	80	73.3
No chemical	0	0	10	3.3
Non-inoculated				
No chemical	70	100	70	80.0

Analysis of variance of table 11.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	17	222.0		
Replications	2	1.3		
Treatments	4	189.3	47.3	16.89**
Error	11	31.4	2.8	

Table 12. Cotton seedling disease test to determine effectiveness among four fungicides after the organism P. debaryanum had been allowed to become established in steamed soil.

Treatment	Rate	Cotton seedlings Total of 3 replications (%) Emerged (12 days)	Survived (18 days)	Free of lesions (22 days)
Inoculated				
Zineb	1: 5,000	83.3	80.0	60.0
Thiram	1:20,000	70.0	70.0	26.6
Captan	1:60,000	50.0	36.6	0.0
Pentachloronitro-benzene	1: 5,000	0.0	0.0	0.0
No chemical	--	0.0	0.0	0.0
Non-inoculated				
No chemical	--	86.6	86.6	86.6

Data for seedlings surviving after 18 days (%)

Treatment	Replicates 1	2	3	Mean
Inoculated				
Zineb	100	60	80	80.0
Thiram	90	40	80	70.0
Captan	50	0	60	36.6
Pentachloronitro-benzene	0	0	0	0.0
No chemical	0	0	0	0.0
Non-inoculated				
No chemical	90	90	70	83.3

Analysis of variance of table 12.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	17	243.5		
Replications	2	17.3		
Treatments	4	223.1	55.8	15.94**
Error	11	3.1	3.5	

DISCUSSION

The foregoing investigation tends to confirm field tests in which certain fungicides controlled cotton seedling diseases. For the sake of convenience and comparison, all rates of the chemicals used in these studies will be discussed according to what their concentration would be when figured on an active ingredient basis. (the first 3 tests were not figured in this manner in the text).

With the exception of one replication of one test in steamed soil, pentachloronitrobenzene was not effective against P. debaryanum up to a rate of 1 part per 5,000 parts of soil. It was consistently effective against R. solani at 1:5,000 and at 1:25,000; however, data on the test where the lower rate was used was not included with the other results as the organism did not remain consistently virulent throughout this particular test. Zineb showed promise against both R. solani and P. debaryanum in both steamed and non-steamed soil only when greenhouse temperatures were relatively high and conditions were less favorable for a severe test. Thiram, like zineb, did not control either P. debaryanum or R. solani when conditions were favorable for a severe test. It exhibited marked toxicity to cotton seedlings above a rate of 1:20,000 as evidenced by delayed germination and stunting. Captan was consistently effective in insuring emergence throughout the entire series of tests even at a rate as low as 1:60,000 and under conditions favoring extreme disease severity. Emergence was usually accelerated at least 24 hours. At the lower rate, however, captan did not control post-emergence injury.

Phytotoxicity, as evidenced by marginal leaf scorching, occurred if the rate of pure captan exceeded 1:40,000.

Since only one type of soil was used in these studies, different results might have been obtained had other types been used. Richardson (28) found that thiram persisted for 2 months in sandy soil, but disappeared from compost soil within one week. According to its manufacturer, captan breakdown under laboratory conditions is a function of pH. Decomposition was relatively rapid above pH 9.5. No comparable information on zineb or pentachloronitrobenzene could be found by the writer.

The type of soil used in these studies, being acid and sandy, would probably favor the persistence of thiram and captan for a considerable period of time; at least long enough to insure satisfactory practical control. At any rate, all chemicals used (except possibly thiram) provided protection to seedlings for at least 4 weeks where seedlings were tested in flats and 6 weeks when tests were made in pots.

There were some discrepancies in the results obtained when flats were used, but most or all of these can be attributed to differences in temperatures in the greenhouse. As these tests were conducted over a considerable time period, differences in length of day and light intensity also appeared to retard the growth of cotton, thus probably augmenting its susceptibility to seed rotting and damping-off fungi. In the test where soil was dried before using, the microflora may have been changed, possibly modifying chemical decomposition; however, there is little evidence from these studies that the balance of microflora was changed enough to alter the disease picture.

The nature of curves plotted from data representing seedling

emergence as a function of inoculum rates of P. debaryanum suggests that soil type, and the amount of inoculum are interacting factors affecting disease severity. A further investigation to determine whether or not this would occur in non-steamed soil under natural conditions would be interesting.

Another interesting point would be to conduct a series of tests varying the inoculum potential while keeping the amount of sorghum seed uniform. One could then observe what effect the medium would have on disease severity. An attempt to determine this relationship during the winter was not successful as infection was very severe at all dosage levels. Had conditions been more favorable to cotton as in previous tests, differences would probably have been evident.

When seeds were subjected to temperatures from 90° to 110° F, 97 to 100 per cent of the seeds germinated; however, if they were first incubated at a temperature of 63° to 68° F for the first 4 days, germination dropped to around 85 to 95 per cent. It was further observed that if seeds were incubated for 6 days, then placed in a greenhouse held at temperatures ranging from 65° to 75° F, a further reduction in germination down to around 65 to 70 per cent was not uncommon. Two possible reasons as to why this occurred are: 1) the seed may have been internally infected with some organism that was detrimental to seed viability at low temperatures but was not as harmful at high temperatures, or 2) physiological process occuring in the seed while in the process of germination may have been adversely effected at lower and not at higher temperatures. Jacobs (18) suggests that alfalfa seeds should be indexed according to their probable response to conditions unfavorable for germination. According to his

view, the likelihood of a seed rotting is determined primarily by the condition of the seed itself (eg. immaturity of a seed when it is harvested and possibly abnormal fractures in the seed coat).

A fungicide showing toxicity to several organisms would be most beneficial as a number of different fungi infect cotton seedlings. The check series in the first test in pots of this study seemed to indicate that zineb gave the best control of the pathogens that existed in soil as it came from the field. Captan showed the most marked control of either R. solani or P. debaryanum in artificially infested soil. Perhaps more investigation will show that other pathogens are as important as R. solani and P. debaryanum in this complex. Possibly a mixture of fungicides will be needed to give effective control.

It is probable that more than one species of Pythium is responsible for reduced stands of cotton in Oklahoma. Further studies are underway at the present time in an effort to determine whether species other than P. debaryanum are involved in the problem.

SUMMARY

Consideration was given to a method of infesting greenhouse soil with a cotton seedling pathogen tentatively identified as Pythium debaryanum Hesse. Soil was satisfactorily infested by either of 2 ways: 1) Standardized hyphal suspensions could be applied at the seed level as seed were planted, or 2) infested sorghum seed could be placed in the soil so as to allow the fungus to become established before planting. Disease severity in steamed soil was found to be affected by temperature, soil type, and amount of inoculum.

P. debaryanum may reduce stands of cotton by rotting the seed or attacking any part of the young seedling before it emerges from the soil. The fungus may also cause post-emergence injury, soreshin being the typical symptom.

Rates of 1:40,000 and 1:60,000 captan, when figured on an active ingredient basis, effectively reduced pre-emergence injury, but fell short of adequate post-emergence protection. Thiram remained fairly uniform in protective value against each organism when used at a rate of 1:20,000. Zineb and pentachloronitrobenzene were consistently effective only when used at a rate of 1:5,000; with one exception, the latter was effective only against R. solani.

Stunting of young seedlings was evident when the rate of pure thiram exceeded 1 part per 20,000 of soil. Leaf burning was expressed when plants were grown in soil containing more than 1:40,000 parts of pure captan. Zineb and pentachloronitrobenzene exhibited no phytotoxicity up to a rate of 1:5,000.

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