

STUDIES WITH SOIL AMENDMENTS FOR THE CONTROL OF VERTICILLIUM WILT
AND SORESHIN OF COTTON

By

ROBERT A. KORISEN

Bachelor of Science

Arizona State College

Tempe, Arizona

1950

Submitted to the Faculty of the Graduate School of
the Oklahoma Agriculture and Mechanical College
in Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

1952

STUDIES WITH SOIL AMENDMENTS FOR THE CONTROL OF VERTICILLIUM WILT
AND SORESHIN OF COTTON

ROBERT A. KORTSEN

MASTER OF SCIENCE

1952

THESIS AND ABSTRACT APPROVED:

L. A. Binkertoff

Thesis Adviser

F. Ben Stubble

Faculty Representative

James W. Hausman

Head of the Department

W. M. Dutocher

Dean of the Graduate School

291976

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Mr. Lloyd A. Brinkerhoff for suggesting the problem and for his help and advice during the investigations. The author is indebted to Dr. Walter W. Hansen and Dr. E. Ben Struble for their guidance in the final preparation of the thesis. Further, the author takes special pleasure in thanking Dr. John Thomas and Dr. Harry C. Young Jr. for their timely advice during the investigations. Lastly, appreciation is expressed to my wife, Dolores, for her help and encouragement.

TABLE OF CONTENTS

	page
INTRODUCTION.	1
LITERATURE REVIEW	2
MATERIAL AND METHODS.	9
Tests of certain chemicals to determine their effectiveness against soil-borne inoculum.	9
Tests to determine the effectiveness of chemicals against inoculum placed in the plant tissues	11
Field and greenhouse tests for the control of cotton seedling diseases.	12
RESULTS	16
Pot tests with soil-borne inoculum	16
Tests to determine the effectiveness of chemicals against inoculum placed directly in the plant tissues.	27
Field and greenhouse tests for the control of cotton seedling diseases.	27
DISCUSSION.	40
SUMMARY	43
LITERATURE CITED.	44

LIST OF TABLES

Table	page
1. Chemicals used against soil-borne inoculum and checked as possible chemotherapeutants.	9
2. Chemicals used in seed row tests in the field and greenhouse . .	12
3. The effect of 8 chemicals applied as drenches once a week for six weeks to Verticillium wilt infested soil for the control of the organism on cotton	17
4. The effect of 2 experimental chemicals applied as drenches once a week for six weeks to Verticillium wilt infested soil for the control of the fungus on cotton.	21
5. The effect of 9 chemicals applied to Verticillium wilt infested soil prior to planting for control of the fungus on cotton . . .	24
6. Data obtained with 8 fungicides tested for their chemotherapeutic control of the Verticillium wilt fungus when placed in the stems of cotton seedlings.	28
7. Data obtained with 2 experimental chemicals tested for their chemotherapeutic control of the Verticillium wilt fungus when placed in the stems of cotton seedlings.	28
8. Data obtained with 8 fungicides tested for their chemotherapeutic control of the Verticillium wilt fungus when placed in stems of cotton seedlings.	29
9. Data obtained with 8 chemicals tested as chemotherapeutants against cotyledonary inoculation of <u>X. malvacearum</u>	30
10. Data obtained with 2 experimental chemicals tested as chemotherapeutants against cotyledonary inoculation of <u>X. malvacearum</u>	31
11. Data obtained with 8 chemicals tested as chemotherapeutants against cotyledonary inoculation of <u>X. malvacearum</u>	31
12. The effect of 13 chemicals applied to the seed row at Paradise, Oklahoma, for the control of cotton seedling diseases.	33
13. The effect of 13 chemicals applied to the seed row at Chickasha, Oklahoma, for the control of cotton seedling diseases.	34
14. The effectiveness of fungicides applied to seed row for the control of the Rhizoctonia seedling disease of cotton under greenhouse conditions	36

List of Tables (Cont'd.).

Table	page
15. Data obtained with fungicides applied to seed row for the control of the <i>Rhizoctonia</i> seedling disease of cotton under greenhouse conditions.	38

LIST OF ILLUSTRATIONS

	page
Fig. 1. Cotton leaf showing chlorosis produced by Dithane D-14. . . .	18
Fig. 2. Cotton leaf showing chlorosis produced by Mersolite-W	20
Fig. 3. Three-day-old cotton seedling showing stem shriveling produced by Experimental Chemical 1182.	22
Fig. 4. Two-week-old cotton seedlings showing phytotoxicity produced by the following chemicals: 1. Systox, 2. TCA, 3. Arasan, 4. Zerlate, 5. Fermate, and 6. Dithane Z-78	25
Fig. 5. Three-week-old cotton seedlings grown in soil treated with TCA and Dithane D-14.	26
Fig. 6. A comparison of seed row applications of fungicides with seed treatment and an inoculated check.	39

INTRODUCTION

Verticillium wilt (Verticillium albo-atrum R. and B.) and soreshin (Rhizoctonia solani Kuhn) are recognized as two important diseases of cotton in the United States. Verticillium wilt is not as wide spread in Oklahoma as soreshin, but its spread is increasing every year, especially in the irrigated region of the State.

Due to the importance of these diseases, it is evident that more adequate control measures should be worked out. There are some varieties of cotton which are more tolerant to Verticillium wilt than others, but none are completely resistant. To develop resistant varieties requires considerable time, effort, and expense, and when obtained, the breeder may be confronted with new races of the causal organism. In view of these difficulties, a soil amendment which would control the organism would be most welcome. Also of importance would be a soil amendment which would control seedling diseases of cotton.

The purpose of the present investigation was to determine whether a number of relatively new organic fungicides and certain herbicides might be of value when used as soil amendments in controlling Verticillium wilt or soreshin. The chemicals were tested: (1) against soil borne inoculum, and (2) against inoculum placed within the plant tissues. The second test was employed to determine if certain chemicals, when absorbed by cotton plants, would control the test organisms when placed in the above ground portions of the plant. The bacterial blight pathogen, Xanthomonas malvacearum (E.F.S.) Dowson, and the wilt fungus, V. albo-atrum were used as the two test organisms.

LITERATURE REVIEW

Intensive effort is now being made to determine what kinds of chemicals are effective, and what types of diseases are controllable through the chemotherapeutic approach. In particular, virus diseases, systemic bacterial infections, and the vascular wilt diseases have been found to yield to certain chemicals when the latter are applied to the soil, and the plant absorbs them through its roots.

The definition of chemotherapy as it is used in this paper is the one postulated by Horsfall and Dimond (18), which is as follows: "Chemotherapy is treatment of the host with a compound so that the action of the compound occurs inside the host when the host is diseased. It may (a) kill the pathogen as it enters the host, (b) rid the host of an established pathogen, or (c) mitigate disease."

One of the early workers in the field of chemotherapy was Howard (20) who in 1941 injected 350 maple trees naturally infected with Phytophthora cactorum (Leb. and Cohn) Schroet. (bleeding canker fungus) with di-hydrochloride salt of di-amino-azo-benzene. The injected trees exhibited stoppage of bleeding and marked improvement in vegetative growth. In 1942 Caroselli and Howard (4) studied the response of maple trees diseased with bleeding canker to chemotherapy and fertilization. Their data showed highly significant differences between treated and untreated trees. Research in 1942 by Horsfall and Zentmeyer (19), indicated that toxins of the Dutch elm fungus and Verticillium wilt fungus of eggplant and maple were weakened and possibly eliminated by internal chemotherapy with 8-hydroxyquinoline sulphate, urea, dihydrochloride of diamino-benzene, and malachite green. Evidence was obtained in 1941 and 1942 by Zentmeyer and Horsfall (37) that 8-hydroxyquinoline sulfate, hydroquinone, ascorbic acid, and 8-hydroxyquinoline

when introduced into the vascular system of eggplants and maples infected with *Verticillium* wilt, had an ameliorating effect on wilting and disease advance. The same was true for Dutch elm disease.

The chemical 8-hydroxyquinoline has long been known to have bacteriostatic and fungistatic properties and is extensively used as an antiseptic. The mechanism of action of this chemical has been postulated (35, 36). The fact that 8-hydroxyquinoline is a useful agent in quantitative analysis for precipitating many minor elements (Cu, Mn, Fe, Zn), suggests the theory that it acts fungistatically by precipitating one or several of these elements so that the microorganisms cannot use them.

In 1944 and 1947, Stoddard (27, 29) working with seedling peach trees inoculated with "X" disease by budding, injected chemicals through the cut upper end of the main stem. In 124 trials p-aminobenzenesulphanilamide, at various concentrations and under various conditions, reduced infection to 21 per cent. This chemical used at a concentration of 1-2000, and injected after inoculation completely prevented infection of 45 trees while the checks averaged 85 per cent infection. Stoddard suggested that the inhibitory effects of the chemical on the virus of X disease is due to artificial immunization. Zentmeyer *et al.* (38) in 1946 reported that 8-hydroxyquinoline benzoate, 8-hydroxyquinoline sulfate, hydroquinone, p-nitrophenol, benzoic acid, and disodium ethylene bisdithiocarbamate when injected or watered in basins at the base of the tree were effective as chemotherapeutants against the Dutch elm fungus. When oxyquinolin benzoate was applied to the soil as a drench at concentrations of 0.1, 0.05, 0.025, and 0.0125 per cent for the control of *Graphium ulmi* Schwarz (28), there resulted a considerable reduction of wilt symptoms at the higher rates. It is suggested by Stoddard that the effect of the oxyquinolin benzoate was due to antidoting

the fungus toxin causing the wilting and was not due to fungicidal action.

Takahashi (32) reported in 1948 the inhibition of virus increase in living cells by low concentrations of malachite green. This inhibition appears to be due to its action as a block in enzyme reactions leading to virus formation. Dimond et al. (9) reported in 1949 that 8-quinoline applied to the soil suppressed symptoms of Dutch elm disease for one year. In 1949 McNew and Sundholm (23) reported the chemotherapeutic value of substituted pyrazoles and related compounds against leaf blight of tomato caused by Alternaria solani (Ell. and G. Martin) L. R. Jones and Grout. The two best chemicals gave 31 and 44 per cent control. It was deduced by these workers that some of the chemical was taken up by the immersed leaf and transported to the other leaves, where it provided protection by chemotherapy.

In 1950 Feldman et al. (15) evaluated chemicals in the laboratory and greenhouse for their control of Dutch elm disease. The chemicals that were effective against the fungus were tested under natural conditions. Results were: (1) hydraulic soil impregnation with lime suppressed wilting for at least one month when soil pH was maintained at 7.0 or slightly above; (2) low magnesium lime gave better disease control than high magnesium lime; and (3) trunk injections of basic chemicals (NaHCO_3 , KHCO_3 , $\text{Ca}(\text{OH})_2$) gave good control.

Chapman et al. (6) reported in 1950 that chemotherapy experiments conducted in the greenhouse have consistently prevented infection of tomato plants with Fusarium wilt. The test compound was applied to sand and apparently was absorbed by the plant. Their experiments showed that effective compounds prevented but did not cure Fusarium wilt of tomatoes.

In 1951 Caroselli and Feldman (3) reported that Carolate and related formulations applied by hydraulic soil impregnation reduced injury from Dutch

elm disease on more than 2,000 woodlot and estate elms. The disease was arrested in approximately 70 per cent of the trees for more than 2 years. These workers suggest that the chemicals were acting fungistatically because the fungus could be cultured from discolored tissue of trees treated 3 years previously.

Chapman (5) reported in 1951 studies on the relation of specific chemotherapeutants applied prior to inoculation. He found that the chemical must be present in the area penetrated by the organism to be effective. Tests for zones of inhibition about filter paper discs indicated that 8-hydroxyquinoline benzoate was not absorbed by cellulosic substances and moved through the plants to the top. In practice it was more effective against the elm twig invading C. ulmi than against the tomato root invading Fusarium lycopersici Sacc. In contrast n-octadecyltriniethylammonium pentachlorophenate was tied up by filter paper and was believed to be similarly held by cellulosic substances in plant roots. It controlled the tomato Fusarium wilt fungus but not the Dutch elm fungus. Ark (1) in 1951 reported that the sodium salt of o hydroxydiphenyl had possibilities as a chemotherapeutant against certain bacterial diseases of orchids. When diseased plants were completely immersed in a 1:2000 solution of the chemical for 60 minutes or longer, successful control of the disease was obtained.

In 1951 Stoddard (30) reported good control with 2-norcamphene methanol, 4-chloro-3, 5-dimethyl phenoxyethanol, and 8-quinolinol sulfate against Fusarium wilt of carnations. The treatments reduced wilt to 2.8, 3.2, and 4.0 per cent respectively, while the checks showed 50.6 per cent. Also in 1951, Dimond and Chapman (8) working with 4-chloro-3, 5-dimethylphenoxyethanol, and 2-norcamphene methanol for control of Fusarium dianthi (Prill. and Del.) Sny. and Han., reported results similar to those of Stoddard (30). The

chemicals were also effective against F. lycopersici.

In 1951 Stoddard (31) reported using disodium ethylene bisdithiocarbamate (Dithane D-14) for the control of strawberry red stele caused by Phytophthora fragariae Hickman. Units of 10 strawberry plants growing in sand received five successive applications of Dithane D-14, 1.5 and 0.75 per cent respectively. The plants were then replanted in infested untreated soil without further treatment. The higher concentration gave 100 per cent control, and the lower 60 per cent; only 10 per cent of the check plants grown in untreated soil remained healthy.

Crowdy (7) in 1951 testing certain aryloxyaliphatic acids as chemotherapeutants against Botrytis fabae Sard. and Botrytis cinerea Pers. infected bean plants, showed diminished rate of growth of the lesions on the treated plants. 2-4-6-trichlorophenoxyacetic acid, pentachlorophenoxyacetic acid, and pentachlorophenoxyisobutyric acid produced average reductions in disease of 30-40 per cent. Dimond et al. (10) in 1951 reported the effect of dyes in retarding the development of crown galls. The dyes were used at a concentration of 0.05 per cent, and none of them prevented gall development. However, gall size was significantly reduced in tomatoes by hypodermically injected dyes prior to inoculation.

The remaining portion of this literature review is concerned with soil amendments applied for the control of soil-borne pathogens of plants. This review only partially covers the subject.

Wilhelm (34) demonstrated that when blood meal, fish meal, cottonseed meal, and ammonium sulfate were applied to infested soil in pots, the inoculum potential of Verticillium albo-atrum was substantially reduced. Rudolph and Harrison (26) reported that when anhydrous ammonia, anhydrous ammonia plus ammonium polysulphide, cyanamid, and sulphur were used on

infested plots for three consecutive years, all failed to control Verticillium wilt of cotton. They also reported that sulfate of ammonia and carbon bisulphide failed to control the disease.

McKeen (22) using tetramethylthiuramdisulfide (Arasan) as a soil treatment, found it effective against damping-off of cucumber, pepper, spinach, and tomato, although control was more complete when seed and soil treatment with Arasan were combined. Heuberger (17) applied disodium ethylene bisdithiocarbamate (Dithane D-14) to the soil at the rate of 75 pounds per acre and received better control of damping-off of peas than when tetra chloro-parabenzquinone (Spergon) was used as a seed treatment. Disodium ethylene bisdithiocarbamate (Dithane D-14), applied as a soil treatment in the planting row at the time of seeding, produced marked reduction in the severity of root rots of bean (21).

Haensler and Moyer (16) greatly reduced seed decay and damping-off with soil applications of calcium cyanamide at the rates of 1,000 to 2,000 lbs. per acre. The same workers reported that calcium cyanamide used at the rate of 5 to 50 lbs. per acre and applied in close proximity to the seed immediately before planting, also gave good control of damping-off and seed decay. Walker and Larson (33) in greenhouse tests using soil with a pH 6.4 and applying calcium cyanamide at the rate of 250 lbs. per acre, prevented club-root infection of cabbage plants. The plants in the untreated soil were all infected.

Nelson (24) using zinc ethylene bisdithiocarbamate (Dithane Z-78) and tetramethyl thiuramdisulfide (Arasan), diluted 1 to 3 with talc, obtained good control of onion smut and damping-off. However, when tetramethyl thiuramdisulfide and formaldehyde were applied to the seed row of soils heavily infested with onion smut, formaldehyde gave better control than did

Arasan. Riker et al. (25) have reported success in the control of damping-off of pine seedlings by soil treatment with calomel. Also soil treatment with tetramethyl thiuremdisulfide was relatively effective not only against damping-off but also against many weed seeds and winter injury.

In greenhouse tests in flats the clubroot of cabbage, onion smut, and damping-off of vegetable seedlings were controlled by fungicides applied to the soil with commercial fertilizer used as a carrier (11). Doran and Sproston (12) have reported that onion smut was controlled by ferric dimethyldithiocarbamate (Fermate) at the rate of 58 pounds per acre applied to soil immediately before seeding. In soil treated with ferric dimethyldithiocarbamate, there was only 1 per cent smut, while in the untreated soil there was 88 per cent. Sodium nitrite applied to soil at the rate of 4-8 oz. per sq. yd. four weeks before seeding was effective in controlling damping-off of lettuce caused by Rhizoctonia (13).

MATERIALS AND METHODS

Tests of certain chemicals to determine their effectiveness against soil-borne inoculum. A heavy clay soil having a pH of 8.0 from Blair, Oklahoma, was used in the tests. The soil was obtained from a cotton field that had been heavily infested with *Verticillium* wilt the previous season.

The different chemicals used in the tests, their active principles, and number of applications are indicated in table 1. The rates at which the chemicals were used are presented in tables 3, 4, and 5.

TABLE 1.- Chemicals used against soil-borne inoculum and checked as possible chemotherapeutants.

Trade Name	Active Principle	% Active Principle	Manufacturer
Actidione ^a	cycloheximide	100.0	Upjohn
Arasen	tetramethylthiuramdisulfide	50.0	DuPont
Bioquin 700 ^a	8 quinolinol benzoate	100.0	Monsanto
Bioquin 850 ^a	8 hydroxyquinoline sulfate	100.0	do
Dithane Z-78	zinc ethylene bisdithiocarbamate	65.0	Rohm & Haas
Dithane D-14 ^a	disodium ethylene bisdithiocarbamate	19.0	do
Dowcide-B	sodium trichlorophenate	50.0	Dow
E. C. ^b 1182 ^a	norcamphanemethanol	100.0	Carbide&Carbon
E. C. ^b 1207 ^a	chlorodimethylphenoxy ethanol	100.0	do
Fermate	ferric dimethyl dithiocarbamate	76.0	DuPont
Maleic hydrazide ^a	maleic hydrazide	-	U. S. Rubber
Mersolite W ^a	phenyl mercuric acetate	96.5	F.W. Berk
O. ^c benzoate ^a	8 hydroxyquinoline benzoate	100.0	Merck
O. ^c sulfate ^a	8 hydroxyquinoline sulfate	100.0	Mallinckrodt
Sodium chlorate	sodium chlorate	100.0	Atlas
Systox	trialkyl thiophosphate	50.0	Geary
T.C.A.	trichloro acetate	90.0	Dow
Zerlate	zinc dimethyldithiocarbamate	76.0	DuPont

^aThese chemicals were applied as drenches once a week for six weeks at the rate of 1 gram per 10,000 grams of soil.

^bE. C. designates experimental chemical.

^cOxyquinoline.

The methods for applying the chemicals were determined by their physical state. The water insoluble powders were mixed thoroughly with all the soil

in the pot to which they were applied. Two of the chemicals used were in granular form and were water soluble. These were applied in 200 ml. of water directly to the soil surface. The chemicals in liquid form were also diluted with 200 ml. of water and applied as drenches to the soil surface. The rates in all instances were applied on a soil weight basis.

Inoculum which was grown on artificial media was added to each pot to make sure that all soil used had essentially the same inoculum potential. In all tests the inoculum was added to the pots before the addition of the chemicals. The source of the inoculum was from a stock culture maintained in the laboratory and isolated from a *Verticillium* wilt infected cotton stalk from Cotton Research Station, Tipton, Oklahoma, July, 1949. The fungus was grown on three different types of media to increase it for inoculation purposes. The inoculum for the first test was grown on test tube slants of potato dextrose agar and used at the rate of one tube per one gallon glazed crock of soil. The inoculum for the second test was grown on 75 ml. of potato broth in 250 ml. flasks which were shaken twice daily for 16 days. The broth was made by cooking 200 grams of peeled potatoes in a liter of water for 15 minutes. One flask was used to inoculate six one gallon glazed crocks. The inoculum for the other four tests was grown on sorghum seeds for 14 days. One hundred grams of sorghum seeds and 75 ml. of water, placed in a 250 ml. flask, were autoclaved once a day on two successive days for 45 and 30 minutes respectively. The inoculum for the remaining four tests was used at the same rate as was indicated in test two. The inoculum for all the tests was incubated at 20° C.

The Waring Blendor was used in all the tests to prepare the inoculum for addition to the pots. The fungal mats of 28 test tubes were removed and placed in the Blendor and macerated for 2 minutes for the first test. The

macerated material was then diluted with 2400 ml. of distilled water, giving 200 ml. of diluted inoculum for each pot. For the second test the fungal mats were removed from the potato broth in the 6 flasks and macerated for 2 minutes in the Blendor. The macerated contents were poured into a five gallon carboy and diluted with 8 liters of distilled water. Each of the 56 one gallon glazed crocks of soil received 200 ml. of diluted inoculum. The preparation of the inoculum for the remaining tests was the same as described for test two.

The inoculum was added to the soil in all tests by removing half of the soil in the pots and adding 100 ml. of the inoculum to the remaining soil. The soil that had been removed was then replaced, and the other 100 ml. was added to the surface of the soil. The pots were then thoroughly watered, placed in the greenhouse, and left for 24 hours. Following this period, six cotton seeds of the variety Acala 4-42 were planted in each pot. All tests were set up in the greenhouse as randomized blocks with each treatment replicated four times.

The plants were first observed for external symptoms of infection such as wilting, mottling and yellowing of the leaves, and abnormal leaf fall.

If no external symptoms were produced when tests were terminated, then the stems were split and checked for vascular discoloration. Tissue isolations were made to determine if the fungus was present when the plants showed none of the above mentioned symptoms.

Tests to determine the effectiveness of chemicals against inoculum placed in the plant tissues. The procedures followed in the pot tests previously described, except for methods of inoculation, were used in setting up these tests. The soil and chemicals used were the same as those indicated in the first pot tests. The methods for culturing the *Verticillium* wilt organism,

preparing the inoculum, and applying the chemicals were the same. The bacterial blight pathogen (X. malvacearum) was cultured on potato dextrose agar.

The method used to inoculate the plants with the *Verticillium* wilt fungus was the needle puncture method described by Evans (14). The technique used for inoculating cotton seedlings with X. malvacearum was a wounding technique described by Brinkerhoff (2).

The criteria for determining the effectiveness of these chemicals as chemotherapeutants against V. albo-atrum are the same as those outlined in the previous test. To determine their effectiveness as systemic bactericides, the inoculated lower epidermis of the cotyledons were checked for typical water soaked lesions. These lesions appear in check plants from six to eight days after inoculation depending on temperature and humidity.

Field and greenhouse tests for the control of cotton seedling diseases.

The chemicals used in the field and greenhouse tests and their active principles are shown in table 2.

TABLE 2.- Chemicals used in seed row tests in the field and greenhouse.

Trade Name	Active Principle	% Active Principle	Manufacturer
Agrox	phenyl mercury urea	6.7	Chipman
Arasan	tetramethylthiuramdisulfide	50.0	DuPont
2% Ceresan	ethyl mercury chloride	2.0	do
Dithane Z-78	zinc ethylene bixdithiocarbamate	65.0	Rohm & Haas
Dow 9B	zinc trichlorophenate	50.0	Dow
Dowcide B	sodium trichlorophenate	50.0	do
F-1112	--	--	do
Ortho-406	N-trichloromethylthis tetrahydrophthalimide	50.0	Calif. Spray
Phygon	2,3-dichloro-1, 4naphthoquinone	50.0	U. S. Rubber
Sperguson	tetrachloro-parabenzquinone	95.0	do
Systox	trialkyl thiophosphate	50.0	Geary
T.C.A.	trichloro acetate	90.0	Dow
4268T	organic arsenic	7.7	Chemagro

The rate at which the chemicals were applied in the field at Paradise, Oklahoma was 15 lbs. per acre except for Spergon, which was also used at three higher rates (30, 60, and 120 lbs. per acre). The same amounts of the chemicals were used at Chickasha, Oklahoma, but instead of using 25 ft. rows as was used at Paradise, they were lengthened to 37 feet to help overcome phytotoxicity. Thus all the rates were reduced by one third in the second experiment.

Each chemical was well mixed with 1 pint of fine sand which was used as a carrier, and placed in one pound paper bags. A total of fourteen different chemicals including Spergon were used. This gave a total of eighteen different treatments, including an untreated check. These treatments were replicated four times and set up as a randomized block experiment with single row plots 25 ft. in length.

The planter boxes on a two row planter were removed and replaced with large funnels. As the planter was drawn along at a slow pace, two men dropped 100 cotton seeds and the sand-diluted chemical in the twenty five foot row space. This left a relatively narrow band of treated sand in the bottom of the drill row. Stand counts, post emergence damping off, and phytotoxicity readings were taken to determine the effectiveness of the treatments.

Tests in the greenhouse which were set up in flats of soil were primarily concerned with the Rhizoctonia seedling disease of cotton. The flats were filled three-fourths full of screened soil from the Perkins Farm. The first test consisted of twenty flats set up as a randomized block experiment, with four replications. All flats of soil were sterilized for six hours in a steam cabinet. The second test consisted of fifty two flats, none of which were sterilized. This test was also set up as randomized blocks and each treatment was replicated four times.

The source of inoculum was from a stock culture of R. solani isolated from an infected cotton seedling, July 15, 1950, from the Cotton Research Station, Chickasha, Oklahoma. The fungus was cultured on sorghum seeds and prepared for use as was previously described in the pot tests for V. albo-atrum. The contents of each 250 ml. flask was used for 3 1/3 flats in the first test and for 7 flats in the second test. The inoculum was incubated for eight days at 20° C.

Two different methods were used in applying the inoculum to the two tests. For test 1 the soil of each flat was placed in a large garbage can, and 200 ml. of inoculum was poured on the soil and then thoroughly mixed. For the second test 200 ml. of inoculum was placed in a Mason jar with the lid punched full of small holes and then sprinkled uniformly on the soil at the seed level.

The chemicals used for the first greenhouse test were as follows: Arasan, Spergon, Phygon and Ortho-406. The same chemicals plus Ceresan-M were used in the second test. Their active principles are shown in table 2. The rates at which these chemicals were used are indicated in table 14. Chemicals for each treatment were weighed at the indicated rate and placed in a glassine bag. Each chemical was then well mixed with 50 ml. of extra fine sand before it was added to the flats. The procedure in applying the chemicals was as follows: each flat was first divided into five evenly spaced furrows, $\frac{1}{2}$ inch deep and $\frac{1}{2}$ inch wide; the sand-chemical mixture was then spread evenly the length and width of each furrow. After chemicals were applied, 100 Acala 4-42 cotton seeds, 20 to each furrow, were planted in each flat. Each flat was then completely covered with $\frac{1}{2}$ inch of soil and watered. The procedure outlined in test 1 for applying chemicals was followed in setting up test 2.

The first test was incubated in the Fiber Laboratory of the Agronomy Department at 70° F. for six days. Afterwards the flats were removed to the

greenhouse. For the second test the flats were incubated in the greenhouse at an average day and night temperature of 77° F.

Stand counts were made two days after the first seedlings emerged. In the first test two different counts were made after the first stand count; the second in two days, and the last in four days. In the second test, the plants were counted every day for eleven days after the first stand count, making a total of twelve counts. Only the surviving seedlings were counted in all of these counts, and after the last count the per cent of post emergence damping off was determined.

RESULTS

Pot tests with soil borne inoculum. Tables 3, 4, and 5 show the results of tests conducted in the greenhouse. The primary purpose of the experiments was to test the fungicidal effect of different chemicals on V. albo-atrum in the soil.

Due to the number of chemicals tested, only those showing indications of controlling wilt will be discussed in any detail. Only four of the eighteen chemicals tested at the rates employed gave indications of possible control of the wilt organism.

Dithane D-14 applied as a drench to cotton plants which were grown in Verticillium infested soil prevented external symptoms of wilt for the length of the experiment (Table 3). The only evidence of phytotoxicity with Dithane D-14 was its affect on the true leaves. The material produced a mild chlorosis which was confined to an area between the major leaf veins. The chlorotic areas were not yellow but a dull white in color. Necrotic areas of about 1/8 to 1/4 of an inch were also produced on the tip of the leaf lobes. These symptoms are shown in figure 1. The plants compared favorably in size, number of leaves, squares, and bolls with the check plants in spite of the chlorosis produced by the Dithane D-14. None of the plants grown in the Dithane D-14 treated soil showed any signs of vascular discoloration at the end of 143 days growth when the test was terminated. The fungus was not recovered in culture from four tissue isolations made from each stem.

Mersolite W., which was also used as a drench, gave good indications of controlling Verticillium wilt (Table 3). However at the rate used, the chemical was highly toxic to cotton seedlings in the cotyledon stage. The plants that emerged died three days later. These pots were left unplanted for one

TABLE 3.- The effect of 8 chemicals applied as drenches once a week for six weeks to Verticillium wilt infested soil for the control of the organism on cotton.

Treatment	Rate	Number of days before wilt symptoms appeared				Treatment Mean
		1	2	3	4	
Actidione	1 p.p.m.	28	41	36	38	35.7
B. 700	1 gr./10,000 gr. soil	28	80	91	90	72.2
B. 850	do	86	85	34	90	73.7
Dithane D-14 ^a	do	143	143	143	143	143.0
O. ^b benzoate	do	85	92	101	96	93.5
O. ^b sulfate	do	63	28	60	48	49.7
M. ^c hydrazide	do	52	38	40	45	43.7
Mersolite W ^a	do	143	143	143	143	143.0
Check (inoculated)	-	31	48	39	66	46.0

Analysis of variance of table 3

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	35	60,672.31		
Blocks	3	592.51	197.503	.790
Treatments	8	54,085.51	6,760.688	27.068**
Remainder	24	5,994.29	249.762	

LSD - at the 1% level 31.2

^aPlants showed no external symptoms in 143 days at which time the experiment was terminated.

^bOxyquinoline

^cMaleic



Fig. 1. Cotton leaf showing chlorosis produced by Dithane D-14.

week after the seedlings died, and then replanted without adding additional chemical. After the plants emerged, the chemical was added when plants formed the first two true leaves. After formation of the true leaves, the plants tolerated the weekly applications of the chemical. The only visible evidence of toxicity was small, irregular, chlorotic spots which developed on many of the leaves after about four weeks. These spots were white in color and closely resembled thrip damage (Figure 2). Except for the toxicity in the seedling stage and the leaf chlorosis, the plants developed normally after overcoming the effects of the chemical. All plants compared favorably in size, number of leaves, squares, and bolls with the checks. Plants treated with Mersolite W. failed to show any external symptoms of *Verticillium* wilt up to 143 days, at which time the plants were removed and examined for vascular discoloration. None of the stems showed vascular discoloration, and the fungus was not recovered in culture from tissue isolations.

Chemical E. C. 1182 used as a drench gave indications of being effective against the *Verticillium* wilt organism (Table 4). This chemical was also phytotoxic to cotton seedlings at the rate used. The first visible evidence of this toxicity was a brownish discoloration of the stem appearing two days after seedling emergence. The following day the discolored area began to shrivel and the cotyledons were wilted. A day later the stems turned black and plants died. A photograph of this phytotoxic reaction in cotton seedlings is shown in figure 3. To overcome this toxicity, the same procedure used for Mersolite-W was followed. The plants treated with E. C. 1182 also tolerated the weekly application after development of true leaves. After formation of the true leaves, the only visible evidence of toxicity was a slight stunting as compared to the check.

The first external symptoms of *Verticillium* wilt in plants treated with

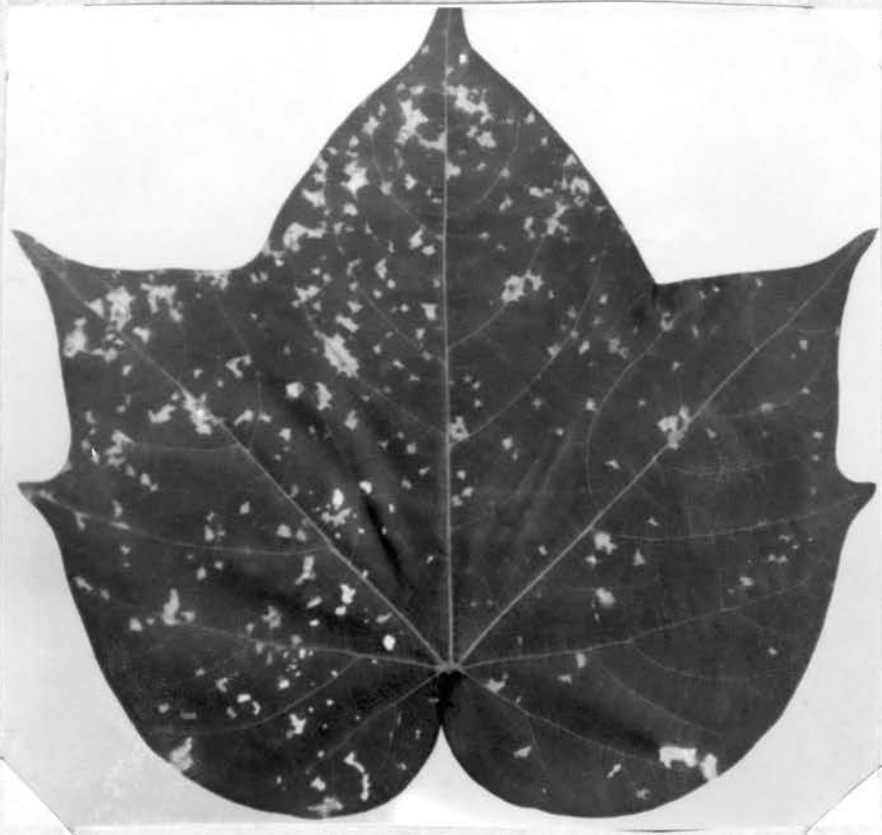


Fig. 2. Cotton leaf showing chlorosis produced by Mersolite-W.

TABLE 4.- The effect of 2 experimental chemicals applied as drenches once a week for six weeks to Verticillium wilt infested soil for the control of the fungus on cotton.

Chemical	Rate	Number of days before wilt symptoms appeared				Treatment Mean
		Replicates				
		1	2	3	4	
E. C. 1182	1/64,000	93	155 ^a	155	155	139.5
E. C. 1207	1/4,000	155 ^a	155	155	155	155.0
Check (inoculated)	-	36	57	55	47	48.7

Analysis of variance of table 4

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	11	29,508.92		
Blocks	3	1,578.92	526.306	2.002
Treatments	2	26,353.17	13,176.585	50.139**
Remainder	6	1,576.83	262.80	

LSD - at the 1% level 42.5

^aPlants showed no external symptoms in 155 days at which time the experiment was terminated.



Fig. 3. Three-day-old cotton seedling showing stem shriveling produced by Experimental Chemical 1182.

E. C. 1182 developed in one replicate after 93 days. The other three replicates remained free for 155 days, at which time this test was terminated. The plants were removed and stems examined for vascular discoloration. Very faint, small, intermittent specks confined to the lower half of the stem were the only evidence of discoloration. Tissue isolations were made from the discolored portions of each stem, and the fungus was recovered in culture.

E. C. 1207 used as a drench gave better control of *Verticillium* wilt than E. C. 1182. However at the rate used it was just as phytotoxic to seedlings in the cotyledon stage as E. C. 1182, and stunting was more evident than with E. C. 1182. To overcome the toxicity of E. C. 1207, the procedure described for Mersolite-W was followed. When the experiment was terminated (after 155 days) none of the replicates with E. C. 1207 showed any external symptoms of *Verticillium* wilt (Table 4). The plants were removed, and all stems examined for vascular discoloration. The stems appeared to be free of discoloration, and the fungus was not recovered in culture from tissue isolations.

Table 5 shows the results of thirteen chemicals applied only once to *Verticillium* wilt inoculated soil. Analysis of these results however shows no statistical differences, but in several replicates the chemicals appeared to be fungistatic. When test was terminated in 92 days most of the plants showing no external symptoms had slight vascular discoloration when stems were examined. The wilt fungus was recovered in culture from all of the slightly discolored stems.

Figure 4 shows the phytotoxic affect of Systox, TCA, Arasan, Fermate, Zerlate and Dithane Z-78 on two-week-old cotton seedlings when applied at the rate of 1 gram per 1,000 grams of soil. Figure 5 is a photograph of 3-week-old cotton plants comparing TCA and Dithane D-14 applied at the rate of

TABLE 5.- The effect of 9 chemicals applied to Verticillium wilt infested soil prior to planting for control of the fungus on cotton.

Treatment	Rate	Number of days before wilt symptoms appeared				Treatment Mean
		1	2	3	4	
Arasan	1/10,000	80	92 ^a	60	64	74.0
Bioquin 850	do	89	92 ^a	92	62	83.7
Dithane Z-78	do	79	45	67	79	67.5
Dowicide-B	do	66	71	78	58	68.2
Fernate	do	44	72	92 ^a	88	74.0
Sodium chlorate	do	81	60	92 ^a	92	81.2
Systox	do	66	87	92 ^a	88	83.2
T.C.A.	do	79	80	92 ^a	92	85.7
Zerlate	do	67	54	67	92 ^a	70.0
Check (inoculated) -		44	78	53	92 ^a	66.7

Analysis of variance of table 5

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	39	9,155.90		
Blocks	3	777.90	259.300	1.093
Treatments	9	1,976.40	219.600	.929
Remainder	27	6,401.60	237.096	

LSD - at the 1% level 30.2

^aPlants showed no external symptoms in 92 days at which time the experiment was terminated.



Fig. 4. Two-week-old cotton seedlings showing phytotoxicity produced by the following chemicals: 1. Systox, 2. TCA, 3. Arasan, 4. Zerlate, 5. Fenamate, and 6. Dithane Z-78. (Note necrosis with Systox, leaf crinkling with TCA, severe stunting with Arasan, Zerlate, and Fenamate, and chlorosis produced by Dithane Z-78.)



Fig. 5. Three-week-old cotton seedlings grown in soil treated with TCA and Dithane D-14. From left to right, first and second pots TCA, third Dithane D-14, and fourth check.

1 gram per 5,000 grams of soil with untreated check.

Tests to determine the effectiveness of chemicals against inoculum placed directly in the plant tissues. The results of the chemicals tested as systemic fungicides against V. albo-atrum and X. malvacearum, are shown in tables 6, 7, 8, 9, 10, and 11. None of the eighteen chemicals added to the soil prevented infection when the two organisms were placed directly in the plant tissues. However some chemicals retarded symptom expression longer than others.

Plants treated with Bioquin 700 showed wilt symptoms in 12 days. Replicates with Bioquin 850 showed wilt symptoms ranging from 11 to 13 days. The Dithane D-14 replicates showed wilt symptoms at 13 to 16 days. Plants treated with Mersolite-W produced wilt symptoms in 13 to 14 days. Plants treated with E. C. 1182 showed typical wilt symptoms at 12 to 13 days. Plants treated with E. C. 1207 showed wilt symptoms at the end of 12 days. The check plants for the above treatments showed symptoms in 8 to 9 days. All the other treatments compared closely with the checks in number of days before wilt symptoms were expressed.

The same chemicals tested against bacterial blight did not differ too much from the check plants in number of days before first symptoms appeared. Plants treated with Dithane D-14 and E. C. 1207 showed symptoms after 10 to 11 days. The rest of the chemicals compared with the check plants which showed typical blight symptoms in 7 to 8 days (Tables 9, 10, and 11).

Field and greenhouse tests for the control of cotton seedling diseases.--

One field test was conducted at Paradise and another at Chickasha, Oklahoma. They were set up to test the effectiveness of the chemicals listed in table 2 against cotton seedling diseases. Of the chemicals applied to the seed row at 15 lbs. per acre in the field, Spergon, Phygon, Ortho 406, Dithane Z-78,

TABLE 6.- Data obtained with 8 fungicides tested for their chemotherapeutic control of the Verticillium wilt fungus when placed in the stems of cotton seedlings.

Chemical	Rate	Number of days before wilt symptoms appeared				Treatment mean
		Replicates				
		1	2	3	4	
Actidione	1 p.p.m.	9	8	8	8	8.2
B. 700	1/10,000	12	12	12	12	12.0
B. 850	do	11	13	11	12	11.7
Dithane D-14	do	16	13	14	13	14.0
O. ^a benzoate	do	8	9	9	8	8.5
O. ^a sulfate	do	9	9	8	8	8.5
M. ^b hydrazide	do	8	8	8	8	8.0
Mersolite W	do	13	14	13	13	13.2
Check (inoculated) -		8	8	9	8	8.2

Analysis of variance of table 6.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	35	203.23		
Blocks	3	1.23	.410	.836
Treatments	8	190.23	23.778	48.526**
Remainder	24	11.77	.490	.702

LSD - at the 1% level 1.4

^aOxyquinoline

^bMaleic

TABLE 7.- Data obtained with 2 experimental chemicals tested for their chemotherapeutic control of the Verticillium wilt fungus when placed in the stems of cotton seedlings.

Chemical	Rate	Number of days before wilt symptoms appeared				Treatment mean
		Replicates				
		1	2	3	4	
E. C. 1182	1/64,000	12	12	12	13	12.2
E. C. 1207	1/4,000	12	12	12	12	12.0
Check (inoculated) -		8	9	9	8	8.5

Analysis of variance of table 7

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	11	36.92		
Blocks	3	.250	.083	.332
Treatments	2	35.167	17.583	70.332**
Remainder	6	1.50	.25	

LSD - at the 1% level .349

TABLE 8.- Data obtained with 8 fungicides tested for their chemotherapeutic control of the Verticillium wilt fungus when placed in the stems of cotton seedlings.

Chemical	Rate	Number of days before wilt symptoms appeared				Treatment mean
		Replicates				
		1	2	3	4	
Arasan	1/10,000	8	8	8	8	8.0
Dithane Z-78	do	8	8	8	9	8.2
Dowicide-B	do	8	9	8	8	8.2
Fernate	do	8	8	9	8	8.2
Sodium chlorate	do	8	8	9	9	8.5
Systox	do	8	8	9	9	8.5
Zerlate	do	8	8	9	9	8.5
TCA	do	8	8	8	9	8.2
Check (inoculated) -		8	8	9	8	8.2

Analysis of variance of table 8

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	35	7.639		
Blocks	3	2.305	.768	4.129*
Treatments	8	.880	.110	.591
Remainder	24	4.454	.186	

LSD - at the 1% level .9

TABLE 9.- Data obtained with 8 chemicals tested as chemotherapeutants against cotyledonary inoculation of X. malvacearum.

Chemical	Rate	Number of days before water soaking appeared				Treatment mean
		Replicates				
		1	2	3	4	
Actidione	1 p.p.m.	7	8	7	8	7.5
B. 700	1/10,000	8	8	8	9	8.2
B. 850	do	7	8	7	7	7.2
Dithane D-14	do	10	11	10	10	10.2
O. ^a benzoate	do	8	8	8	8	8.0
O. ^a sulfate	do	7	8	7	8	7.5
M. ^b hydrazide	do	8	8	8	7	7.7
Mersolite W	do	8	8	8	8	8.0
Check (inoculated) -		7	7	8	8	7.5

Analysis of variance of table 9

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	35	32.00		
Blocks	3	1.39	.463	2.424
Treatment	8	26.00	3.250	17.015**
Remainder	24	4.61	.191	

LSD - at the 1% level .9

^aOxyquinoline

^bMaleic

TABLE 10.- Data obtained with 2 experimental chemicals tested as chemotherapeutants against cotyledonary inoculation of X. malvacearum.

Chemical	Rate	Number of days before water soaking appeared				Treatment mean
		1	2	3	4	
E. C. 1182	1/64,000	8	8	9	8	8.2
E. C. 1207	1/4,000	10	9	11	10	10.0
Check (inoculated) -	-	7	8	8	8	7.7

Analysis of variance of table 10

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	11	14.667		
Blocks	3	2.00	.666	.266
Treatment	2	11.167	5.583	22.532**
Remainder	6	1.500	.250	
LSD - at the 1% level .354				

TABLE 11.- Data obtained with 8 chemicals tested as chemotherapeutants against cotyledonary inoculation of X. malvacearum.

Chemical	Rate	Number of days before water soaking appeared				Treatment mean
		1	2	3	4	
Arasan	1/10,000	7	9	7	8	7.7
Dithene Z-78	do	8	8	8	7	7.7
Dowicide-B	do	8	7	9	8	8.0
Fernate	do	7	7	8	7	7.2
Sodium chlorate	do	7	7	7	8	7.2
Systox	do	8	8	8	8	8.0
Zerlate	do	7	7	7	8	7.2
TGA	do	8	7	7	7	7.2
Check (inoculated) -	-	7	8	7	7	7.2

Analysis of variance of table 11

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	35	12.98		
Blocks	3	.09	.03	.0078
Treatment	8	3.73	.466	1.223
Remainder	24	9.16	.381	
LSD - at the 1% level 1.2				

Arasan, and Systox produced the best stands. The results of the tests at the two locations are shown in tables 12 and 13.

In the test conducted at Paradise, Oklahoma, Dithane Z-78 gave the highest per cent of surviving seedlings with an average of 49.0 per cent for the four replications. Systox, a systemic insecticide, had an average of 45.5 per cent. Ortho 406 had 41.5 per cent surviving seedlings. Spergon was next with 38.7 per cent, while Phygon had 37.0 and the check 36.7 per cent. Dowicide-B, F-1112, TCA, Ceresan-M, Dow-9B, and Agrox produced considerable toxicity in cotton seedlings when used at the rate of 15 lbs. per acre.

The same chemicals tested at Chickasha, Oklahoma, but used at a lower rate, gave somewhat different results. In this test Systox produced the highest per cent of surviving seedlings with 81.0, an average of four replicates. The only visible evidence of phytotoxicity was small, necrotic lesions produced on the first four or five true leaves. Replicates treated with Ortho-406 had an average of 79.2 per cent seedling survival when stand counts were made. Replicates treated with Phygon, Arasan, Dithane Z-78, and Spergon had 75.0, 72.5, 65.7, and 62.0 per cent respectively for seedling survival. The check replicates had a relatively high per cent of seedling survival, 72.0. TCA was the only chemical in this test to produce any amount of visible phytotoxicity other than the necrosis produced by Systox. Replicates treated with TCA had poor stands, and seedlings were severely stunted. The poorer germination with the majority of the chemicals in both tests indicated that toxicity might be an important factor. Therefore, lower rates were used in subsequent tests.

The experiments in the greenhouse were conducted to obtain further information on some of the chemicals that were used in the field tests.

Some interesting data were obtained from the first greenhouse test of

TABLE 12.- The effect of 13 chemicals applied to the seed row at Paradise, Oklahoma, for the control of cotton seedling diseases.

Treatment	Rate	Total surviving seedlings				Treatment mean
		Replicates				
		1	2	3	4	
Spergon	1X ^a	31	45	48	31	38.7
do	2X	31	22	8	16	19.2
do	4X	16	6	10	21	13.2
do	8X	4	7	8	9	7.0
Phygon	1X	39	35	44	30	37.0
Ortho 406	do	37	56	38	35	41.5
Dithane Z-78	do	53	58	47	38	49.0
Agrox	do	14	23	18	8	15.7
Arasan	do	24	34	19	34	27.7
Check (untreated)	-	39	35	31	42	36.7
4268T	1X	16	12	25	16	17.2
Dow 9B	do	4	10	0	1	3.7
Ceresan	do	9	30	12	14	16.2
TCA	do	17	10	8	3	9.5
Systox & Spergon	do	32	23	37	30	30.5
Systox	do	40	49	46	47	45.5
F-1112	do	5	4	0	11	5.0
Dowicide B	do	1	0	0	0	.025

Analysis of variance of table 12

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	71	18,878.00		
Blocks	3	168.77	56.25	1.303
Treatment	17	18,508.50	971.00	22.504**
Remainder	51	2,200.73	43.15	

LSD - at the 1% level 12.

^a13 grams per 25 ft. row space or 15 lbs. per acre with 40" row spacings.

TABLE 13.- The effect of 13 chemicals applied to the seed row at Chickasha, Oklahoma, for the control of cotton seedling diseases.

Treatment	Rate	Total surviving seedlings				Treatment mean
		Replicates				
		1	2	3	4	
Spergon	1X ^a	39	76	66	67	62.0
do	2X	70	67	28	73	59.5
do	4X	47	49	53	65	53.5
do	8X	44	11	44	27	31.5
Rhygon	1X	83	73	72	72	75.0
Ortho 406	do	81	79	79	78	79.2
Dithane Z-78	do	78	74	81	30	65.7
Agrox	do	50	69	48	37	51.0
Arasen	do	76	71	61	73	72.5
Check (untreated)	-	82	56	79	71	72.0
4268T	1X	58	63	63	40	56.0
Dow 9B	do	55	26	56	55	48.0
Ceresan	do	71	69	63	65	67.0
TCA	do	17	8	15	12	13.0
Systox & Spergon	do	64	44	59	50	54.0
Systox	do	83	75	80	86	81.0
F-1112	do	67	59	13	15	38.5
Dowicide B	do	15	21	88	17	35.2

Analysis of variance of table 13

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	71	35,629.99		
Blocks	3	702.37	234.12	.929
Treatment	17	22,080.71	1,298.86	5.156**
Remainder	51	12,846.91	251.90	

LSD - at the 1% level 20

^a13 grams per 37 ft. row space or 11.3 lbs. per acre with 40" row spacings.

fungicides applied to the furrow for the control of the *Rhizoctonia* disease of cotton seedlings. The results of these data are indicated in table 14.

The following chemicals were used in the first test: Arasan, Ortho 406, Phygon, and Spergon. Flats treated with Spergon had the highest per cent of seedling survival with 56.7, an average of the four replicates. There was no visible evidence of phytotoxicity in any of the flats treated with Spergon. The four replicates treated with Arasan had an average of 54.5 per cent seedling survival when last stand count was made, 6 days after the first stand count. There was also no visible evidence of toxicity produced by Arasan in any of the flats. Flats treated with Ortho 406 were 1 day earlier in seedling emergence than the other treatments. The four replicates treated with Ortho 406 had an average of 52.0 per cent seedling survival. Phygon used at the indicated rate seemed to be toxic to germinating seedlings. In each flat treated with Phygon, there seemed to be a considerable number of seeds that failed to germinate, and many of those that did germinate had difficulty in shedding their seed coats. The check flats had only 12 surviving seedlings when the final stand count was made. This gave an average of 3.0 per cent for the four replications.

The second test of fungicides applied to the seed row consisted of Ceresan-M, Arasan, Spergon, Phygon, and Ortho-406. Seed treatment with Ceresan-M was included to compare it with the fungicides applied to the seed row. Ceresan-M applied to the seed row was quite toxic. Germination in flats treated with Ceresan-M was two days later than the other four treatments. As the germinating seeds came in contact with the Ceresan, a bulbous condition was produced in the hypocotyl region. However the seedling developed normally after the radical penetrated the treated zone. Ceresan had the highest per cent of surviving seedlings upon termination of the test.

TABLE 14.- The effectiveness of fungicides applied to seed row for the control of the Rhizoctonia seedling disease of cotton under greenhouse conditions.

Treatment	Rate	Total surviving seedlings				Treatment mean
		1	2	3	4	
Arasan	.23 grams/ft. ^a	51	62	38	67	54.5
Ortho 406	do	84	28	25	73	52.0
Phygon	do	25	30	15	30	25.0
Spargon	do	58	35	66	68	56.7
Check (inoculated)	-	0	0	3	9	3.0

Analysis of variance of table 14

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Total	19	13,066.55		
Blocks	3	1,418.95	472.983	2.073
Treatment	4	8,909.80	2,227.450	97.630**
Remainder	12	2,737.80	228.150	

LSD - at the 1% level 32.6

^aEquivalent to 7.5 lbs./acre with 40" row spacings.

Flats treated with Arasan gave the highest total of emerged seedlings, and there was no evidence of phytotoxicity in the four replicates. When the test was terminated after 12 days, Arasan was second to Ceresan-M in total surviving seedlings. Flats treated with Spergon or Ortho 406 had the same number of emerged seedlings, and there was no evidence of phytotoxicity with either chemical at the rates employed. Spergon controlled post-emergence damping-off better than did Ortho 406. Ortho 406 treatments had the highest per cent of post-emergence damping-off of all the chemicals applied to the seed row. Phygon, at the high rate, showed evidence of being phytotoxic. Seedlings in flats treated with Phygon had difficulty in shedding their seed coats and were retarded in growth as a result. Seed treatment with Ceresan-M gave the lowest total of emerged seedlings of all the chemical treatments. There was an average of 48 emerged seedlings for the four replicates, and 75 per cent of these damped-off. When the first stand counts were made, the four check flats had a total of 8 emerged seedlings, and after the seventh day all of these had damped-off (Table 15). A photograph comparing Ceresan-M, Arasan, Spergon, and Ortho-406 with check and seed treatment is shown in figure 6.

TABLE 15.- Data obtained with fungicides applied to seed row for the control of the Rhizoctonia seedling disease of cotton under greenhouse conditions.

Treatment	Rate	Mean of four replicates		
		Total emerged seedlings Per cent	Total surviving seedlings after 12 days Per cent	Post-emergence damping-off Per cent
	High rate ^a			
Phygon	.25 grams/ft.	67	40	40
Arasan	do	80	53	34
Spargon	do	78	48	38
Ceresan-M	do	73	66	10
Ortho-406	do	76	34	55
	Low rate ^b			
Phygon	.13 grams/ft.	76	41	47
Arasan	do	82	53	35
Spargon	do	71	41	41
Ceresan-M	do	76	61	19
Ortho-406	do	78	32	58
Seed treated with Ceresan-M 2 gr/kilo.gr.seed		48	12	75
Check (inoculated -)		8	0	100

Analysis of variance of table 15

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
<u>High rate</u>				
Total	19	5,534.55		
Blocks	3	2,395.35	798.450	11.799**
Treatment	4	2,327.20	581.800	8.598**
Remainder	12	812.00	67.666	
<u>Low rate</u>				
Total	19	6,936.95		
Blocks	3	4,038.95	1,402.850	22.606**
Treatment	4	1,983.75	495.938	7.992**
Remainder	12	744.65	62.054	
		High rate LSD - at the 1% level	17	
		Low rate LSD - at the 1% level	17	

^aEquivalent to 7.5 lbs/acre with 40" row spacings.

^bEquivalent to 3.7 lbs/acre with 40" row spacings.



Fig. 6. A comparison of seed row applications of fungicides with seed treatment and an inoculated check. Top row left to right, Ortho 406, Spergon, and seed treatment. Bottom row, check, Ceresan-M, and Arasan.

DISCUSSION

Several amendments, when applied as drenches to pots of soil inoculated with V. albo-atrum, were effective in bringing about a substantial reduction of wilt in cotton plants. Dithane D-14, Mersolite-W, and E. C. 1207 completely eliminated wilt symptoms in all replicates, and it would appear that the amendments acted fungicidally. It was not determined whether the amendments eliminated the organism in the soil or merely inactivated the fungus so that it was unable to penetrate the host. If the fungus penetrated the host, it was prevented from developing because the organism was not isolated from plant tissues. It is conceivable that these amendments may have effected control of the disease by improving the nutrition of the susceptible, thus augmenting its resistance.

Chapman (5) reported that when certain chemicals were applied to the soil they would be translocated to the top of the plant if not absorbed by cellulosic substances, and if absorbed by cellulosic substances, would be held by the roots. In view of this, it is possible the above three chemicals were absorbed and held by the roots, thereby preventing penetration. Then the question arises, why is the chemical only effective against the organism while in the roots and not effective in the soil? One possible answer could be that when the chemical is being absorbed by the roots and held, it is in a greater concentration than when mixed thoroughly with the soil. On the other hand, if the chemicals were absorbed and translocated to the plant extremities, they would be of little value in controlling root invading fungi unless they were fungitoxic when applied to the soil. A chemical of this type would be more effective against foliage attacking organisms. If the same pots had been replanted upon termination of the experiments, it would be possible to determine if the organism had been eliminated by the soil

amendments.

Oxyquinoline benzoate, E. C. 1182, Bioquin 850, Systox (a systemic insecticide), and TCA showed evidence of being fungistatic against V. albo-atrum at the rates employed. In several replicates treated with the above amendments, only slight external and internal symptoms of wilt were produced as compared to the checks. It is conceivable that the amendments were being absorbed and translocated, thereby suppressing fungal development. Also the possibility exists that the amendments were partially inactivating the toxins produced by the wilt fungus.)

When the same soil amendments were tested against tissue inoculations of V. albo-atrum and X. malvacearum, only a few showed inhibiting effects on symptom expression. It appears from the results obtained that some chemicals were being absorbed and translocated, while others were not. At any rate, several of the amendments suppressed symptom expression from one to several days longer than the checks. When Dithane D-14, Mersolite-W, and E. C. 1207 were used as drenches against soil-borne inoculum, they gave excellent control, but used as systemics, they were only partially effective. It is probable from this evidence that the amendments, when being absorbed, were rendered partially ineffective by the plant tissues and juices. It is also probable that the absorbed chemical is not present in the area of inoculation in large enough quantities to be fungitoxic. When the inoculum was placed in the above ground portions of the plant, the wounding of the tissue could have interfered with translocation in that area long enough for the organism to become established.

There is evidence that E. C. 1207 is absorbed by the plant when applied to the soil. The chemical has a characteristic odor, and the leaves of

treated cotton plants take on this odor shortly after application.

A note of interest is that only those chemicals applied as drenches were effective in the above investigations at the rates employed.

Seed row applications of chemicals for the control of cotton seedling diseases should be investigated further because seed treatment has not given adequate control in many cases. ¶ When conditions are optimum for growth of the damping-off fungi, seed treatment is usually not too effective. One possible reason why it is not too effective is that when conditions are optimum for growth of the damping-off fungi, soil moisture is such that the chemical is readily leached away from the seed coat. To overcome this situation, a chemical will have to have good residual and fungitoxic qualities for 2 to 4 weeks after planting. In order to get more adequate control, a larger amount of chemical should be present in the area of the germinating seed. An ideal situation would be one where the chemical protected the seedling from the seed bed to the soil surface. Also, further experimentation on rates and methods of application should be made.

SUMMARY

Of 18 chemicals tested only Dithane D-14, Mersolite-W, and E. C. 1207, applied at six weekly intervals as drenches to inoculated soil, were effective in controlling *Verticillium* wilt of cotton. No symptoms, external or internal, were observed in plants grown in soil treated with Dithane D-14 or Mersolite-W when the test was terminated after 143 days. Plants treated with E. C. 1207 produced no external or internal symptoms of wilt in 155 days at which time the test was terminated.

In inoculated soil with E. C. 1182 used as a drench as above, there were external wilt symptoms in one plant 93 days following planting. The remaining 3 plants in this test showed no external symptoms in 155 days when the test was terminated. However, slight vascular discoloration was observed in these plants, and the wilt fungus was recovered in culture.

Bioquin 700, Bioquin 850, Dithane D-14, E. C. 1182, E. C. 1207, and Mersolite-W mixed with soil suppressed wilt symptoms in cotton plants 3 to 8 days longer than no treatment. The cotton plants had been manually inoculated above ground with *Verticillium albo-atrum*.

Cotton seedlings grown in soil treated with Dithane D-14 or Mersolite-W, retarded the development of bacterial blight lesions 3 days longer than the untreated plants. The cotton seedlings had been inoculated with *Xanthomonas malvacearum* in the cotyledonary stage.

Arasan, Ceresan-M, Ortho 406, Phygon, and Spergon applied to the seed row in flats of inoculated soil for the control of the soreshin disease of cotton, produced highly significant results. Flats treated with the above chemicals had considerably higher seedling survival when the tests were terminated than did seed treatment with Ceresan-M or check flats.

LITERATURE CITED

1. Ark, P. A. Sodium salt of *o*-hydroxydiphenyl, a promising chemotherapeutant. U. S. Dept. Agr., Pl. Dis. Repr. 35:44. 1951.
2. Brinkerhoff, L. A. Annual report of cotton disease investigations. Okla. Agric. Exp. Sta. and Div. of Cotton and Fiber Crops and Diseases, BPISAE, U.S.D.A. Page 16. 1950. (On file with the Botany and Plant Path. Dept., Okla. A&M College).
3. Caroselli, N. E., and A. W. Feldman. Dutch elm disease chemotherapy with Carolate and related formulations. (Abst.) Phytopath. 41:6. 1951.
4. _____, and F. L. Howard. Response of diseased maple trees to chemotherapy and fertilization. Phytopath. 32:21. 1942.
5. Chapman, R. A. Relation of specific chemotherapeutants to the infection court. (Abst.) Phytopath. 41:6. 1951.
6. _____, A. E. Dimond, and E. M. Stoddard. Assaying chemotherapeutants in the greenhouse. (Abst.) Phytopath. 40:4. 1950.
7. Crowdy, S. H. Chemotherapeutant effect of certain aryloxyaliphatic acids. (Abst.) Phytopath. 41:8. 1951.
8. Dimond, A. E. and R. A. Chapman. The chemotherapeutic properties of two compounds against Fusarium wilt. (Abst.) Phytopath. 41:11. 1951.
9. _____, G. H. Plumb, E. M. Stoddard, and J. G. Horsfall. An evaluation of chemotherapy and vector control by insecticides for combating Dutch elm disease. Conn. Agr. Exp. Sta. Bul. 531:1-61. 1949.
10. _____, E. M. Stoddard, and Saul Rich. The effect of dyes in retarding the development of crown galls. Phytopath. 41:911-14. 1951.
11. Doran, W. L. The control of some soil-borne diseases of plants by fungicides applied to the soil in fertilizer. Mass. Agric. Exp. Sta. Bul. 455. 1950.
12. _____, and T. Sproston Jr. The control of onion smut by fungicides applied to the soil. Phytopath. 35:654. 1945.
13. Ellis, D. E. Soil treatment with sodium nitrite for controlling damping-off and root knot. Phytopath. 33:1110-1111. 1943.
14. Evans, S. Greenhouse test of methods used in the inoculation of cotton plants with Verticillium albo-atrum. (Thesis) Okla. A&M College. 1949.
15. Feldman, A. W., N. E. Caroselli, and F. L. Howard. Chemicals for Dutch elm disease therapy. (Abst.) Phytopath. 40:8. 1950.

16. Haenseler, C. M., and T. R. Moyer. Effect of calcium cyanamide on the soil microflora with special references to certain plant parasites. *Soil Sci.* 43:133-149. 1937.
17. Heuberger, J. W. Preliminary note on the use of dithiocarbamates as soil treatments for the control of soil-borne diseases. U. S. Dept. Agr., Pl. Dis. Repr. 29:295-297. 1945.
18. Morsfall, J. G., and A. E. Dimond. Plant chemotherapy. *Annual Review of Microbiology* 5:209-222. 1951.
19. _____, and G. A. Zentmeyer. Antidoting the toxins of plant diseases. *Phytopath.* 32:22. 1942.
20. Howard, F. L. Antidoting toxins of Phytophthora cactorum as a means of plant disease control. *Science* 94:345. 1941.
21. Leach, L. D., and W. C. Snyder. Localized chemical applications to the soil and their effects upon root rots of beans and peas. *Phytopath.* 37:363. 1947.
22. McKeen, C. D. Soil treatment with Arasan for the control of damping-off of certain vegetables. *Phytopath.* 39:15. 1949.
23. McNew, G. L., and N. K. Sundholm. The fungicidal activity of substituted pyrazoles and related compounds. *Phytopath.* 39:736. 1949.
24. Nelson, Ray. Comparison of dust fungicides and formaldehyde in the control of onion smut. *Phytopath.* 39:16. 1949.
25. Riker, A. J., R. H. Gruenhagen, and L. F. Roth. Some chemical treatments and their influence on damping-off, weed control, and winter injury of red pine seedlings. *Jour. Agr. Res. (U.S.)*, 74:87-95. 1947.
26. Rudolph, B. A., and G. J. Harrison. Attempts to control *Verticillium* wilt of cotton and breeding for resistance. *Phytopath.* 29:753. 1939.
27. Stoddard, E. M. Immunization of peach trees to X disease by chemotherapy. (Abst.) *Phytopath.* 34:1011-1012. 1944.
28. _____. Soil applications of Oxyquinolin Benzoate for the control of foliage wilting in elms caused by Graphium ulmi. *Phytopath.* 36:682. 1946.
29. _____. The X disease of peach and its chemotherapy. *Conn. Agr. Exp. Sta. Bul.* 506:1-18. 1947.
30. _____. Chemotherapeutic control of *Nusarium* wilt of carnations. (Abst.) *Phytopath.* 41:33. 1951.

31. _____ . A chemotherapeutic control of strawberry red stele. (Abst.) *Phytopath.* 41:34. 1951.
32. Takahashi, W. N. The inhibition of virus increase by Malachite green. *Science* 107:226. 1948.
33. Walker, J. G., and R. H. Larson. Calcium cyanamid in relation to control of club root of cabbage. *Jour. Agr. Res. (U.S.)*, 51:183-189. 1935.
34. Wilhelm, S. Effect of various soil amendments on the inoculum potential of the *Verticillium* wilt fungus. *Phytopath.* 41:684-690. 1951.
35. Zentmeyer, G. A. Mechanism of action of 8-hydroxyquinoline. (Abst.) *Phytopath.* 33:1121. 1943.
36. _____ . Inhibition of metal catalysis as a fungistatic mechanism. *Science* 100:294-295. 1944.
37. Zentmeyer, G. A. Jr., and J. G. Horsfall. Internal therapy with organic chemicals in treatment of vascular diseases. (Abst.) *Phytopath.* 33:16. 1943.
38. Zentmeyer, G. A., J. G. Horsfall, and P. P. Wallace. Dutch elm disease and its chemotherapy. *Conn. Agr. Exp. Sta. Bul.* 498:1-68. 1946.

STUDIES WITH SOIL AMENDMENTS FOR THE CONTROL OF VERTICILLIUM WILT
AND SCRESHIN OF COTTON

ROBERT A. KORTSEN

Mr. Lloyd A. Brinkerhoff

The content and form have been checked and approved by the author and thesis adviser. Changes or corrections in the thesis are not made by the Graduate School office or by any committee. The copies are sent to the bindery just as they are approved by the author and faculty adviser.

Typist - Mrs. Dolores G. Kortsen

THESIS TITLE: STUDIES WITH SOIL AMENDMENTS FOR THE
CONTROL OF VERTICILLIUM WILT AND SORE-
SHIN OF COTTON

AUTHOR: ROBERT A. KORTSEN

THESIS ADVISER: MR. LLOYD A. BRINKERHOFF

The content and form have been checked and approved
by the author and thesis adviser. Changes or correc-
tions in the thesis are not made by the Graduate
School office or by any committee. The copies are
sent to the bindery just as they are approved by the
author and faculty adviser.

TYPIST: MRS. DOLORES G. KORTSEN