ABSTRACT OF THESIS

FUNGICIDAL EFFECTS OF SYNTHETIC PLANT HORMONES ON *ACTINOMYCES* SPECIES

Submitted by

Merle

ABSTRACT

The many attempts to control common scab of potatoes have not been entirely satisfactory. Synthetic plant hormones which are being used to eliminate weeds affect certain of the soil microorganisms, but little is known concerning their effects on soil pathogens.

The problem

What are the fungicidal effects of synthetic plant hormones on the potato scab organism?

Problem analysis.--1. Is there an inhibitory effect of synthetic plant hormones on the potato scab organism separate from the inhibitory effect of a high hydrogen ion concentration?

2. Are there differences in the effect of these chemicals on various races of Actinomyces?

3. What is the lowest effective concentration of these chemicals for the inhibition of the growth of Actinomyces?

Delimitation.--This investigation has been limited to the effects of 25 synthetic plant hormones on four races of Actinomyces.

Definition of terms.--Synthetic plant hormones refer to 2,4-dichlorophenoxyacetic acid, its salt and esters, and related derivatives of other phenoxyacetic acids.

METHODS AND MATERIALS

The 25 synthetic plant hormones tested were obtained from four sources.

The isolates of Actinomyces were obtained from soil samples and from potato tubers. Four soil isolates, each from a different soil, were obtained by dilutions of a one gram soil sample with sterile water in the ratio 1:10,000 and plated on potato dextrose agar. These cultures were not pathogenic on Bliss Triumph and Irish Cobbler potato tubers growing in the greenhouse in sterile soil when tested by standard techniques. The cultures from potatoes were isolated from deep scab pustules on tubers of the Irish Cobbler variety. This was done by sterilizing the surface of the potato, peeling off the scab pustule, removing a small piece of host tissue beneath the scab pustule and suspending it in melted agar and later transferring individual scab colonies to a sterile agar plate.

Modified potato dextrose agar was the medium used throughout the study. This medium is the same as standard potato dextrose agar with but five grams of dextrose instead of 20 grams per liter. The chemicals were added to the medium before any adjustment in pH was made with NaOH or HCl. The medium was then autoclaved for 15 minutes under 15 pounds pressure. Exploratory

tests had shown that autoclaving a medium containing 500 p.p.m. of the sodium salt of 2,4-D under these conditions did not alter the pH or change the apparent effect of the chemical.

All tests were made in Petri dishes kept at room temperature. When the agar in the plates was hard and when the surface of the agar was free of moisture, the plates were inoculated with four different cultures of Actinomyces. The plates were read for positive growth or complete inhibition seven days after inoculation.

The purpose of the first experiment was to study the effect of different levels of pH on Actinomyces species with and without 500 p.p.m. of the sodium salt of 2,4-D in the medium. A series of three plates each for pH 5.0, 6.0, 7.0 and 8.0 was used.

In order to determine the toxic limits of various forms of 2,4-D and related compounds, the chemicals were divided into two groups. Those compounds of known molecular weight and purity were tested on a molar basis in order to have as accurate a basis as possible for comparing the toxic effects of the chemicals. This information was available for 2,4-dichlorophenoxyacetic acid, its sodium and ammonium salts, and all of its esters. The concentrations were 15, 29, 59, 119, 238, 476, 953, 1906, 3812, 7625, and 15,250 X 10^{-6} M. with two plates for each concentration. As the purity of the other compounds was unknown, they were

tested on a parts per million basis. The p.p.m. concentration levels were 4, 8, 16, 32, 63, 125, 250, 500, 1000, 2000, and 4000 p.p.m. These approximate the equivalent p.p.m. of the molar concentrations of the sodium salt of 2,4-D

The solubilities of these chemicals varied considerably. The following were sufficiently water soluble at the concentrations employed to be added directly to the medium: the sodium, ammonium, magnesium, manganese, nickel, and cobalt salts of 2,4-D. All but one of the esters and the cupric salt were emulsified in Carbowax 1500. The chemicals were added to 15 parts by weight of the melted Carbowax; three drops of Triton X-100 were used as a wetting agent. This was added to hot water to form an emulsion. The emulsion was added to the melted agar. Since Carbowax was unsatisfactory for some chemicals, five c.c. of lanolin were used to incorporate into the medium the calcium, cuprous, and ferrous salts and the cyclohexyl ester of 2,4-D. Neither of the emulsifiers was satisfactory for the ferric salt of 2,4-D. Five c.c. of acetone were used to dissolve the ferric salt. When this solution was added to water a good emulsion was formed. This emulsion was heated to drive off excess acetone. When no further odor of acetone was noted, the emulsion was added to modified potato dextrose agar. Triethanolamine was used to emulsify 2,4-dichlorophenoxyacetic acid; parachlorophenoxyacetic acid; 2,4,5-trichlorophenoxyacetic acid; 2,4,5,6-tetrachlorophenoxyacetic acid; and pentachlorophenoxyacetic acid.

Using ten parts to one of chemical by weight, triethanolamine was used in the same manner as Carbowax. Controls containing only Carbowax, triethanolamine, and lanolin were used.

RESULTS

The results of the first experiment to determine the effect of 500 p.p.m. of the sodium salt of 2,4-D at various pH levels showed that there is an effect of the chemical separate from that of the pH of the medium. All isolates grew at pH 7.0 and 8.0 and did not grow at pH 5.0 with or without the sodium salt of 2,4-D. At a pH of 6.0, 500 p.p.m. of the sodium salt of 2,4-D inhibited the growth of all the cultures.

The results of the second experiment showed that there were differences in the effects of the various synthetic plant hormones on the growth of Actinomyces sp. All but three chemicals had the same inhibitory effect on the pathogenic as on the nonpathogenic isolates. The methyl ester of 2,4-D inhibited the growth of nonpathogenic culture 105 at a concentration level one below that which inhibited the growth of the two pathogenic and the other nonpathogenic culture of Actinomyces. The pentachlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid inhibited the growth of culture 105 at a concentration level one higher than that which inhibited the other isolates.

The lowest concentration of the various chemicals for the complete inhibition of the growth of Actinomyces sp. showed

considerable variation. Most of the chemicals tested on a molar basis had about the same inhibitory effect on the growth of Actinomyces sp. as did 2,4-dichlorophenoxyacetic acid. The n- and iso-butyl and the n- and iso-amyl esters of 2,4-D had little or no inhibitory effect. The methyl ester of 2,4-D was outstanding for it inhibited the growth of all four cultures of Actinomyces at a low concentration, i.e., 238 X 10^{-6} M. The toxicity of the esters decreased with an increase in the length of the side chain. The effect of those chemicals tested on a p.p.m. basis form a similar pattern. Most of the chemicals exhibited approximately equal inhibitory effects. Pentachlorophenoxyacetic acid was outstanding in that it inhibited all growth at a concentration of 63 p.p.m. The toxicity of the acid compounds increased with an increase in the number of chlorine atoms in the ring.

In general the n- and iso- forms of the esters and the two forms of the salts with different valences inhibited the cultures at the same concentration.

Triethanolamine and lanolin did not inhibit the growth of Actinomyces, but the two highest concentrations of Carbowax had an inhibitory effect.

SUGGESTIONS FOR FURTHER STUDY

Further tests of these chemicals should be conducted in the greenhouse and in the field for their effect on Actinomyces scabies. Since several of these synthetic plant hormones at low concentrations inhibited the growth of Actinomyces sp., they might have similar effects on other soil-borne pathogens. Tests should be conducted to determine the effects of synthetic plant hormones on such soil-inhabiting pathogens as Fusarium, Pythium, and Rhizoctonia.

T H E S I S

FUNGICIDAL EFFECTS OF SYNTHETIC PLANT HORMONES ON *ACTINOMYCES* SPECIES

Submitted by

Merle E. Michaelson

In partial fulfillment of the requirements

for the Degree of Master of Science

Colorado

Agricultural and Mechanical College

Fort Collins, Colorado

December, 1948

COLORADO AGRICULTURAL AND MECHANICAL COLLEGE

December 1 1948

WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY MERLE E. MICHAELSON

ENTITLED FUNGICIDAL EFFECTS OF SYNTHETIC PLANT HORMONES ON ACTINOMYCES SPECIES

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

CREDITS 10

Committee on Graduate Work

L. W. Durrell — Major Professor
Jess L. Suits — Minor Professor
W. D. Thomas Jr
R. E. Atkinson

L. W. Durrell — Head of Department
L. W. Durrell — Dean of Division

Committee on Final Examination

Examination Satisfactory

L. W. Durrell
Jess L. Suits
Laurence A. Schaal
Robert L. Whitney
W. D. Thomas, Jr.

David K. Morgan
Dean of the Graduate School

This is to certify that Merle E. Michaelson has demonstrated his ability to read scientific German literature, both with preparation and at sight.

Respectfully submitted,

Bruno Klinger

Bruno Klinger
Associate Professor of
Botany and Plant Pathology

ACKNOWLEDGMENT

The writer wishes to express his appreciation to the following members of the faculty of Colorado Agricultural and Mechanical College, Fort Collins, Colorado, for their assistance in conducting this study and in the preparation of this manuscript:

Dr. L. W. Durrell, Dean of Division of Science and Arts and Head of Department of Botany and Plant Pathology; Dr. Jess L. Fults, Dr. W. D. Thomas, Jr., and Professor R. E. Atkinson of Department of Botany and Plant Pathology.

The writer also wishes to acknowledge the assistance of Dr. L. A. Schaal, Pathologist, United States Department of Agriculture.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter I

INTRODUCTION

One of the serious diseases of potato is common scab caused by Actinomyces scabies (Thaxt.) Guss. This organism probably lives in all arable soils although not all races are pathogenic to all potato varieties. There have been many attempts to control this disease with chemicals, but the results have not been entirely satisfactory. Within the last ten years considerable work has been done with synthetic plant hormones in the elimination of weeds (14). These chemicals also affect certain of the soil microorganisms (7, 8, 12, 16), but little is known concerning their effects on soil pathogens.

The problem

What are the fungicidal effects of synthetic plant hormones on the potato scab organism, Actinomyces scabies (Thaxt.) Guss.?

Problem analysis.--1. Is there an inhibitory effect of synthetic plant hormones on the potato scab

organism separate from the inhibitory effect of a high hydrogen ion concentration?

2. Are there differences in the effect of these chemicals on various races of Actinomyces?

3. What is the lowest effective concentration of these chemicals for the inhibition of the growth of Actinomyces?

Delimitation.--This investigation has been limited to the effects of 25 synthetic plant hormones on four races of Actinomyces.

Definition of terms.--Synthetic plant hormones refer to 2,4-dichlorophenoxyacetic acid, its salts and esters, and related derivatives of other phenoxyacetic acids.

Chapter II

REVIEW OF LITERATURE

Many attempts have been made to control common scab of potato with chemicals, but reports on the value of seed and soil treatments have been conflicting.

Cairns et al (1) in Ireland obtained satisfactory control of scab by the disinfection of the affected seed tubers with organic mercurials before planting if a sufficient interval had elapsed between the growing of successive potato crops on the same land.

Mercury salts have been effective in killing the scab organism on the seed piece, but when used to eliminate it in the soil, the results have been inconclusive. Under Long Island conditions, Cunningham and Wessels (3) found that four pounds of either yellow oxide of mercury or of calomel added to each ton of 5-8-5 fertilizer mixture on soils having a pH of 5.5 or lower reduced scab. Taylor (13) found that the addition of mercury to the limestone soils of New York increased scab infection. Schaal (10) reported that potato scab was not controlled on sandy alkaline soil of northern

Colorado with mercuric chloride, yellow oxide of mercury, sulfamic acid, aluminum sulfate, or sulfur.

Probably the best control of potato scab at present is the use of resistant varieties adapted to local areas, but these varieties may lose their resistance for new races of Actinomyces are apparently being formed due to variations. Schaal (11) has shown that variants differ from their parent cultures in pathogenicity.

Since enormous quantities of synthetic plant hormones, i.e., 2,4-D weed killers, are being used in the control of weeds, it is important to know more about their effects on the soil inhabiting fungi and bacteria.

In 1944 Stevenson and Mitchell (12) reported that 0.02 percent 2,4-dichlorophenoxyacetic acid or its sodium salt in potato dextrose agar had an inhibitory effect on the growth of Bacillus subtilis, Aerobacter cloacae, Staphylococcus aureus, and Phytomonas tumefaciens but had no apparent effect on the growth of Fusarium sp. and Penicillium sp. At 0.08 percent concentration of the chemical, the growth of all bacteria but A. cloacae was prevented, but there was no apparent effect on the growth of the fungi.

Martin (8) reported that at low concentrations (below 10 p.p.m.) 2,4-dichlorophenoxyacetic acid did not appreciably affect either soil bacteria or soil fungi. When the concentration was increased to 100 p.p.m. and above, some soil microorganisms were inhibited. The 2,4-dichlorophenoxyacetic acid was more toxic to common soil fungi under acid than under alkaline conditions.

Lewis and Hamner (7) used the method of Vincent and Vincent (15) to test five different samples of 2,4-dichlorophenoxyacetic acid. Filter paper discs were dipped into the solutions to be tested and placed on plates which had been inoculated with a spore suspension of the organism. The plates were incubated at 28° C. and the zone of inhibition surrounding the pads was measured after one or two days of incubation. The bacteria were grown on malt agar at pH 5.5. The most noticeable result was the lack of inhibition at concentrations of 1,000 p.p.m. However, in this method of testing the effects of 2,4-D, the chemical is diluted as it diffuses out of the pad into the agar. Phytomonas phaseoli and Bacillus brevis were only slightly inhibited by saturated solutions of some of the samples of 2,4-D. Rhizobium leguminosarum was not affected by any of the 2,4-D

samples even at saturated concentrations. They tentatively concluded that under normal rates of application for killing of weeds, the amount of 2,4-D which reaches the soil would have no important effect on the soil microorganisms or on plant pathogens present in the soil.

According to Carlyle and Thorpe (2) species of Rhizobium differ in their sensitivity to the ammonium and sodium salts of 2,4-D. They also found that the ammonium salt was more toxic than the sodium salt, and that none of the test species was seriously inhibited in sand culture by concentrations equivalent to less than 200 pounds of the sodium salt per acre. They concluded that ordinary field applications of the herbicide probably would be harmful to legumes but would have very little effect on Rhizobium sp. living free in the soil.

Recently Worth and McCabe (16) reported the effects of different concentrations of sodium 2,4-dichlorophenoxyacetate on bacteria with various oxygen requirements. The concentrations of the chemical ranged from two p.p.m. to 20,000 p.p.m. in six different concentrations. In general the aerobic bacteria were inhibited by 2,4-D, whereas the facultative anaerobic

organisms showed no inhibitory effects. Clostridium tetani, an anaerobe, responded to the 2,4-D like the facultative anaerobes, while Clostridium welchii and Clostridium botulinum varied in their response making it impossible to come to a definite conclusion. They concluded that those organisms requiring free oxygen for respiration are smothered by 2,4-D and those organisms capable of anaerobic respiration only are not affected to any significant degree by 2,4-D.

Fults and Payne (6) have demonstrated that bacteria populations in nodule smears from the common bean plant were decreased by 2,4-D and that the bacteria rod lengths were changed. Payne and Fults (9) reported that nodulation of the common bean plant was prevented by 2,4-D at a concentratiom as low as 0.009 pounds per acre. This rate is only from 0.3 to 5.8 percent of the amount ordinarily used as a weed killer.

Thompson, Swanson, and Norman (14) have reviewed the effects of these chemicals on higher plants. They also tested 1,160 new compounds and compared them with 2,4-D. Six of the compounds tested are in the group reported in this paper. As expressed by the

kidney-bean single-droplet water test, the n-amyl ester of 2,4-D was 83 percent as effective as 2,4-dichlorophenoxyacetic acid; the iso-amyl, 38 percent; the n-butyl ester, 50 percent; the iso-butyl ester, 133 percent; the iso-propyl ester, 93 percent; and 2,4,5-trichlorophenoxyacetic acid, 93 percent. As expressed by the kidney-bean single-droplet oil test the n-amyl ester of 2,4-D was 81 percent as effective as 2,4-dichlorophenoxyacetic acid; the iso-amyl ester, 85 percent; the n-butyl ester, 111 percent; the iso-butyl ester, 64 percent; the iso-propyl ester, 68 percent; and 2,4,5-trichlorophenoxyacetic acid, 236 percent.

Fults and Payne (5) using the split pea stem method of testing the effects of synthetic plant hormones reported that at all concentrations between 0.000029 M. and 0.122000 M. the ammonium and sodium salts of 2,4-D and parachlorophenoxyacetic acid were superior to 2,4-D and the ethyl and butyl esters were inferior. As determined by the castor bean test, the sodium and ammonium salts and the butyl ester of 2,4-D were about equal to 2,4-D whereas the ethyl ester and parachlorophenoxyacetic acid were inferior.

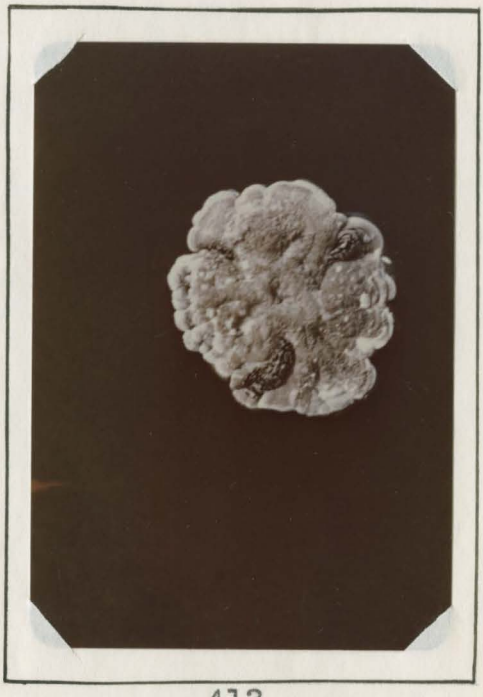
Chapter III

METHODS AND MATERIALS

The 25 chemicals tested to determine the effects of synthetic plant hormones on Actinomyces species were obtained from four sources. The 2,4-dichlorophenoxyacetic acid and its sodium salt were obtained from the J. T. Baker Chemical Company. The ammonium salt of 2,4-D came from the Dupont laboratory. Dr. A. R. Ronzio, formerly Associate Chemist, Chemistry Section, Colorado Agricultural Experiment Station, synthesized and purified the methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, n-amyl, iso-amyl, and cyclohexyl esters of 2,4-D. The calcium, cobalt, cupric, cuprous, ferric, ferrous, magnesium, manganese, and nickel salts of 2,4-D, parachlorophenoxyacetic acid; 2,4,5-trichlorophenoxyacetic acid; 2,4,5,6-tetrachlorophenoxyacetic acid; and pentachlorophenoxyacetic acid were obtained from the American Chemical Paint Company.

The isolates of Actinomyces were obtained from soil samples and from potato tubers. Four soil isolates, each from a different soil, were obtained by dilutions

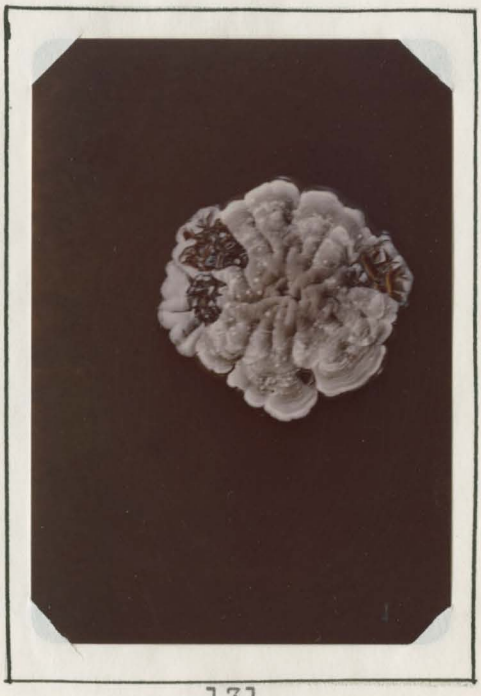
403



412

Fig. 1.--Color photographs of Actinomyces cultures 403 and 412, which were pathogenic on Bliss Triumph and Irish Cobbler potatoes, used in the fungicidal tests discussed in this thesis.

105



131

Fig. 2.--Color photographs of Actinomyces cultures 105 and 131, which were nonpathogenic on Bliss Triumph and Irish Cobbler potatoes, used in the fungicidal tests discussed in this thesis.

of a one gram soil sample with sterile water in the ratio 1:10,000 and plated on potato dextrose agar. These cultures were not pathogenic on Bliss Triumph and Irish Cobbler potato tubers growing in the greenhouse in sterile soil when tested by standard techniques. The cultures from potatoes were isolated from deep scab pustules on tubers of the Irish Cobbler variety. This was done by sterilizing the surface of the potato, peeling off the scab pustule, removing a small piece of host tissue beneath the scab pustule and suspending it in melted agar and later transferring individual scab colonies to a sterile agar plate.

Modified potato dextrose agar was the medium used throughout the study. This medium is the same as standard potato dextrose agar with but five grams of dextrose instead of 20 grams per liter. The chemicals were added to the medium before any adjustment in pH was made with NaOH or HCl. The medium was then autoclaved for 15 minutes under 15 pounds pressure. Exploratory tests had shown that autoclaving a medium containing 500 p.p.m. of the sodium salt of 2,4-D under these conditions did not alter the pH or change the apparent effect of the chemical.

All tests were made in Petri dishes kept at room temperature. When the agar in the plates was hard and when the surface of the agar was free of moisture, the plates were inoculated with four different cultures of Actinomyces. The plates were read for positive growth or complete inhibition seven days after inoculation.

The purpose of the first experiment was to study the effect of different levels of pH on Actinomyces species with and without 500 p.p.m. of the sodium salt of 2,4-D in the medium. A series of three plates each for pH 5.0, 6.0, 7.0 and 8.0 was used.

In order to determine the toxic limits of the various forms of 2,4-D and related compounds, the chemicals were divided into two groups. Those compounds of known molecular weight and purity were tested on a molar basis in order to have as accurate a basis as possible for comparing the toxic effects of these chemicals. This information was available for 2,4-dichlorophenoxyacetic acid, its sodium and ammonium salts, and all of its esters. The concentrations were 15, 29, 59, 119, 238, 476, 953, 1906, 3812, 7625, and 15,250 X 10^{-6} M. with two plates for each concentration. As the purity of the

other compounds was unknown,[1] they were tested on a parts per million basis. The p.p.m. concentration levels were 4, 8, 16, 32, 63, 125, 250, 500, 1000, 2000, and 4000 p.p.m. These approximate the equivalent p.p.m. of the molar concentrations of the sodium salt of 2,4-D.

The solubilities of these chemicals varied considerably. The following were sufficiently water soluble at the concentrations employed to be added directly to the medium: the sodium, ammonium, magnesium, manganese, nickel, and cobalt salts of 2,4-D. All but one of the esters and the cupric salt were emulsified in Carbowax 1500. The chemicals were added to 15 parts by weight of the melted carbowax. Three drops of Triton X-100 were used as a wetting agent. This was added to hot water to form an emulsion. The emulsion was added to the melted agar. Since carbowax was unsatisfactory for some chemicals, five c.c. of lanolin were used to incorporate into the medium the calcium, cuprous, and

---

[1] After all tests were completed, a letter received from Mr. Robert Beatty, American Chemical Paint Company, Ambler, Pa., stated that all chemicals furnished by their laboratory were "CP chemicals."

ferrous salts and the cyclohexyl ester of 2,4-D. Neither of the emulsifiers was satisfactory for the ferric salt of 2,4-D. Five c.c. of acetone were used to dissolve the ferric salt. When this solution was added to water a good emulsion was formed. This emulsion was heated to drive off excess acetone. When no further odor of acetone was noted, the emulsion was added to modified potato dextrose agar. Triethanolamine was used to emulsify 2,4-dichlorophenoxyacetic acid; parachlorophenoxyacetic acid; 2,4,5-trichlorophenoxyacetic acid; 2,4,5,6-tetrachlorophenoxyacetic acid; and pentachlorophenoxyacetic acid. Using ten parts to one of chemical by weight, triethanolamine was used in the same manner as carbowax. Controls containing only carbowax, triethanolamine, and lanolin were used.

Chapter IV

RESULTS

The results of the study of the effects of synthetic plant hormones on the growth of Actinomyces species show a great variation in the effects of these materials.

The results of the first experiment to determine the effect of 500 p.p.m. of the sodium salt of 2,4-D at various pH levels are given in Table 1. All isolates grew at pH 7.0 and 8.0 but did not grow at pH 5.0 with or without the sodium salt of 2,4-D in the medium. However, at pH 6.0, 500 p.p.m. of the sodium salt completely inhibited the growth of all the Actinomyces cultures. This indicates there is an effect of the chemical separate from that of the pH of the medium.

The results of testing 25 synthetic plant hormones on two soil isolates and two tuber isolates of Actinomyces are given in Table 2 and Table 3. Most of the chemicals tested on a molar basis (Table 2) had about the same effect on the growth of Actinomyces as did 2,4-dichlorophenoxyacetic acid. The n- and iso-butyl

Table 1.--THE EFFECTS OF THE SODIUM SALT OF 2,4-D (500 P.P.M.) ON THE GROWTH OF FOUR *ACTINOMYCES SP.* AT pH 5.0, 6.0, 7.0 and 8.0 ON MODIFIED POTATO DEXTROSE AGAR

Culture Number	pH 5.0		pH 6.0		pH 7.0		pH 8.0	
	Control	2,4-D	Control	2,4-D	Control	2,4-D	Control	2,4-D
105	0[1]	0	+	0	+	+	+	+
112	0	0	+	0	+	+	+	+
122	0	0	+	0	+	+	+	+
131	0	0	+	0	+	+	+	+

[1] 0 = complete inhibition

\+ = growth

Table 2.--THE EFFECTS OF 12 DIFFERENT FORMS OF 2,4-D ON THE GROWTH OF TWO PATHOGENIC[1] AND TWO NONPATHOGENIC[2] RACES OF ACTINOMYCES SP. AT pH 6.5

Chemical	Concentration X 10^{-6} M										
	15	29	59	119	238	476	953	1906	3812	7625	15250
2,4-dichlorophenoxy-acetic acid	+[3]	+	+	+	+	+	+	+	0	0	0
sodium 2,4-dichloro-phenoxy acetate	+	+	+	+	+	+	0	0	0	0	0
ammonium 2,4-dichloro-phenoxy acetate	+	+	+	+	+	+	+	0	0	0	0
methyl 2,4-dichloro-phenoxy acetate	+	+	+	+[4]	0	0	0	0	0	0	0
ethyl 2,4-dichloro-phenoxy acetate	+	+	+	+	+	+	0	0	0	0	0
n-propyl 2,4-dichloro-phenoxy acetate	+	+	+	+	+	+	+	0	0	0	0
iso-propyl 2,4-dichloro-phenoxy acetate	+	+	+	+	+	+	+	0	0	0	0
n-butyl 2,4-dichloro-phenoxy acetate	+	+	+	+	+	+	+	+	+	+	0
iso-butyl 2,4-dichloro-phenoxy acetate	+	+	+	+	+	+	+	+	+	+	0
n-amyl 2,4-dichloro-phenoxy acetate	+	+	+	+	+	+	+	+	+	+	0
iso-amyl 2,4-dichloro-phenoxy acetate	+	+	+	+	+	+	+	+	+	+	0
cyclohexyl 2,4-dichloro-phenoxy acetate	+	+	+	+	+	+	+	+	0	0	0

[1] Cultures 403 and 412

[2] Cultures 105 and 131

[3] 0 = complete inhibition
+ = growth

[4] Culture 105 was inhibited

Table 3.--THE EFFECTS OF 13 DIFFERENT FORMS OF 2,4-D ON THE GROWTH OF TWO PATHOGENIC[1] AND TWO NONPATHOGENIC[2] RACES OF ACTINOMYCES SP. AT pH 6.5

Chemical	Concentration in p.p.m.										
	4	8	16	32	63	125	250	500	1000	2000	4000
calcium 2,4-dichlorophenoxy acetate	+[3]	+	+	+	+	+	+	0	0	0	0
cobalt 2,4-dichlorophenoxy acetate	+	+	+	+	+	+	+	0	0	0	0
cupric 2,4-dichlorophenoxy acetate	+	+	+	+	+	+	0	0	0	0	0
cuprous 2,4-dichlorophenoxy acetate	+	+	+	+	+	+	0	0	0	0	0
ferric 2,4-dichlorophenoxy acetate	+	+	+	+	+	+	0	0	0	0	0
ferrous 2,4-dichlorophenoxy acetate	+	+	+	+	+	+	+	0	0	0	0
magnesium 2,4-dichlorophenoxy acetate	+	+	+	+	+	+	+	+	0	0	0
manganese 2,4-dichlorophenoxy acetate	+	+	+	+	+	+	+	+	0	0	0
nickel 2,4-dichlorophenoxy acetate	+	+	+	+	+	+	+	+	0	0	0
para chlorophenoxy acetic acid	+	+	+	+	+	+	+	+	+	0	0
2,4,5-trichlorophenoxy acetic acid	+	+	+	+	+	+	+	0[4]	0	0	0
2,4,5,6-tetrachlorophenoxy acetic acid	+	+	+	+	+	+	+	0	0	0	0
pentachlorophenoxy-acetic acid	+	+	+	0[4]	0	0	0	0	0	0	0

[1]Cultures 403 and 412
[2]Cultures 105 and 131
[3] 0 = complete inhibition
+ = growth
[4]Growth for culture 105

and the n- and iso-amyl esters of 2,4-D had little or no inhibitory effect. The methyl ester of 2,4-D was outstanding for it inhibited the growth of all four cultures of Actinomyces at a low concentration, i.e., 238×10^{-6} M. The effect of those chemicals tested on a p.p.m. basis form a similar pattern. Most of the chemicals exhibited approximately equal inhibitory effects. Pentachlorophenoxyacetic acid was outstanding in that it inhibited all growth at a concentration of 63 p.p.m.

All but three chemicals had the same inhibitory effect on the pathogenic as on the nonpathogenic isolates. The methyl ester of 2,4-D inhibited the growth of nonpathogenic culture 105 at a concentration level one below that which inhibited the growth of the two pathogenic and and the other nonpathogenic culture of Actinomyces. The pentachlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid inhibited the growth of culture 105 at a concentration level one higher than that which inhibited the other isolates.

Triethanolamine and lanolin did not inhibit the growth of Actinomyces, but the two highest concentrations of carbowax had an inhibitory effect.

Chapter V

DISCUSSION

The results of the study of the effects of synthetic plant hormones on Actinomyces species indicated that several of these compounds may be useful in the control of common scab of potato.

The results of the first experiment to determine the effect of pH of the medium on the action of the sodium salt of 2,4-D on four isolates of Actinomyces clearly showed that the synthetic plant hormone had an effect separate from that of pH. Martin (8) found in California that common soil fungi were unaffected by 2,4-D under neutral conditions but were inhibited with an acid pH. The studies of Schaal (11) have indicated that most races of Actinomyces scabies prefer a neutral medium and that most races will not grow on media with a pH of less than 5.2. If results under field conditions follow the trend of those obtained in the laboratory, there is a possibility of control of potato scab with these compounds, particularly if the soil is slightly acid.

In general the two pathogenic isolates of Actinomyces and the two nonpathogenic isolates used in these tests reacted in the same manner to synthetic plant hormones. Nonpathogenic isolate 105 was inhibited by three compounds at a concentration different from the concentration inhibiting the growth of the other isolates. Perhaps, in general all species of Actinomyces would react the same to these synthetic plant hormones. If the synthetic plant hormones would inhibit many races of Actinomyces, perhaps most potato varieties could be grown with reasonable assurance of being scab-free. Perhaps it would not be necessary to kill the soil Actinomyces to control scab. If one could inhibit the growth of the scab organism during a short period in the development of the tubers, the potato probably would be free of scab because infection occurs between the time the tubers are forming until they are two-thirds to three-fourths developed (4). Thus by holding the scab organism in check for three to five weeks, common scab of potato might be controlled.

In Table 2 the esters of 2,4-D are arranged in order of increased length of the side chain. It is

interesting to note that the results showed that the toxicity of the compounds decreased with the increase in the length of the side chain. The methyl ester of 2,4-D was most toxic and the butyl and amyl esters least toxic with the ethyl and propyl esters of 2,4-D intermediate. Possibly this side chain is the determining factor in the toxicity of the compounds. The sodium salt and the ethyl ester of 2,4-D and the ammonium salt and the propyl esters were toxic at the same concentrations respectively. The sodium salt and the methyl and ethyl esters of 2,4-D were more toxic than 2,4-dichlorophenoxy-acetic acid. Perhaps the substitution in the side chain determines this. It should also be noted that the n- and iso- forms of the esters exhibited equal toxicity.

The effective concentration for 2,4-D and its cyclohexyl ester was 1906 X 10^{-6} M.; for the ammonium salt, n- and iso-propyl ester, 953 X 10^{-6} M.; for the sodium salt and the ethyl ester, 476 X 10^{-6} M.; and for the methyl ester of 2,4-D, 238 X 10^{-6} M.

In Table 3 the acid forms are arranged in order of increased number of chlorine atoms in the ring. The toxicity of the compounds increased with an increase in

the number of chlorine atoms present. Although these compounds were not tested on a molar basis but on a p.p.m. basis, the toxic effects exhibited suggest that the increased toxicity was due to the increase in the number of the chlorine atoms in the ring. Although the valence of copper is different in the cuprous and cupric salts of 2,4-D, the compounds were toxic at the same concentration. However, the ferric salt of 2,4-D was more toxic than the ferrous salt.

Pentachlorophenoxyacetic acid was toxic at a concentration of 63 p.p.m.; the cuprous and cupric salts, 250 p.p.m.; the calcium, cobalt, and ferrous salts, 2,4,5-trichlorophenoxyacetic acid, and 2,4,5,6-tetrachlorophenoxyacetic acid, at 500 p.p.m.; the magnesium, manganese, and nickel salts, at 1,000 p.p.m.; and parachlorophenoxyacetic acid, at 2,000 p.p.m.

Several of these chemicals warrant testing in the greenhouse first and then under field conditions. The methyl ester of 2,4-D and pentachlorophenoxyacetic acid were toxic at the lowest concentrations and at concentrations decidedly lower than the other compounds. The ethyl ester of 2,4-D, and the cuprous, cupric, ferrous, ferric, calcium, cobalt, sodium, and ammonium

salts of 2,4-D were toxic at moderately low concentrations.

Carlyle and Thorpe (2) reported in their studies with species of Rhizobium that the ammonium salt was more toxic than the sodium salt of 2,4-D. On the isolates of Actinomyces, the sodium salt was more toxic than the ammonium salt. In this study the n- and iso-propyl esters, the calcium, cobalt, and ferrous salts of 2,4-D; 2,4,5-trichlorophenoxyacetic acid were toxic at approximately the same concentration as the ammonium salt. The cyclohexyl ester, the magnesium, manganese, and nickel salts were about equally toxic as 2,4-dichlorophenoxyacetic acid. Thus the compounds more toxic than 2,4-D and those of equal toxicity warrant testing in the greenhouse and in the field.

Suggestions for Further Study

Further tests of these chemicals should be conducted in the greenhouse and in the field for their effects on Actinomyces scabies. Since several of these synthetic plant hormones at low concentrations inhibited the growth of Actinomyces species, they might have

similar effects on other soil-borne pathogens. Tests should be conducted to determine the effects of synthetic plant hormones on such soil-inhabiting pathogens as Fusarium, Pythium, and Rhizoctonia.

Chapter VI

SUMMARY

One of the serious diseases of potato is common scab caused by Actinomyces scabies (Thaxt.) Guss. This organism probably lives in all arable soil although not all races present are pathogenic to all potato varieties. Chemicals have been used to control this disease with varying results. With the introduction of synthetic plant hormones such as 2,4-D have come reports of their effects on soil microorganisms. Little is known concerning the effect of these chemicals on soil pathogens. Therefore the problem arises: what are the fungicidal effects of the materials on the potato scab organism?

In determining the effect of these chemicals on Actinomyces species, one is confronted with the problem of separating the effect of pH from the effect of the chemical. The results of the present study showed that the fungicidal effect of synthetic plant hormones was separate from that of pH.

Twenty-five different and related compounds of 2,4-D were tested at 11 different concentrations on soil and tuber isolates of Actinomyces. The soil isolates

were obtained from four different soils and were non-pathogenic. The two pathogenic cultures were isolated from tubers.

All tests were conducted in the laboratory by inoculating plates of modified potato dextrose agar containing the chemicals. Four different isolates of Actinomyces were placed on each plate, one in each quadrant. Positive growth or complete inhibition was recorded for each test seven days after inoculation.

The effective concentration of the synthetic plant hormones varied considerably. The methyl ester of 2,4-D and pentachlorophenoxyacetic acid stood out as being toxic at low concentrations. The n- and iso-butyl and n- and iso-amyl esters had little or no inhibitory effect at the concentrations tested. The toxicity of the esters decreased with the increase in the length of the side chain. The toxicity of the related acid forms increased with an increase in the number of chlorine atoms present in the ring.

In general all isolates reacted in a similar manner to the synthetic plant hormones. The nonpathogenic isolate 105 was inhibited by three compounds at a

concentration different from the concentration inhibiting the growth of the other isolates.

B I B L I O G R A P H Y

BIBLIOGRAPHY

1. Cairns, H., Greeves, T. N., and Muskett, A. E. The control of common scab Actinomyces scabies (Thaxt.) Guss. of the potato by tuber disinfection. Annals of Applied Biology, 23:718-742, November 1936.

2. Carlyle, R. E., and Thorpe, John D. Some effects of ammonium and sodium 2,4-dichlorophenoxyacetates on legumes and the Rhizobium bacteria. American Society of Agronomy. Journal, 39: 929-936, October 1947.

3. Cunningham, H. S., and Wessels, P. H. Controlling common scab of the potato on Long Island by the addition of mercury compounds to the fertilizer mixture and the relation of soil reaction to the treatment. New York. Agricultural Experiment Station, Geneva. Bulletin, 685:1-20, April 1939.

4. Fellows, Hurley. Relation of growth in potato tuber to the potato scab disease. Journal of Agricultural Research, 32:757-781, April 15, 1926.

5. Fults, Jess L., and Payne, Merle G. A biometric evaluation of the growth-regulating and herbicidal properties of some organic compounds. American Society of Agronomy. Journal, 39: 667-681, August 1947.

6. Fults, Jess L., and Payne, Merle G. Some effects of 2,4-D, DDT, and Colorado 9 on the bacteria Rhizobium leguminosarum Frank in the root nodules of the common bean. American Journal of Botany, 34:245-248, May 1947.

7. Lewis, R. W., and Hamner, C. L. Effects of 2,4-D on some microorganisms. Michigan. Agricultural Experiment Station. Quarterly bulletin, 29:112-114, November 1946.

8. Martin, J. P. The hormone weed killer 2,4-D. California Citrograph, 39:248-264, July 1946.

9. Payne, Merle G., and Fults, Jess L. Some effects of 2,4-D, DDT, and Colorado 9 on root nodulation in the common bean. American Society of Agronomy. Journal, 39:52-55, January 1947.

10. Schaal, L. A. Seed and soil treatment for the control of common scab. American Potato Journal, 23:163-170, May 1946.

11. Schaal, L. A. Variation and physiologic specialization in the common scab fungus (Actinomyces scabies). Journal of Agricultural Research, 69:169-186, September 1944.

12. Stevenson, E. C., and Mitchell, John W. Bacteriostatic and bactericidal properties of 2,4-dichlorophenoxyacetic acid. Science, 101: 642-644, June 22, 1945.

13. Taylor, C. F. Field experiments on potato scab control in western New York. American Potato Journal, 11:40-45, February 1934.

14. Thompson, H. E., Swanson, Carl P., and Norman, A. G. New growth-regulating compounds. I. Summary of growth inhibitory activities of some organic compounds as determined by three tests. Botanical Gazette, 107:476-507, June 1946.

15. Vincent, J. G., and Vincent, H. W. Filter paper disc modification of the Oxford cup penicillin determination. Society of Experimental Biology and Medicine. Proceedings, 55:162-164, March 1944.

16. Worth, Winfield A., Jr., and McCabe, Anne M. Different effects of 2,4-D on aerobic, anaerobic, and facultative anaerobic microorganisms. Science, 108:16-18, July 2, 1948.