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ANTIBIOTIC ACTIVITY OF CERTAIN SPORE-FORMING BACTERIA AND OTHER MICROORGANISMS AGAINST SOME PLANT PATHOGENIC FUNGI IN THE SOIL

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THE UNIVERSITY OF ALBERTA

ANTIBIOTIC ACTIVITY OF CERTAIN SPORE-FORMING BACTERIA AND OTHER MICROORGANISMS AGAINST SOME PLANT PATHOGENIC FUNGI IN THE SOIL

A DISSERTATION
SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES
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by

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ABSTRACT

Aerobic spore-forming bacteria failed to reduce the severity of attack on wheat seedlings by Ophiobolus graminis, Fusarium culmorum and Helminthosporium sativum under the conditions of greenhouse experiments, in spite of the fact that they showed marked antagonism towards these fungi on potato dextrose agar. On the basis of these results, it appears possible that microorganisms other than spore-forming bacteria may be responsible for the suppression of these pathogenic fungi in natural soil. Results similar to those obtained with the spore-forming bacteria were obtained with several actinomycetes studied.

By means of a sensitive-microorganism technique, the persistence in soil of streptomycin and actidione was determined. Streptomycin was immediately inactivated in soil, whereas, actidione was found to remain in an active state for about a week. This method appears to be applicable to other water soluble, filterable antibiotics.

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Part II

ANTIBIOTIC ACTIVITY OF CERTAIN SPORE-FORMING BACTERIA AND OTHER MICROORGANISMS AGAINST SOME PLANT PATHOGENIC FUNGI IN THE SOIL

Edwin A. Peterson

INTRODUCTION

A number of microorganisms have been reported to exhibit antagonism towards various root-disease fungi. However, little attention has been given to the activity of aerobic spore-forming bacteria as antagonists of these pathogens in the soil. Yet, these spore-formers are widely distributed in soil and several are known to possess definite antibiotic properties. It was thought that a special study of such spore-forming bacteria might yield results which would help to explain the suppression of plant pathogenic fungi in normal soils.

Although several antibiotics have been isolated in pure form and have been found to be antagonistic to plant

pathogens, there is relatively little information available on the behavior of such substances in the soil. If antibiotics are to find a place in the control of plant diseases caused by soil- and seed-borne plant pathogens, it is likely that ability to persist in the soil will be a character of considerable importance in determining their usefulness.

OBJECTIVES

The investigations reported here have been divided into two parts. Those covered under Part I were undertaken in an attempt to determine the importance of aerobic spore-forming bacteria, including strain A32 of Bacillus subtilis Cohn, emend. Prazmowski and Bacillus polymyxa (Prazmowski) Migula, as antagonists of the cereal root-disease fungi Ophiobolus graminis Sacc., Fusarium culmorum (W. G. Sm.) Sacc. and Helminthosporium sativum P.K.B. in soil. Another objective was to study the effects of several actinomycetes on these fungi. Included among the former was Streptomyces griseus (Krainsky) comb. nov., an

organism known to produce the antibiotics streptomycin and actidione on suitable media.

Under Part II, studies on the persistence in the soil of two purified antibiotics, streptomycin and actidione, which are known to be active against certain seed-borne pathogens, are reported. Microorganisms used to measure antibiotic activity were B. subtilis (A32), sensitive to streptomycin, and Polyspora lini Lafferty, sensitive to actidione.

PART I

Previous studies on antibiosis at this laboratory

In 1931, Henry (11) reported that the growth of Helminthosporium sativum in sterilized soil was largely suppressed by the addition of a small amount of unsterile soil. He found that bacteria, actinomycetes and fungi each had a suppressing effect on this pathogen in soil and reduced the severity of root infection caused by it. Fungi were more effective than the bacteria and actinomycetes, but a mixture of all these organisms had the most pronounced effect. According to Ludwig and Henry (17), recontamination of sterilized soil, previously infested with the "take-all" fungus Ophiobolus graminis, by the addition of a small quantity of unsterilized soil resulted in a marked reduction in severity of wheat seedling infection by that fungus. They found that the saprophytic soil fungus Trichoderma viride became rapidly dominant in the recontaminated soil and concluded that it probably played an important role in the suppression of the pathogenic fungus.

Other work at this laboratory has indicated that the soil microflora may be of considerable importance in the suppression of certain seed-borne plant pathogens. Henry and Campbell (13) found a high percentage infection of flax seedlings by Polyspora lini, the fungus causing the "browning" disease, when infested seed was sown in sterilized soil. However, when similar seed was sown in natural soil, the percentage of seedling infection was significantly lower. They obtained similar results with the "anthracnose" pathogen Colletotrichum lini.

SOURCES OF KNOWN PATHOGENIC FUNGI AND ANTIBIOTIC MICROORGANISMS

The following test organisms of known identity were used in these studies.

Pathogenic fungi

Ophiobolus graminis (No. 77), a virulent strain isolated from wheat collected near Wetaskiwin, Alberta, by F. R. Davies in 1931.

Ophiobolus graminis (S.P.-1), a virulent strain isolated by the author from wheat collected near Stony Plain, Alberta, in 1951.

Fusarium culmorum (F), a virulent strain isolated by the author from wheat collected near Fleet, Alberta, in 1949.

Helminthosporium sativum (No. 6), a virulent strain isolated from wheat collected near Brooks, Alberta, in 1928.

Antibiotic microorganisms

Bacillus subtilis (A32), a strain isolated from Edmonton black soil by A. W. Jackson, formerly of this laboratory.

Bacillus subtilis (N.R.C. No. 4), a strain obtained in 1951 from the Prairie Regional Laboratory of the National Research Council of Canada, Saskatoon, through the courtesy of Dr. A. C. Blackwood.

Bacillus polymyxa (A.T.C. 7070). American Type Culture Collection number 7070. Obtained in 1942 from the National Research Council of Canada, through the courtesy of Dr. G. A. Ledingham.

Streptomyces griseus (A.T.C. 10137). American Type Culture Collection number 10137. Obtained in 1951 from the Division of Bacteriology and Dairy Research, Dominion Department of Agriculture, through the courtesy of Dr. A. G. Lochhead.

ANTIBIOTIC ACTIVITY OF BACILLUS SUBTILIS (A32) AND BACILLUS POLYMYXA (A.T.C. 7070) AGAINST OPHIOBOLUS GRAMINIS, FUSARIUM CULMORUM AND HELMINTHOSPORIUM SATIVUM

Introduction

Bacillus subtilis and Bacillus polymyxa are each known to produce a number of antibiotic substances (5, 8, 16, 20). One of these substances produced by B. subtilis, namely, subtilin, was first described by Jansen and Hirschman (16) who found it to be antagonistic to Gram-positive bacteria chiefly, but also to a number of pathogenic fungi. The action of subtilin on Xanthomonas translucens, the seed-borne agent of bacterial-stripe of barley, was studied by

Goodman and Henry (10). They found that subtilin reduced infection of barley by X. translucens when applied to seed that had been artificially infested with this pathogen.

During further work with the bacterial stripe organism, Goodman (9) observed that a medium staled by a strain (A32) of B. subtilis, isolated from soil, reduced the severity of bacterial stripe of barley seedlings when used to treat seed that had previously been infested with X. translucens. However, he concluded that the active substance produced by B. subtilis (A32) was different from subtilin since methods for the isolation of pure subtilin were ineffective when applied to the A32 cultures. Apparently A32 does not produce subtilin.

In view of the known antibiotic activity of the spore-forming bacteria B. subtilis and B. polymyxa and of their occurrence in soil, studies were undertaken to determine their effects on the growth of the root disease fungi Ophiobolus graminis, Fusarium culmorum and Helminthosporium sativum on an agar medium and on the ability of these fungi to attack wheat seedlings in sterilized soil.

Pure Culture Studies

Methods

Three series of 6 Petri plates containing potato dextrose agar were inoculated with the test fungi; one series with O. graminis (No. 77), one with F. culmorum (F) and one with H. sativum (No. 6). In each plate there was planted a small portion of an agar slant-culture of one of the pathogenic fungi. The plates were kept at room temperature for several days until the fungus colonies reached a diameter of about 2 cm. Each series of plates was then divided into two groups. In one, the plates were streaked with B. subtilis (A32) at a distance of about 3 cm. from the edge of the fungal colonies, in the other, with B. polymyxa (A.T.C. 7070) in a similar manner. After incubation for an additional 7 days, ratings for degree of antagonism, based upon the width of the zone of inhibition between the fungus and the bacterium, were made.

Results

As shown in Table I, B. subtilis (A32) exhibited marked antagonism to the three root-rot fungi when tested on

potato dextrose agar at room temperature. On the other hand, B. polymyxa (A.T.C. 7070) showed no antagonism towards O. graminis or H. sativum and only slightly inhibited F. culmorum.

TABLE I

RELATIVE DEGREE OF ANTAGONISM* OF B. SUBTILIS (A32) AND B. POLYMYXA (A.T.C. 7070) AGAINST SOME ROOT-DISEASE FUNGI ON POTATO DEXTROSE AGAR

Antagonist	O. graminis (No. 77)	F. culmorum (F)	H. sativum (No. 6)
B. subtilis (A32)	+ +	+ + +	+ + +
B. polymyxa (A.T.C. 7070)	o	+	o

* o = no inhibition
+ = slight inhibition — inhibition zone - up to 3 mm.
+ + = moderate inhibition — inhibition zone - 3 to 6 mm.
+ + + = pronounced inhibition — inhibition zone - 6 to 10 mm.

Greenhouse Studies

An attempt was made to determine whether B. subtilis and B. polymyxa could influence the severity of attack by Ophiobolus graminis, Fusarium culmorum and Helminthosporium sativum on wheat seedlings in sterilized soil.

Methods

Inoculum of the pathogenic fungi was produced by growing them on Edmonton black soil plus 10 percent cornmeal (by weight). Erlenmeyer flasks (200 ml.), each containing 50 gm. of soil cornmeal mixture plus 30 ml. of distilled water, were sterilized for 3 hours at 15 pounds steam pressure. A culture was started by transferring a small portion of a young fungus colony from a potato dextrose agar slant to a flask of the soil-cornmeal medium. Where a bacterium was to be included in addition to the fungus, 1 ml. of a 1 : 1,000,000 dilution of the bacterium in water was added to each flask at the time the fungus was added. All flasks were incubated at room temperature for 4 weeks. After 3 weeks, flasks were sampled to determine the presence of the organisms added. At the end of 4 weeks, flasks containing contaminants were discarded. Flask contents were cut up into small particles with

a sterile spatula. This inoculum was then added to 4" pots containing a 3 : 1 mixture of Edmonton black soil and sand, which had been sterilized for 8 hours at 15 pounds steam pressure, at the rate of one flask per pot. To each pot were added, at the inoculum level, 25 Red Bobs wheat seeds which had been previously surface sterilized by immersion for 3 minutes in a 1 : 1000 mercuric chloride solution and rinsed in sterilized water. About an inch of sterilized soil was used to cover the seed. The pots were randomized on a greenhouse bench and watered, each with 200 ml. of sterilized distilled water. During the period of the experiment, the pots were watered with sterilized distilled water as required and a greenhouse temperature of approximately 20°C. was maintained.

After 4 weeks, the plants were removed from the pots and soil was washed from their roots with running water. The height of each plant was measured, from the seed to the tip of the longest leaf. Severity of infection was determined by examination of each plant (the portion extending an inch on either side of the point of seed attachment) and assigning to it a numerical rating of from 0 to 5 based on the amount of necrosis present. The ratings for each pot were converted to the percentage of the maximum rating that was possible.

Results

When sampled after 3 weeks it was found that in flasks to which bacteria and a fungus had been added, the bacteria had multiplied tremendously. The fungi in all flasks had penetrated throughout the medium except in those to which O. graminis (No. 77) had been added along with B. subtilis (A32). Here the development of the fungus O. graminis was greatly reduced.

Summarized infection and height data are presented in Table II.

TABLE II

THE EFFECTS OF B. SUBTILIS (A32) AND B. POLYMYXA (A.T.C. 7070) ON THE SEVERITY OF INFECTION OF WHEAT SEEDLINGS BY OPHIOBOLUS GRAMINIS, FUSARIUM CULMORUM AND HELMINTHOSPORIUM SATIVUM IN STERILIZED SOIL

Fungus	Percent infection			Average height in cm.		
	B. subt.	B. poly.	Control	B. subt.	B. poly.	Control
O. graminis (No. 77)	1.9	19.8	26.9	40.7	39.9	38.7
F. culmorum (F)	33.4	31.6	34.1	42.1	40.0	41.1
H. sativum (No. 6)	66.6	60.1	67.8	25.2	30.2	31.0
None	10.0	8.3	8.5	36.4	39.9	38.3

Except for O. graminis in the presence of B. subtilis (A32), no statistically significant differences in the degree of infection between the bacterial treatments and the controls were obtained.

Discussion

The striking reduction in severity of infection by O. graminis in the presence of B. subtilis (A32) as compared with the fungus alone may be accounted for by the fact that growth of the fungus in flasks to which A32 had been added was greatly reduced. Consequently, only a limited amount of inoculum, as compared with that in control flasks containing the fungus alone, had been produced by the time of seeding.

A possible explanation of the results with O. graminis is that this fungus, normally a slow growing organism, was unable to compete with the rapidly multiplying bacteria for available food. Moreover, considering the antagonistic action of B. subtilis (A32) against O. graminis on potato dextrose agar, perhaps products of bacterial metabolism, possibly antibiotics, may have directly

inhibited the development of the fungus under the conditions described. However, the fact that F. culmorum and H. sativum appeared to be unimpeded in their development in the presence of A32 suggests that these fungi, because of their ability to grow rapidly, were able to compete successfully with the bacterium.

ANTIBIOTIC ACTIVITY OF MISCELLANEOUS ISOLATES OF SPORE-FORMING BACTERIA FROM LOCAL SOILS AGAINST OPHIOBOLUS GRAMINIS, FUSARIUM CULMORUM AND HELMINTHOSPORIUM SATIVUM

Introduction

It has been reported that certain bacteria, Bacillus spp. in particular, have an appreciable inhibitory effect on various soil-borne plant pathogens under certain conditions. According to Waksman (20), Novogrudsky observed that certain bacteria were able to destroy and dissolve the mycelium and spores of different phytopathogenic

fungi both in artificial media and in soil. He reported that Fusarium graminearum in sterilized soil caused the death of wheat plants grown in it, whereas in the presence of lysogenic bacteria, the pathogen was much less active. Furthermore, the addition of bacteria to unsterilized "flax sick" soil infested with F. lini markedly reduced the percentage of wilted flax plants.

Anwar (1) conducted experiments to determine the survival of H. sativum and F. lini in soil. In pure culture work, he found that soil isolates of B. subtilis were antagonistic to both fungi. When steamed soil was infested with inoculum of H. sativum, grown on a wheat-oat mixture, and seeded with barley, the resulting plants were severely diseased and yield was significantly reduced. However, after infesting steamed soil with a mixture of inoculum of H. sativum and inoculum of certain isolates of B. subtilis, also grown on a wheat-oat mixture, the barley plants grew almost as well as plants in non-infested soil and yield was not lowered significantly. Moreover, in field tests, barley in rows inoculated with the root-rot fungus and B. subtilis grown on a wheat-oat medium, showed a much lighter infection than the barley in rows inoculated with the fungus alone. No control of flax wilt, following the addition of F. lini and B. subtilis to soil, was obtained.

Cordon and Haenseler (7) isolated a strain of Bacillus simplex which was found to produce a thermostabile substance that inhibited growth and even caused the death of Rhizoctonia solani. When the substance was added to soil, marked control of seed decay and damping-off of cucumbers and peas was observed.

Since in the previous section it was shown that B. subtilis (A32) and B. polymyxa (A.T.C. 7070) are generally ineffective as antagonists against O. graminis, F. culmorum and H. sativum in sterilized soil, it seemed advisable to determine how other aerobic spore-forming bacteria in the soil behave towards these pathogens. For this purpose, isolations were made from a large number of soil samples collected from the main soil zones and from widely separated localities in the province of Alberta during the summer of 1950.

Isolation of Spore-forming Bacteria

Spore-forming bacteria were readily obtained by subjecting a soil suspension to pasteurization, thereby eliminating non-spore-forming bacteria, actinomycetes and fungi. Approximately 1 gm. of soil from each sample was suspended in 10 ml. of distilled water in a test tube.

The tubes were immersed in water at temperatures ranging from 80° to 83°C. for a period of 10 minutes and then immediately cooled. One ml. quantities of the pasteurized suspensions were mixed with approximately 10 ml. lots of potato dextrose agar in plates. The plates were incubated at room temperature for 4 days when bacterial transfers from individual colonies were made to potato dextrose agar slants, one to each slant. All isolates were subjected to pasteurization and reisolated to eliminate any possible non-sporing contaminants.

Pure Culture Studies

Methods

Petri plates containing potato dextrose agar were inoculated with the test fungi O. graminis (No. 77), F. culmorum (F) and H. sativum (No. 6) as previously described. The plates were incubated at room temperature for several days until the fungus colonies reached a diameter of about 2 cm. Duplicate plates of each fungus were treated, each with a bacterial isolate. The bacterium was streaked on the surface of the medium on two opposite sides of the fungus colony and at a distance of about 2 cm. from its edge.

Plates were incubated for an additional 7 days at room temperature whereupon ratings of the degree of antagonism, based on the width of the zone of inhibition, were made.

B. polymyxa (A.T.C. 7070) and several named strains of B. subtilis including A32 were also tested at the same time and under the same conditions as the above isolates for comparative purposes.

Results

As shown in Table III, a relatively high proportion of the spore-forming bacterial isolates exhibited varying degrees of antagonism towards the three root-rotting fungi when tested on potato dextrose agar. Out of a total of 66 isolates tested, 51 were antagonistic to O. graminis (No. 77), 40 to F. culmorum (F) and 36 to H. sativum (No. 6). In addition, it was found that 31 isolates were antagonistic to all three of these fungi.

TABLE III

RELATIVE NUMBERS OF ANTAGONISTIC AND NON-ANTAGONISTIC SPORE-FORMING BACTERIA ISOLATED FROM DIFFERENT SOILS WITH REFERENCE TO THEIR DEGREE OF ANTAGONISM TOWARDS SEVERAL ROOT DISEASE FUNGI ON POTATO DEXTROSE AGAR

Fungus	No. of isolates tested	No. of isolates showing the degree of antagonism indicated				Total number of isolates showing antagonism	Percentage of isolates showing antagonism
		None	Slight	Moderate	Pronounced		
O. graminis (No. 77)	66	15	18	12	21	51	77.3
F. culmorum (F)	66	26	18	10	12	40	60.6
H. sativum (No. 6)	66	30	8	16	12	36	54.5

Greenhouse Studies

A number of the spore-forming bacterial isolates included in the test reported in Table III were selected for study in sterilized soil on the basis of their marked antagonism towards Ophiobolus graminis, Fusarium culmorum and Helminthosporium sativum on potato dextrose agar. Also included, for comparison, were three spore-formers of known identity, namely, B. subtilis (A32), B. subtilis (N.R.C. No. 4) and B. polymyxa (A.T.C. 7070).

Methods

Four-inch pots containing a 3 : 1 mixture of Edmonton black soil and sand were sterilized for 8 hours at 15 pounds steam pressure. Fungus inoculum, grown in flasks on a sterilized soil-cornmeal mixture, was added at the rate of 1 flask per pot. After seeding with 25 Red Bobs wheat seeds, previously surface sterilized with a 1 : 1000 mercuric chloride solution, 50 ml. of a heavy bacterial suspension was distributed uniformly over the inoculum and seed by means of a pipette. Control pots received fungal inoculum, seed and 50 ml. of sterile distilled water in place of the bacterial suspension. Seed was covered with about an inch of sterilized soil.

The experiment included 4 pots for each of 11 bacterial treatments and 4 pots for a sterile water (control) treatment. The pots were randomized on a greenhouse bench and were watered, each with 200 ml. of sterile distilled water. During the course of the experiment, pots were watered as required. The greenhouse temperature varied between 20° and 25°C.

Four weeks after seeding, plants were removed from the pots and the soil was washed from their roots with running water. The height of each plant was measured and disease severity was determined as previously described.

Results

Results summarizing the effects of spore-forming bacterial isolates on O. graminis (No. 77) were to be reported at this time. However, a number of marked discrepancies in the data make them unreliable. Considerable variation of the same treatment, with regard to severity of infection occurred among replications, namely, from 0 to 40 percent. Moreover, controls (the fungus alone) showed an unusually low degree of infection as compared with normal infection of wheat seedlings by O. graminis observed in previous tests where similar techniques were employed.

For these reasons the results are considered to be unreliable, and hence, are omitted. It may, however, be mentioned that there was no indication of inhibition of O. graminis (No. 77) by any of the bacterial isolates tested.

The tests involving F. culmorum (F) and H. sativum (No. 6) were uniform with regard to replications and the data are summarized in Table IV.

The spore-forming bacterial isolates tested showed little, if any effect on the infection of wheat seedlings by F. culmorum (F) or H. sativum (No. 6) under the conditions of the outlined experiments. It may be noted (Table IV) that although the differences obtained were not significant, as determined by an analysis of variance, there appears to be a tendency for slight inhibition of F. culmorum by two isolates, namely, culture numbers 13a - 50 and 32 - 50 as compared with the fungus alone. On the other hand, there appears to be some stimulation of this fungus by isolates 13b - 50, 41 - 51 and 42 - 51. There also appears to be a slight tendency for reduction in the severity of attack by H. sativum in the case of several isolates, but again, these differences, as compared with this fungus alone, are not statistically significant.

TABLE IV

THE EFFECTS OF INDIVIDUAL SPORE-FORMING BACTERIAL ISOLATES ON THE INFECTION OF WHEAT SEEDLINGS BY FUSARIUM CULMORUM AND HELMINTHOSPORIUM SATIVUM IN STERILIZED SOIL

Culture No.	Identity	Average length of leaves in cm.*		Average percent root infection*	
		F. culmorum	H. sativum	F. culmorum	H. sativum
8a - 50	Unidentified	37.1	30.2	31.2	70.4
12a - 50	"	36.3	30.2	35.6	65.4
13a - 50	"	36.8	31.0	28.4	66.3
13b - 50	"	35.0	39.9	38.4	67.0
19b - 50	"	35.7	30.4	35.2	67.7
28 - 50	"	38.9	29.1	32.5	70.5
30 - 50	"	37.9	36.0	33.0	65.6
32 - 50	"	35.7	30.3	28.8	71.8
40 - 51	B. subtilis (A32)	36.4	31.2	32.4	67.5
41 - 51	B. polymyxa (A.T.C. 7070)	36.2	26.5	39.2	72.4
42 - 51	B. subtilis (N.R.C. No. 4)	32.9	24.9	38.6	74.2
Control		35.6	29.9	34.5	70.7

* Differences not significant

Discussion

The results of the agar plate tests suggest a relatively large proportion of the spore-forming bacteria may be potential antagonists of Ophiobolus graminis, F. culmorum and Helminthosporium sativum in the soil. Yet, in spite of their marked antagonism (with the exception of B. polymyxa A.T.C. 7070) towards these fungi on potato dextrose agar, the spore-forming isolates used in greenhouse studies had little inhibitive effect on the ability of the pathogenic fungi to attack wheat seedlings under the conditions described. These observations are in agreement with those of Broadfoot (4) who emphasized that the antagonism of a saprophytic microorganism to a plant pathogen on artificial media is not a reliable indication of the effect it may have on the parasitic activity of the same pathogen in the soil.

ANTIBIOTIC ACTIVITY OF A MIXTURE OF SPORE-FORMING BACTERIA FROM THE SOIL AGAINST *OPHIOBOLUS GRAMINIS* AT DIFFERENT SOIL TEMPERATURES

Introduction

Since various aerobic spore-forming bacteria are known to occur in soils, there is a possibility that these organisms, as a group, may influence the activity of certain soil-borne plant pathogens. As previously mentioned, Henry (11) found that a mixture of soil fungi was more effective in suppressing *Helminthosporium sativum* in soil than a mixture of either bacteria or actinomycetes. In view of these results, it would be of interest to know what effects a mixed population of spore-forming bacteria might have on root-disease fungi in the soil.

The question of soil temperature may be of importance in determining the activity of spore-forming bacteria. Soil temperature is always a factor influencing the development of plant diseases caused by soil-borne plant pathogens. In addition to its effect on the hosts and on the parasites, temperature may affect the development of soil microorganisms which are naturally antagonistic to

such pathogens and hence, indirectly alter the parasitic activities of these pathogens.

Henry (12) studied the influence of soil temperature on the reaction of wheat seedlings to Ophiobolus graminis. He found that in sterilized soil the fungus caused severe damage at temperatures ranging from 13° to 27°C. However, in unsterilized soil, while damage was severe at temperatures below 20°C., a marked reduction of damage occurred at temperatures above 20°C. Although most of the seedlings grown in sterilized soil were dead after 25 days at 27°C., the seedlings grown in unsterilized soil at the same temperature were almost as vigorous as those of the non-inoculated controls.

In the following investigation the effects of a mixture of spore-forming bacteria, common to Edmonton black soil, on the ability of O. graminis to attack wheat seedlings in sterilized soil were studied at several different soil temperatures.

Greenhouse Studies

Methods

Fungus inoculum was grown on a soil-cornmeal medium as previously described. A mixture of spore-forming bacteria from soil was obtained as follows. Ten gm. of Edmonton black soil were added to 100 ml. of distilled water, shaken vigorously for about 3 minutes and allowed to settle for 1 hour. The soil suspension was then added to test tubes at the rate of 10 ml. per tube. The tubes were immersed in water at a temperature of 80°C. for 10 minutes then immediately cooled. The pasteurized soil suspension was added to small Erlenmeyer flasks containing 50 gm. of sterile Edmonton black soil (approximately 30 percent moisture content) at the rate of 1 ml. per flask. After 9 days incubation at room temperature, flasks were sampled to determine the presence of the bacteria added by streaking soil particles on the surface of potato dextrose agar.

Gallon crocks containing a 3 : 1 mixture of moist Edmonton black soil and sand were covered with several thicknesses of newspaper and sterilized for 12 hours at 15 pounds steam pressure. After cooling, 8 crocks were

placed in each of 4 controlled temperature tanks at the temperatures 15°, 20°, 25° and 30°C., respectively. Seeding was delayed for 24 hours to allow soil temperatures to become constant. The paper coverings were left on the crocks until the time of seeding to minimize contamination. The crocks in each tank were divided into 2 series. In one series, each crock received 1 flask of fungus inoculum and 1 flask of bacterial inoculum. In the other, the control series, each crock received 1 flask of fungus inoculum and 1 flask of sterilized soil in place of the bacterial inoculum. The crocks were then seeded at the inoculum level with 25 Red Bobs wheat seeds, previously surface-sterilized with a 1 : 1000 mercuric chloride solution. Seed was covered with about an inch of sterilized soil and all crocks were watered with approximately 200 ml. of sterile distilled water.

During the period of the experiment, the crocks were watered as required. Temperatures were maintained by thermostat control.

After 4 weeks plants were removed from the crocks and their roots were washed in running water. The height of each plant was measured and ratings of the severity of disease were made as described previously.

Results

Agar plate tests indicated that the spore-forming bacteria had increased greatly in numbers in the flasks of sterilized soil when the soil was sampled after 9 days.

The summarized height and root infection data are given in Table V. No statistically significant differences between the reaction of wheat seedlings to O. graminis (S.P. -1) in the presence of the spore-forming bacteria and their reaction to the pathogenic fungus alone were found at any temperatures used. The fungus caused severe damage to the seedlings at the temperatures 15°, 20° and 25°C. At 30°C., plants appeared vigorous and normal in size. Although on most of the plants grown at 30°C. the seminal roots and sub-coronal internodes showed severe lesioning, vigorous secondary roots that were almost free from infection had developed at the crowns. This was not observed on plants grown at the three lower temperatures.

TABLE V

REACTION OF WHEAT SEEDLINGS TO OPHIOBOLUS GRAMINIS (S.P.-1) IN THE PRESENCE AND IN THE ABSENCE OF A MIXTURE OF SPORE-FORMING BACTERIA IN STERILIZED SOIL AT DIFFERENT SOIL TEMPERATURES

Soil temp. °C.	Inoculum added to the soil	Av. height in cm.	Av. percent root infection
15	O. gram. + bacteria*	23.1	79.7
	O. gram.	21.3	86.3
20	O. gram. + bacteria*	24.0	82.9
	O. gram.	27.1	81.0
25	O. gram. + bacteria*	22.2	87.7
	O. gram.	24.2	86.8
30	O. gram. + bacteria*	39.2	56.6
	O. gram.	43.4	59.4

* A mixture of spore-forming bacteria from a sample of Edmonton black soil.

Discussion

Under the conditions of this investigation, the mixture of spore-forming bacteria used had little, if any effect on the ability of O. graminis (S.P.-1) to attack wheat seedlings. Moreover, it is interesting to note that this strain of the fungus exhibited marked parasitic activity in sterilized soil over a relatively wide range of temperatures and that it was not appreciably suppressed by the bacteria at any of the temperatures tested.

The increased vigor of the plants grown at 30°C., as compared with those grown at the lower temperatures, suggests that this temperature closely approaches the upper limit for parasitic activity by O. graminis (S.P.-1).

In view of these results and those reported by Henry (12), it seems possible that microorganisms, other than the aerobic spore-forming bacteria, are involved in the suppression of Ophiobolus graminis in natural soils.

ANTIBIOTIC ACTIVITY OF CERTAIN ACTINOMYCETES AGAINST *OPHIOBOLUS GRAMINIS*, *FUSARIUM CULMORUM* AND *HELMINTHOSPORIUM SATIVUM*

Introduction

Many actinomycetes, antagonistic to bacteria, fungi and other actinomycetes in artificial culture have been isolated from soil (2, 20). Although they occur abundantly in various soils, there is little knowledge of their role as antagonists there.

Cooper and Chilton (6) studied the antibiotic activity of a large number of actinomycetes, isolated from the sugar-cane soils of Louisiana. They found that an average of 23.4 percent of their isolates were antagonistic to *Pythium arrhenomanes* in pure culture. Similarly, Meredith and Semeniuk (18) found that 21 percent of actinomycetes which had been isolated from Iowa soils were antagonistic to *Pythium graminicola*. However, they reported that when spores of a species of *Streptomyces* were added to unsteamed soil, there was no apparent effect on the growth of soil fungi.

In the present studies, experiments were made to determine the antagonism of a number of actinomycetes isolated from soil against the root-disease fungi Ophiobolus graminis, Fusarium culmorum and Helminthosporium sativum in pure culture. Furthermore, an attempt was made to determine their ability to antagonize O. graminis and H. sativum in sterilized soil.

Pure Culture Studies

A relatively large number of actinomycetes, isolated from soil, are maintained as part of the stock culture collection of this laboratory. These were tested for antagonism against O. graminis, F. culmorum and H. sativum in pure culture.

Methods

The methods used for determination of the antagonism of actinomycete isolates against the pathogenic fungi O. graminis, F. culmorum and H. sativum were essentially the same as those previously described for the testing of spore-forming bacterial isolates. Potato dextrose agar was

selected as a culture medium on the basis of preliminary tests with some of the actinomycetes. The pathogenic fungi grew well on this medium and the antagonistic effects of certain actinomycete isolates on them were easily recognized.

Three series of Petri plates containing potato dextrose agar were inoculated with the pathogenic fungi, one series with O. graminis (S.P.-1), one with F. culmorum (F) and one with H. sativum (No. 6). In most cases, a single streak of spores from each actinomycete isolate was made on the surface of the agar in 2 plates of each series, at a distance of 3 cm. from the fungus inoculum. However, a number of the isolates did not sporulate in culture. Here, bits of mycelium were used to make the streaks. Plates were kept at room temperature for 10 days and counts were made of the isolates that were antagonistic to each of the fungi.

Results

Although some of the actinomycetes tested did not grow satisfactorily on potato dextrose agar, out of a total of 44 isolates that did grow on this medium, 8 were antagonistic to O. graminis, 5 were antagonistic to F. culmorum and 13 were antagonistic to H. sativum.

Greenhouse Studies

Two actinomycetes were selected, on the basis of their pronounced antagonism towards O. graminis and H. sativum in pure culture, for study in sterilized soil. In addition, Streptomyces griseus (A.T.C. 10137) was included in this study. However, since this species failed to grow on potato dextrose agar, its effects on the pathogenic fungi in pure culture were not determined.

Methods

The procedure followed in this experiment was the same as that described for the studies with isolates of spore-forming bacteria. Fungus inoculum was grown on a soil plus 10 percent cornmeal medium. Actinomycetes were grown on a glycerol-yeast extract medium* described by Bottcher and Conn (3), solidified by the addition of agar.

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* Bottcher and Conn medium for actinomycetes modified by the addition of 15 gm. of agar per liter.

Glycerol	-	5 ml.
Yeast extract (Difco)	-	2 gm.
K_2HPO_4	-	1 gm.
Agar	-	15 gm.
Water	-	1000 ml.

The experiment was conducted in 2 series of pots containing a sterilized mixture of soil and sand, one series for each of the pathogenic fungi, O. graminis (S.P.-1) and H. sativum (No. 6). Each series included the following:

(a) 3 pots containing fungus inoculum for each of 3 actinomycete treatments,

(b) 3 pots containing fungus inoculum for a sterile water control treatment,

(c) 3 pots containing sterilized soil-cornmeal medium (non-inoculated) for a sterile water control treatment.

Fungus inoculum was added at the rate of one flask per pot. After seeding with 25 surface sterilized Red Bobs wheat seeds, an actinomycete spore suspension was distributed uniformly over the seeded layer, by means of a pipette, at the rate of 50 ml. per pot. Control pots each received 50 ml. of sterile distilled water. Seed was then covered with about an inch of sterilized soil. Pots of each series were randomized on a greenhouse bench and watered, each with 200 ml. of sterile distilled water. During the experiment, the greenhouse temperature was maintained at approximately 20°C. and pots were watered as required.

After 4 weeks plants were removed from the pots and notes were taken on heights and severity of root infection.

Results

Height and root infection data are summarized in Table VI. Differences between the actinomycete treatments and the sterile water treatment of infested soil were not statistically significant. Similar results were obtained for both O. graminis (S.P.-1) and H. sativum (No. 6). Severe damage was caused by each of the pathogenic fungi. It may be seen in Plate I, Figures 1 and 2, that all plants grown in infested sterilized soil were severely stunted as compared with the vigorous plants grown in uninfested sterilized soil.

TABLE VI

THE EFFECTS OF CERTAIN ACTINOMYCETES ON THE SEVERITY OF INFECTION BY OPHIOBOLUS GRAMINIS AND HELMINTHOSPORIUM SATIVUM IN STERILIZED SOIL

Fungus	Average percent root infection				Average height in cm.			
	S. gris.	Act. V9	Act. 107	Control	S. gris.	Act. V9	Act. 107	Control
O. graminis (S.P.-1)	85.4	90.0	93.1	91.6	13.6	12.2	13.0	13.2
H. sativum (No. 6)	87.8	74.9	73.8	80.2	9.0	10.8	12.3	11.0
None	-	-	-	2.4	-	-	-	26.6

PLATE I

THE EFFECTS OF CERTAIN ACTINOMYCETES ON THE SEVERITY OF INFECTION OF WHEAT SEEDLINGS BY *OPHIOBOLUS GRAMINIS* AND *HELMINTHOSPORIUM SATIVUM* IN STERILIZED SOIL

Figure 1. Sterilized soil infested with *O. graminis* (S.P.-1)

Figure 2. Sterilized soil infested with *H. sativum* (No. 6)

Treatments from left to right: fungus + *S. griseus*, fungus + Act. V9, fungus + Act. 107, fungus alone, non-inoculated control.

Discussion

Although the actinomycetes used in these studies were antagonistic in pure culture to Ophiobolus graminis and Helminthosporium sativum, they showed little, if any influence on the parasitic activity of these pathogens towards wheat seedlings under the conditions described.

Generally, actinomycetes grow slowly in the soil. Ludwig and Henry (17), were unable to show the presence of actinomycetes until 8 days after sterilized soil was re-contaminated with a small amount of normal soil. This slow growth of the actinomycetes might explain the absence of their influence on the pathogenic fungi in soil.

PART II

THE BEHAVIOR OF CERTAIN PURIFIED ANTIBIOTICS FROM *STREPTOMYCES GRISEUS* IN THE SOIL

Introduction

The value of an antibiotic for the control of soil- or seed-borne plant pathogens might well depend largely on its ability to remain in an active state when in contact with soil. According to Weindling *et al* (21), the persistence of antibiotics in soil depends on such factors as their stability, adsorption, and resistance to decomposition by the soil microflora. Siminoff and Gottlieb (19) pointed out that antibiotics such as streptomycin and subtilin, whose reactions are on the alkaline side of neutrality, are adsorbed and effectively inactivated by colloidal complexes such as clays and soil organic matter, and thus are probably biologically inactive in the soil.

In the following studies an attempt was made to determine the persistence of antibiotic activity in the soil of two purified antibiotics, namely, streptomycin and

actidione. These two antibiotics have previously been used in seed treatment investigations at this laboratory. Streptomycin was found to be ineffective for the control of covered smut of oats when used in liquid form for treatment of infested seed (15). On the other hand, actidione yielded promising results in smut control, both in liquid and dust form (14). Complete control of covered smut of wheat was obtained when infested seed was immersed in a 10 ppm. solution of actidione for 1 minute. Furthermore, a finely divided clay dilution of this antibiotic also effectively controlled covered smut of wheat when used to treat infested seed.

More recently, it was noticed that actidione, when diluted with the clay, lost little, if any activity after 9 months storage in rubber-stoppered test tubes at room temperature.

Seedling Technique

Phytotoxicity of certain concentrations of streptomycin and actidione have been observed on numerous occasions at this laboratory during seed treatment investigations both in the greenhouse and in the field. Wheat seed soaked in harmful water solutions of streptomycin gave rise to a high percentage of albino seedlings. Similar solutions of actidione markedly reduced germination of the soaked seed. On the basis of these observations, an experiment was conducted to determine whether the harmful effects of streptomycin and actidione on wheat seedlings might be used to indicate the persistence of these compounds in the soil.

Methods

A quantity of Edmonton black soil was adjusted to a moisture content of 30 percent and added to Petri plates at the rate of 50 gm. per plate. The plates were divided into two groups, one group was sterilized for 2 hours at 15 pounds steam pressure, the other was left unsterilized. Each group was separated into four series of 10 plates each, which received the following treatments:

series 1, 20 ml. of a 10,000 ppm. sterile water solution of streptomycin per plate;

series 2, 20 ml. of a 5,000 ppm. sterile water solution of actidione per plate;

series 3, 20 ml. of a 1,000 ppm. sterile water solution of actidione per plate; and

series 4, 20 ml. of sterile distilled water per plate.

One plate in each series of the two groups (sterilized and unsterilized) was seeded immediately with 25 Red Bobs wheat seeds, previously surface sterilized with a 1 : 1000 mercuric chloride solution. The remainder of the plates of each series was similarly seeded periodically, beginning after an interval of 3 days. As the experiment progressed, the interval between seedings was extended.

All plates were kept at room temperature during the course of the experiment. Sterile distilled water was added to the plates regularly to maintain the soil moisture content. In an attempt to minimize contamination and to prevent rapid drying of the soil, the plate covers were left on until emergence of the seedlings was noticed. Percentage emergence was recorded 14 days after seeding.

Results

Wheat seedlings grown in soil to which a solution of streptomycin had been added appeared to be normal, as compared with those grown in untreated soil. In view of the high concentration of streptomycin used, the results suggest that this antibiotic was immediately inactivated in the soil. As shown in Table VII, germination of wheat, seeded up to 60 days after a solution of actidione had been added to sterilized soil, was completely inhibited. With reference to the lower concentration of actidione in unsterilized soil, complete inhibition was apparent for only 6 days. Wheat sown 9 days after the addition of this antibiotic to unsterilized soil resulted in a very limited germination, but seeding 14 days after the addition of the antibiotic resulted in little, if any effect on germination as compared with control plates. It may be noted that the inhibitory effect of the higher concentration of actidione was maintained in unsterilized soil somewhat longer than that of the lower concentration.

The results for actidione (1,000 ppm.) in unsterilized soil are illustrated graphically in Figure 1.

TABLE VII

PERCENTAGE EMERGENCE OF WHEAT SEEDED AT INTERVALS AFTER ADDITION OF ANTIBIOTIC SOLUTIONS TO SOIL

Soil	Antibiotic	Conc.	Days after addition of antibiotic to the soil until seeding									
			0	3	6	9	14	19	27	35	45	60
Sterile	Streptomycin	10,000 ppm.	68	96	100	80	80	92	92	80	76	80
	Actidione	5,000 ppm.	0	0	0	0	0	0	0	0	0	0
	Actidione	1,000 ppm.	0	0	0	0	0	0	0	0	0	0
	None	-	92	92	100	96	96	84	80	84	76	84
Unsterile	Streptomycin	10,000 ppm.	52	60	52	52	52	72	52	52	84	76
	Actidione	5,000 ppm.	0	0	0	0	0	0	32	64	84	76
	Actidione	1,000 ppm.	0	0	0	12	72	92	72	68	88	84
	None	-	44	48	-	88	68	84	56	-	84	80

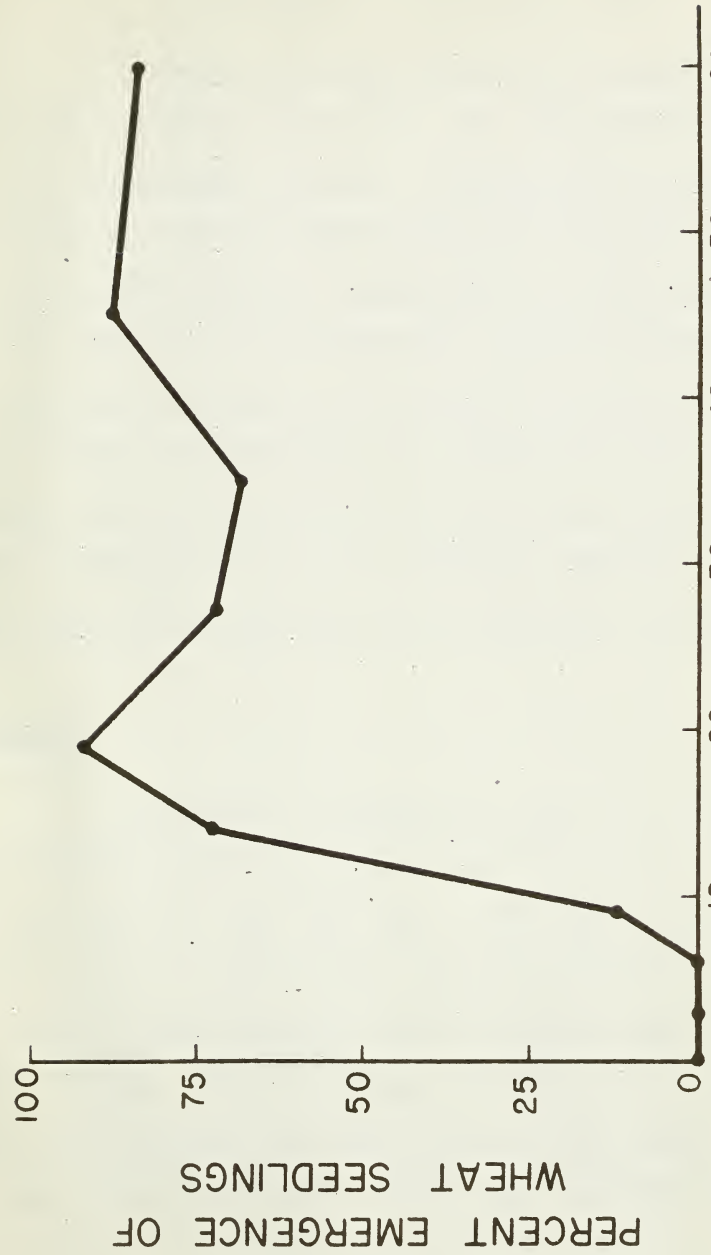

Figure 1. Persistence in unsterilized soil of a 1000 ppm. solution of actidione as measured by its effect on germination of wheat seed

Sensitive-microorganism Technique

A number of difficulties were encountered while using the seedling method for the determination of antibiotic persistence in soil. Considerable attention was required to prevent rapid drying of the soil contained in shallow Petri plates. Germination of wheat seeds was not always uniform in the different control plates. Plates containing sterilized soil became contaminated with various fungi after a short time on a laboratory table. In an attempt to overcome these difficulties, a bacterium, sensitive to streptomycin, and a fungus, sensitive to actidione were used to measure antibiotic activity of these compounds.

Methods

A quantity of Edmonton black soil was adjusted to a 25 percent moisture content and was added to test tubes at the rate of 5 gm. per tube. The tubes were plugged with cotton and one half of them was sterilized for 1 hour at 15 pounds steam pressure while the other half was left unsterilized. The two groups (sterilized and unsterilized) were each divided into two series of 10 tubes. One series of each group received a 10,000 ppm. sterile distilled

water solution of streptomycin at the rate of 2 ml. per tube. The other series of each group received a 1,000 ppm. sterile distilled water solution of actidione at the rate of 2 ml. per tube.

One tube from each series of the two groups was tested for antibiotic activity immediately and then at intervals over a period of 50 days. Antibiotic activity was determined as follows. The contents of a tube were extracted with 10 ml. of distilled water by vigorous shaking for 3 minutes. The water extract was then filtered through a Seitz bacterial filter. A one-half inch sterile absorbent paper disc was saturated with the filtrate and placed at the center of a Petri plate on the surface of a potato dextrose agar dilution of the sensitive organism. Duplicate plates were used for each filtrate. Plates were incubated at room temperature for 4 days and the widths of the inhibition zones were measured.

Of several bacteria tested for sensitivity to streptomycin, B. subtilis (A32) was selected as a suitable test organism on the basis of its rapid growth and wide zone of inhibition by this antibiotic. Similarly, Polyspora lini (194-b) was selected from a number of fungi tested for sensitivity to actidione. Since this strain of P. lini

sporulates profusely on potato dextrose agar, heavy spore suspensions were readily obtained for preparation of the agar test plates.

It was thought that filtration might result in a decrease in activity of one or both antibiotic solutions and hence, influence the accuracy of results. This possibility was investigated. A 10 ml. aliquot of a 10,000 ppm. sterile distilled water solution of streptomycin was filtered through a Seitz bacterial filter and tested for antibiotic activity against B. subtilis (A32) by the paper disc method. Similarly, a 10 ml. aliquot of 1,000 ppm. sterile water solution of actidione was filtered and tested against P. lini (194-b). The activity of the filtrates of each antibiotic was compared with the activity of similar unfiltered solutions. As shown in Table VIII no difference between filtered and unfiltered solutions was found for either streptomycin or actidione. This experiment was repeated with almost identical results. Both antibiotics, in water solution, evidently pass freely through a Seitz filter. Hence, this type of filter can be used safely to remove microorganisms from such solutions.

TABLE VIII

ANTIBIOTIC ACTIVITY OF FILTERED AND UNFILTERED SOLUTIONS OF STREPTOMYCIN AND ACTIDIONE AS MEASURED BY THE SENSITIVE-MICROORGANISM METHOD

Antibiotic	Sensitive Organism	Treatment	Average width of inhibition zone in mm.*
Streptomycin 10,000 ppm.	B. subtilis (A32)	Filtered	13.2
		Unfiltered	13.2
Actidione 1,000 ppm.	P. lini (194-b)	Filtered	22.0
		Unfiltered	21.8

* Averages are each of 5 plates.

Results

As shown in Table IX, the filtered water extracts from tubes containing sterilized soil and from tubes containing unsterilized soil to which streptomycin had been added, failed to inhibit B. subtilis (A32), even when tested immediately following the addition of the antibiotic.

Antibiotic activity of actidione in sterilized soil remained constant for at least 50 days (the period of the experiment). However, in unsterilized soil, activity remained constant for only 6 days. A slight decline in activity was apparent at 9 days and by the end of 17 days only a trace of inhibition of the sensitive fungus was noticed. The results for actidione are illustrated graphically in Figure 2.

TABLE IX

THE PERSISTENCE OF ANTIBIOTIC ACTIVITY OF STREPTOMYCIN AND ACTIDIONE IN STERILE AND UNSTERILIZED SOIL*

Antibiotic and Sensitive Organism	Soil	Days after addition of antibiotic to soil until extraction									
		0	3	6	9	13	17	24	32	40	50
Streptomycin 10,000 ppm. against B. subtilis (A32)	Sterile	0	0	0	0	0	0	0	0	0	0
	Unsterile	0	0	0	0	0	0	0	0	0	0
Actidione 1,000 ppm. against P. lini (194-b)	Sterile	16.5	15.0	15.0	15.5	15.0	15.5	13.5	16.0	16.0	15.0
	Unsterile	15.0	14.5	15.5	10.5	4.5	Trace	0	0	0	0

* Data presented are duplicate plate averages of inhibition widths measured in mm.

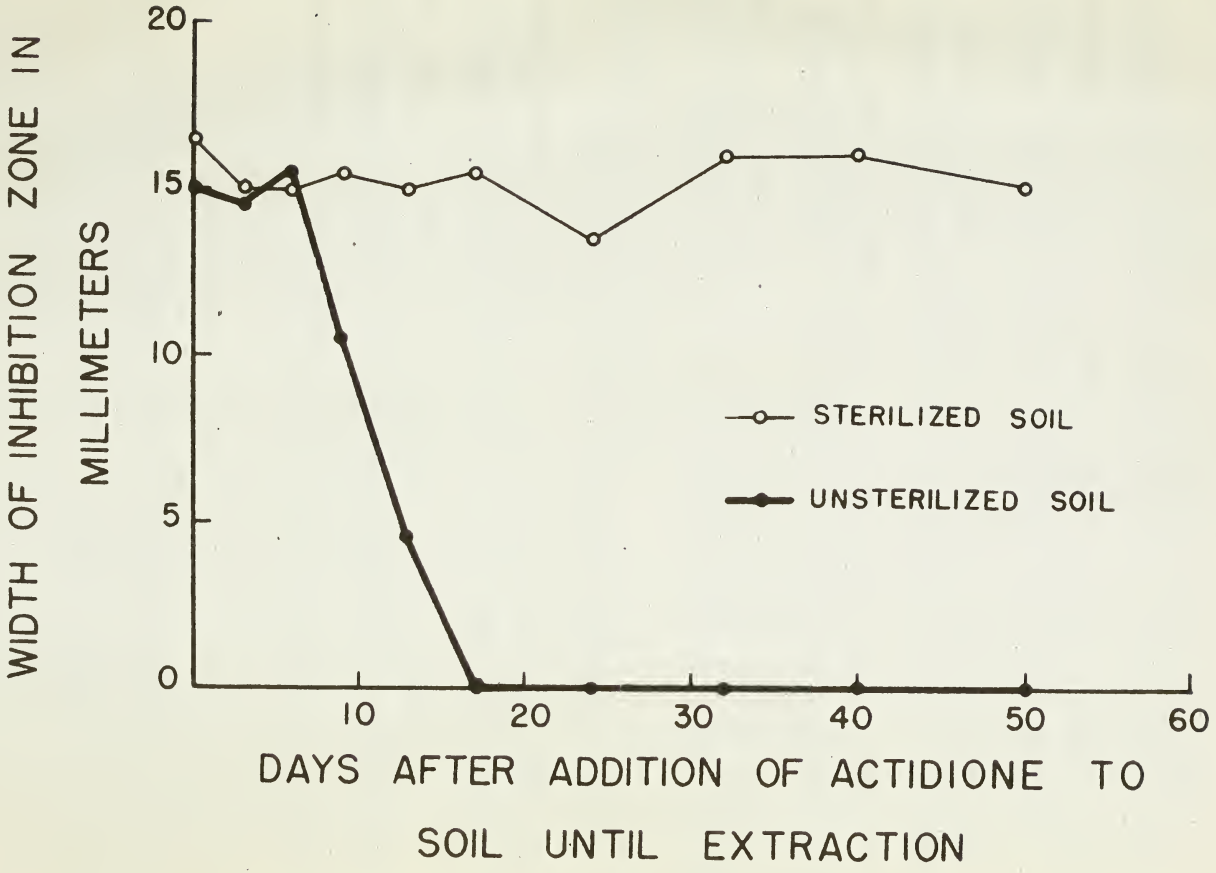

Figure 2. Persistence in sterilized and unsterilized soil of a 1000 ppm. solution of actidione as measured by its activity against P. lini (194-b)

Discussion

The results of the seedling and sensitive-microorganism techniques indicate that streptomycin was immediately inactivated when added to soil. This is in agreement with the work of Siminoff and Gottlieb (19). On the other hand, actidione appears to maintain a high proportion of its activity for at least 9 days in normal unsterilized soil and for a considerably longer period in sterilized soil. The apparent decline of antibiotic activity of actidione in unsterilized soil after about a week is believed to be the result of decomposition of this compound by the soil microflora.

The sensitive-microorganism technique for the determination of antibiotic persistence in soil was found to be more sensitive and easier to use than the seedling technique. It should be possible, by the sensitive-microorganism method, to determine quickly whether other water-soluble filterable antibiotics may persist in soil.

GENERAL DISCUSSION

In pure culture studies, a high proportion of aerobic spore-forming bacteria isolated from a number of different soils showed marked antagonism towards the root-disease fungi Ophiobolus graminis, Fusarium culmorum and Helminthosporium sativum. Yet, under the conditions of the greenhouse studies, the spore-formers used, including strains of Bacillus subtilis, failed to reduce the severity of attack by these fungi on wheat seedlings grown in sterilized soil. From these results, it would appear that spore-forming bacteria have little to do with the suppression of such pathogens in natural soils. This might well be the case in view of the results of Henry (11), who found that fungi were more effective in suppressing H. sativum than either bacteria or actinomycetes. On the other hand, Anwar (1) reported that certain B. subtilis isolates could reduce the severity of disease of barley caused by H. sativum in the greenhouse and in the field. It should, however, be noted in Anwar's experiments that inoculum of both the pathogenic fungus and the bacteria was increased on a wheat-oat medium. Fungus and bacterial inocula were then mixed and added to the soil. It seems possible that toxic

products resulting from bacterial growth in the wheat-oat mixture might have been responsible for the suppression of this fungus.

In the studies reported here, the individual spore-forming bacteria used were added to the soil at the time of seeding as a spore suspension. Hence, some time would be required before accumulation of toxic products would be sufficient to influence the pathogenic fungus. This might possibly account, at least in part, for the lack of agreement between these results and those reported by Anwar (1).

In an experiment in which a mixture of spore-forming bacteria were increased on and added to sterilized soil along with the pathogen O. graminis there was no apparent influence on the parasitic activity of the fungus towards wheat seedlings. This lends support to the results of tests with individual spore-forming bacteria which have indicated that these organisms, when present in soil which has not been enriched with organic matter, are not very active in the suppression of O. graminis.

The effects of several actinomycetes on the fungi O. graminis and H. sativum in sterilized soil were very similar to those reported for the spore-forming bacteria. A possible explanation of the results with the actinomycetes is that the pathogenic fungi, each being well

established in the soil-cornmeal medium and in the vicinity of the germinating seed, may have been able to gain entrance and cause infection in host tissue before the actinomycetes had developed sufficiently to influence fungal growth.

Of two methods used to determine the persistence of streptomycin and actidione in the soil, namely, the seedling technique and the sensitive-microorganism technique, the latter proved to be the most practical as a laboratory procedure. The sensitive-microorganism technique may also be applicable to other water soluble, filterable antibiotics.

If antibiotics are to find a place in the control of soil- and seed-borne plant pathogens, their ability to remain in an active state for some time may be an important character. Considering that seedlings are most susceptible to infection by certain microorganisms during the interval between germination and emergence, it seems likely that if adequate protection can be provided at this time, the chances for the production of healthy plants would be greatly increased. Since the antibiotic activity of actidione is maintained in the soil for several days whereas that of streptomycin is not, the former antibiotic may have a wider field of usefulness than the latter in the control of plant disease.

SUMMARY

1. Two spore-forming bacteria of known identity, namely, Bacillus subtilis (A32) and Bacillus polymyxa (A.T.C. 7070) were tested for antibiotic activity against Ophiobolus graminis, Fusarium culmorum and Helminthosporium sativum on potato dextrose agar and in sterilized soil.

2. B. subtilis (A32) was antagonistic to O. graminis, F. culmorum and H. sativum while B. polymyxa (A.T.C. 7070) failed to inhibit O. graminis and H. sativum and only slightly inhibited F. culmorum on potato dextrose agar.

3. B. subtilis (A32) suppressed O. graminis, but had no apparent effect on the growth of F. culmorum and H. sativum on a sterilized soil-cornmeal medium. B. polymyxa (A.T.C. 7070), on the other hand, had no effect on the growth of any of these fungi on this medium.

4. Out of 66 isolates of spore-forming bacteria obtained from different local soils, 51 were antagonistic to O. graminis, 40 were antagonistic to F. culmorum and 36 were antagonistic to H. sativum on potato dextrose agar.

5. A number of individual spore-forming bacterial isolates failed to reduce the severity of attack by O. graminis, F. culmorum and H. sativum on wheat seedlings grown in sterilized soil under the conditions of the greenhouse experiments.

6. A mixture of spore-forming bacteria isolated from a sample of Edmonton black soil, had little if any effect on the ability of O. graminis (S.P.-1) to attack wheat seedlings at the temperatures 15°, 20°, 25° and 30°C.

7. Infection of wheat seedlings by O. graminis (S.P.-1) occurred at 15°, 20°, 25° and 30°C., the most severe damage being caused at 15°, 20° and 25°C. However, plants grown at 30°C. showed severe lesioning of seminal roots and sub-coronal internodes but vigorous secondary roots that were almost free from infection developed at their crowns.

8. Out of 44 actinomycetes isolated from soil and tested on potato dextrose agar, 8 were antagonistic to O. graminis, 5 to F. culmorum and 13 to H. sativum. Three isolates were antagonistic to all three fungi.

9. The persistence in soil of the antibiotics streptomycin and actidione was measured by a seedling

method and by a sensitive-microorganism method. The latter was found to be the more practical as a laboratory procedure.

10. Streptomycin was immediately inactivated when added to soil.

11. Actidione retained a high proportion of its activity for 9 days in natural soil and for at least 50 days in sterilized soil.

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