STUDIES ON POTATO SCAB CAUSED BY ACTINOMYCES SCABIES

THESIS FOR THE DEGREE OF M. S.

C. W. Frutchey

1932

atoes - Diseases + pests

STUDIES ON POTATO SCAB CAUSED BY ACTINOMYCES SCABIES

Thesis Presented for Degree of

Master of Science

Michigan State College

By

C. W. Frutchey

1932

THESIS

ACKNOWLEDGEMENTS

The writer wishes to express his appreciations to Dr. J. H. Muncie for advice and suggestions given throughout this study and for criticism and correction of the manuscript. The writer is also grateful to Dr. Muncie for help in carrying on some of the experiments.

The writer is indebted to Dr. E. A. Bessey for helpful suggestions and for criticism and correction of the manuscript.

Acknowledgement is made to the following for cooperation with the field experiments: Frank Williams of Milford, Michigan, Walter Kraus of Monroe, J. C. Pedpath of Kalamazoo, and W. L. Tyler of Coldwater.

TABLE OF CONTENTS

I. Introduction

II. Literature Review

1. Tuber borne scab

2. Soil borne scab

3. Taxonomy of the scab group

III. Statement of the Problem

IV. Scope of Investigation and Presentation of Data

1. Soil treatments

(a) Field treatments

(b) Greenhouse treatments

2. Reaction of Actinomyces to the mercury compounds

3. Effect of chemicals on the soil microflora under different moisture conditions

4. Morphology and pathogenicity

(a) Pathogenicity tests

(b) Key to Actinomyces strains

V. Discussion of Results

VI. Conclusion

VII. Bibliography

INTRODUCTION

The disease "potato scab" is a constant menace in certain sections of the potato growing districts of Michigan. Prevention by an acid reaction in the soil or by seed treatment is not successful in most instances due to the fact that the rotation crops grown require a high lime content, and that the soil is heavily infested with the scab organism, a condition making seed treatment nearly useless. With this condition present, experiments for the control of potato scab by disinfecting the soil with chemicals were thought to be worthy of effort.

The nature of the work has been extensive rather than intensive, partially because a large number of factors are concerned in experiments dealing with soil disinfection, and partially because the results of the field experiments were the opposite of what might normally be expected. Various phases of the work were undertaken to help explain the manner in which the scab organism reacted to the chemical treatments, and it is from these phases that the greater part of the conclusions are drawn.

LITERATURE CITED

Tuber borne scab.

The disease of potatoes generally known as "common scab" which is caused by a species of Actinomyces, named by Thaxter (61) Oospora scabies and by Güssow (20) Actinomyces scabies, is probably as old as potato culture. Prior to the latter part of the ninteenth century considerable literature had accumulated pertaining to scab, and several theories were advanced as to its cause by both scientific and popular writers. These included: (1) mechanical irritation, (2) damage resulting from insect agencies, (3) chemical erosion or irritation, (4) excess of moisture, and (5) action of Fungi. W. G. Smith (58) of England was the chief supporter of the mechanical irritation theory and thought rubbish to be the cause.

The insect theory was largely supported by popular writers and the cause of scab generally attributed to the wire-worm. The chemical agency theory was advocated by Sorauer (59) of Germany; the substances considered important were lime and ashes both of which seemed to be proportional in amounts to the degree of scab. A much later contribution to this theory was made by Humphrey (23) whose results as well as those of other workers, although of value, failed to indicate the true cause of the disease. He was unable to associate any organism constantly or even frequently with the disease and listed environmental factors as the cause of the disease. Many of these factors since have been shown to affect the number of scab organisms present in the soil to the same extent that his results showed increase or decrease in the

percentage of scabby tubers.

The fourth theory, was that excess moisture caused an increase in sap with a rupturing of the lenticels and cork formation, and the resultant production of scab spots. Among the writers favoring this theory were, Frank, Giersberg, Schacht, Arther, Nobbe, and Beckwith, cited by Humphrey (23). The theory of causation by true fungi, had its origin in the announcement by Wallroth (69) that the disease was due to the action of the fungus Erysibe subterranea Wallr. This statement was reaffirmed by Julius Kuhn (33), and assented to by Jubainville and Vesque (30), in 1878. Sorauer (59) and many other writers denied the causal nature of this or any other similar fungus and stated that if present, it was only an accompaniment of the real cause. Brunchorst (4) ascribed the cause of scab to the direct action of a myxomycetous fungus named by him Spongospora solani. The scab disease of Wallroth and Brunchorst is powdery scab, and the organisms named by them are synonymous. Spongospora subterranea (Wallr.) John is the final name given to powdery scab, and therefore the citations above do not pertain to the common scab of potato.

It was not until about 1890 that much of scientific value in regard to potato scab appeared in the literature. Most of the available accounts deal with the control of the disease because of its constant menace in potato regions where it often caused rather heavy losses in decreasing the market value of infected tubers.

Thaxter (61) was the first to show that the scabby, corky lesions on the tubers were due to an invading organism and was the first to isolate this organism from scab lesions on tubers, grow it in pure

culture, and then inoculate uninfected tubers and reproduce the disease. He recognized that the organism was tuber borne and contained within the scab lesions after digging and that the use of disease-free seed for planting was advisable. Other early workers who investigated scab were, Bolley (1 and 2), Humphrey (23), Kinney (31), Galloway (16), Jones and Edson (29), and others. Of these workers, Bolley (1 and 2) carrying on experiments in Indiana and North Dakota probably did more extensive and intensive work than any other investigator before 1900. Although he did not classify the organism as did Thaxter (61) he did discover that there was a definite plant parasite responsible for the disease and that this organism was tuber and soil borne. He believed the disease to be due to a bacterium and reproduced scab with an organism that he isolated from scabby potatoes. He also made some progress in determining the environmental factors necessary for the development of scab and discovered that the disease could be somewhat prevented by soaking the seed tubers in a 1-1000 solution of corrosive sublimate for one and one-half hours. Jones and Edson (29), and Kinney (31), conducted experiments for the prevention of scab and obtained some information on the value of seed treatment before planting, and of soil treatment with sulfur.

Several writers have endeavored to separate the different types of scab by the effect upon the host. Types of scab were recognized before it was known that the disease was caused by an organism. Humphrey (24) separated the disease into deep and superficial scab. Both Thaxter (61) and Bolley (1) recognized the two types of symptoms but thought them to be variations of the same disease. Frank and Kruger (15) claimed to be able to distinguish morphological differences in the

different types, namely, (1) shallow scab, (2) deep scab, (3) bulging scab, and (4) bulging deep scab. However, Lutman (38) believed these different types of lesions to be stages in the advance of the same disease. He also supports Thaxter's view point as to the type of organism, stating that "the distribution of the hyphae is very irregular, occurring on any part of the potato, but always in the outer layer of the periderm", and he makes the statement that "scab is due to the hypertrophy of the cells of the cork cambium, the cells of which are much thickened due to their suberization". According to Lutman and Cunningham (40) "the ordinary scab of potato is due to an increase in growth in the cork layer, the production of which is due to a stimulation on its surface and in its outer cell layers by the growth of an organism which forms chemical substances which are absorbed and which cause the cork cells to increase both in size and number". Willard (47), pointed out the fact that several types of scab existed but believed them to be caused by variations of the scabies group. Later in another report (46) he listed six different types of scab, and tested 24 isolations of Actinomyces for pathogenicity, 11 of which proved to be pathogenic. These he classed as distinct species. Jones (26) worked with different types of scab studying the structure of the potato cells affected. His conclusions were that the type of scab lesions produced depends upon the activity of the organism and that deep scab resulted from the organism penetrating the cells so rapidly that a corky layer could not be built up at the surface to prevent penetration.

Soil borne scab.

Bolley (2) was among the first investigators to recognize that the organism causing the disease "potato scab" was soil borne. He determined that the organism was capable of living at least four years in the soil and then causing scab of potatoes. Jones and Edson (28) showed that the organism could remain in the soil 25 or more years and then cause a heavy scabbing of tubers. They also unintentionally showed that the organism is present in new soil. Lutman (37) proved that potatoes grown in virgin soil were often scabby and that the longer potatoes were grown in a field the more scab would be present, disregarding the fact that all of the seed used was disinfected with formaldehyde. Lutman and Cunningham (40) obtained results to show that the potato scab organism, named by Gasperini, Actinomyces chromogenus and which they identified as a scab producing organism, is wide spread and is found in practically all soils, so far as known, but is most numerous in those soils which are rich in humus. Literature on soil borne scab takes up a wide field of investigation. Due to the research of soil bacteriologists it has been determined that the Actinomyces group make up a comparatively large percentage of the soil microflora. Waksman (63) found that on an average 17% of soil borne organisms were Actinomyces. Heavy soils and those rich in undecomposed organic substances are relatively richer in Actinomycetes than corresponding lighter soils. In an earlier account (62) he found and named thirty species of Actinomyces, but later grouped some of these together into one species. He discovered that the Actinomyces group were not extensive ammonifiers although they reduced nitrites to nitrates, and that they were strong cellulose decomposers forming humus from organic matter. According to

Waksman (62) Globig, Rossi-Dori, Rullman, and Madsen isolated and studied Actinomyces from different sources. Beijerinck (22) stressed the importance of organic matter for Actinomyces growth. Fousek (14) found more Actinomycetes in heavy than in light soils. Krainsky (32) found 30% of the soil microflora to be Actinomycetes. Conn(8), (7), and (6) observed a greater number of Actinomycetes, 39.4%, in old sod soil, and concluded that they play a role in the decomposition of grass roots. Millard (47) states that "scab occurs most commonly and with greater virulence on light sandy or gravelly soils especially those of a hungry nature, and it can be inhibited by green-manuring of the soil.

The fact that the scab organism is soil borne is closely associated with several factors affecting its development. Martin (41 and 42) observed that the pH reaction is far more concerned than is the soil moisture in affecting the amount of scab. Soils varying in moisture content, but with a similar reaction gave equal amounts of scab. When sulfur was added and a pH of 5.1 produced, scab was greatly reduced. Variation in the amount of rainfall caused no difference in the percentage of scab until sulfur was added. Addition of sulfur resulted in a decrease of scab in proportion to the lowering of the pH value. The effectiveness of a given quantity of sulfur depends upon its ability to produce an acid reaction when added to the soil. In another article (43) he states that part of the inhibitory effect of sulfur is probably due to its toxicity and not altogether to the pH reaction. Gillespie (18 and 19) using two synthetic and one potato extract media, concluded that the potato scab organism would not grow

in a pH lower than 4.7. The strains varied somewhat in their reaction to a given pH, but the variation was not appreciable, and none of them gave good growth at a pH lower than 5.8. He did not find scabby potatoes on soils with a pH value of less than 5.1. This was true of all soils, sand, muck, or peat. Wheeler and Adams (71) working over a period of three years showed that all forms of lime tend to promote scab, and flowers of sulfur tend to inhibit it, but may injure the potatoes if applied in too large amounts. Sulfate of ammonia proved even better than sulfur. Wheeler, Hartwell, and Moore (72) found the effect of sulfur for the prevention of potato scab to depend on the acidity derived from its application. Halsted (21) obtained good control of scab by the application of sulfur. Lime gave 100% scab. Corrosive sublimate, kainit, and copper sulfate when applied to the soil, decreased the crop yield as well as the amount of scab. Sherbakoff (57) found that injury on various crops including potatoes resulted if sulfur were added at the rate of more than 900 pounds per acre. When added with commercial fertilizer, it was less injurious. The injury was more marked in light soils which were low in humus. In another article (56) he reports that scab is greatly reduced with heavy applications of sulfur, and that the number of scab lesions was more reduced than the number of scabby tubers although the latter were also greatly reduced by the treatment. Garman (17) states that "the use of flour of sulfur applied to the soil gave little or no results and in some cases gave more scab than the untreated". Lint (43) observed that sulfur reduced the amount of scab appreciably, and the smaller applications of 300 pounds seemed as effective as the larger applications of 600 pounds.

Wedgworth (70) reported that the additions of sulfur usually increased the percentage of clean tubers, and that 600 pounds of sulfur per acre showed the greatest amount of clean tubers.

Other factors which have been thought to regulate if not control scab are, moisture, temperature, and crop rotation. Shapovalov (55) concluded that there is always enough cellulose in the soil to perpetuate the organism from year to year. Crop rotation would not eradicate the scab fungus from the soil. Sanford (52) believes moisture the main factor controlling scab in the soil which he used, a dark organic sandy loam. Scab developed abundantly in dry soil while moist soil produced almost all clean tubers. The H. ion concentration varied but little. Shapovalov (54) conducted temperature germination tests with the scab organism and found that temperature from 35° to 40° C. were the most favorable for the germination of the conidia, though unfavorable for long continued growth of the mycelium. The maximum temperature for growth is about 40.5° C., the optimum 25° to 30° C., and the minimum about 5° C. Involution forms are not the result of temperature conditions. Jones, McKinney, and Fellows (27) conducted experiments in the "Wisconsin tanks", the temperature ranging from 11° to 30.5° C. They determined that the development of scab is influenced by soil temperature, the optimum being about 22° C. This is in accord with field experiments of various investigators showing that there is a greater prevalence of scab in hot summers than in cooler ones. Jones and McKinney (28) stressed the point that potato scab is not so prevalent in Northern Europe as it is in North America where the summer temperatures are much higher.

Judging from the data given by these investigators, it would

seem logical to believe that scab development is inter-dependent on a combination of factors, one of which may be dominant for a given condition.

Taxonomy of the scab group.

A great deal of study has been made of the group of organisms causing "potato scab", but as yet it is not definitely known whether or not those organisms causing the disease can be placed in one distinct species. Thaxter (61) was the first to associate and prove that an organism was directly the cause of the disease and after a great deal of study he named it Oospora scabies. His description is very specific and while it is probable that several types of scab organisms will fit in generally with the description, it is also true that an organism would have to be grown under the same conditions as was Thaxter's original organism before it could be proved to be identical. His reasons for placing the organism in the genus Oospora were that it appears more nearly allied to certain forms included in this genus by Saccardo. However, he adds that "it is needless to remark that the genus Oospora, as given by Saccardo has no scientific value, and the reference of a form to this genus is merely, as in the present case, a confession of ignorance concerning its true position". Bolley (1) believed the organism to be a bacterium. However, he did not name the organism and later in his report (2) he states that it agrees with the description given by Thaxter. Cunningham (11) isolated three forms of an organism from potato scab lesions and reproduced the disease upon inoculation. He believed then to be identical with Thaxter's, but disagrees with the generic

name because the two characters, true branching and aerial fruiting, separated this organism from the bacteria, and are recognized as important characters of the family Mycobacteriaceae and of the genus Streptothrix. Upon this basis Cunningham thought the organism should be placed in the genus Streptothrix. Gussow (20) disagrees with Cunningham in the use of the name Streptothrix because it was used by Corda in 1839 for another genus and according to the Vienna code cannot be applied to a second one. He states that other names such as Cladothrix, Nocardia, and Actinomyces have been loosely used for the organism causing potato scab. Cladothrix cannot be used because of its false branching and ciliated spores. Nocardia is also untenable because the name was applied to other organisms by Saccardo, and Gussow therefore changed the name to Actinomyces scabies (Thaxter) Gussow. Lutman and Cunningham (40) identified the scab organism as Actinomyces chromogenus Gasperini. McKinney (45) states that he was also able to isolate forms of Actinomyces identical with Actinomyces chromogenus in pigment formation but adds that various workers have demonstrated that Actinomyces chromogenus is a group of various species differing in physiology and morphology. He maintains with Drechsler that the binomial Actinomyces scabies (Thaxter) Gussow should be accepted as the name of the organism which causes common scab of the potato tuber.

Drechsler (12) criticizes other workers on the genus Actinomyces because they have been too quick to form conclusions on the taxonomy of the group from details only slightly studied. His study includes 18 groups or types of Actinomyces which he carefully describes

morphologically. He believes that Actinomyces does not represent a transition between the Hyphomycetes and the Schizomycetes. He determined rather definitely that Actinomyces is a fungus belonging to the Fungi Imperfecti. Conn (9) working with various culture media and 75 culture found it very difficult to distinguish between the various type of Actinomyces unless extreme care had been taken in preparation of a protein-free media and unless the organism was repeatedly grown in the same medium.

Lehmann and Newmann cited in Waksman (62) consider the Actinomyces a special group which stand between the Hyphomycetes and the Schizomycetes; related to the latter by their slender hyphae and protoplasmic properties, and to the former by the branching formation of aerial hyphae with conidia like structures. Claypole (10) believes that the higher sporing fungi, the true bacteria, and the acid-fast bacteria have all arisen from the Streptothrix group which is highly variable both morphologically and physiologically. Within the group Actinomyces, Waksman (62), (63), (64), (65), (66), (67) and (68) has probably contributed more than any other worker to the knowledge of species and forms. He classifies the organisms according to morphology and physiology depending in many cases on chromatic characteristics. Millard and Burr (46) studied the pathogenicity on potatoes of 24 strains of Actinomyces, naming 11 pathogenic species.

There has been a great deal of work within the group Actinomyces and as has been stated by many investigators, the variation is so great that separation into distinct species is extremely difficult. Perhaps there has been too much work separating the variations into

distinct species. This criticism has been offered by some writers. According to Buchanan (5) the following classification is the one to which the potato scab organism belongs: genus, Actinomyces; family, Actinomycetaceae; order, Actinomycetales; and class, Schizomycetes. Hyphomycetes is sometimes given as the class depending upon the investigator.

STATEMENT OF THE PROBLEM

The control of scab is a well known problem confronting the potato growers in Michigan, and especially so in the southern part of the state. In times past when the growers knew very little about potato scab, little or no care was taken to prevent the spread of the organism, and no thought was given toward keeping the original infestation down to a minimum. Due to the lack of this knowledge and to the methods of cultivation and fertilizing practiced, the amount of scab producing organism in the soil has greatly multiplied. It is of common practice in many of the potato districts to grow cover crops, mainly clover and alfalfa, for a period of from two to four years, then plow them under and again plant the field to potatoes. To make the conditions more favorable for these crops, lime is used to produce a neutral or slightly alkaline soil reaction. These conditions are also favorable for the growth of the potato scab organism, Actinomyces scabies. Millard (47) stated that by the addition of green manure, scab could be controlled by keeping it as a saprophyte rather than a parasite, a condition brought about by the scarcity of food material. However, it has been shown by, Lutman (37), Waksman (62), Conn (8) and others that an abundance of organic matter, other factors being optimum, produced an abundance of scabby potatoes.

The above condition has occurred in certain districts in the southern part of the lower peninsula of Michigan, and the Actinomyces content of the soil has been greatly increased by liming, and turning

under green manure crops. Another source of contamination which is often of importance and which it is well to mention, is the feeding of uncooked potatoes to farm animals, and the application of the manure to the field as fertilizer. Horse (48) has shown that the scab organism is capable of passing through the digestive tract of animals in the manure, which when applied to the soil will increase the number of scabby tubers. These sources of contamination have greatly increased the scab menace in certain districts, producing a problem of great importance to the growers.

The work taken up here has special reference to the control of scab by treating the soil with chemicals. Considerable work has been done on the control of scab by seed treatment, changing the soil reaction by the use of sulfur and other chemicals, and by the study of environmental factors such as, moisture and temperature. However, little work has been done to control scab in the soil by chemical treatment.

Although sulfur treatment of the soil, according to Waksman (67) and Wheeler, Hartwell and Moore (72) acts primarily as an inhibitor of the organism due to the acid reaction produced in the soil; it has also been shown by other writers, Cherbakoff (56) in particular, that the continuous addition of large quantities of sulfur to the soil for scab control proved markedly injurious to other crops in rotation as well as to potatoes.

In certain regions where potatoes are grown the soil if not originally infested with the scab organism, has with the continued planting of the crop, become so heavily infested that whenever

conditions such as moisture, temperature, soil reaction, and organic matter are favorable, a heavily scabbed crop will be produced. Tuber treatment before planting while effective for the seed piece does not reduce the amount of scab in the soil that is badly infested. Due to the fact that scab causes considerable damage and economic loss in potato growing regions, that the organism is soil borne, living in the soil as a saprophyte year after year, and that it is most virulent in neutral or slightly alkaline soil, a practical control to be applied in the field would be of commercial value.

Attempts were therefore made to find a practical means of inhibiting the scab organism by the application of chemicals in small quantities to the soil. To be of practical value, the chemical used should have the following properties: namely, that it will not injure the productive value of the soil, that it will not injure the growth of crops, that it is rather inexpensive and that it can be applied with a fertilizer or as a fertilizer, and that it is effective in reducing the number of scab producing organisms in the soil. If all these properties could be found in one chemical, the losses caused by soil borne scab could be reduced to a minimum by a simple routine of soil treatment.

SCOPE OF INVESTIGATION AND PRESENTATION OF DATA

It has already been stated that the main object of this work was to determine if it were possible to disinfect the soil with chemicals in so far as the scab organism was concerned and thus control the disease "potato scab". The results obtained from this phase of the work, however, have made it necessary to study the problem from different angles, namely, to isolate many strains of Actinomyces scabies, to test their pathogenicity; to determine the effect of the disinfecting chemicals employed upon these strains; to study the relation of soil moisture content to the effect of the chemicals on the soil microflora, and to a limited extent study the mycological phase or taxonomy of the strains.

Previous work on soil treatment other than sulfur for the prevention of scab is very limited. Halsted (21) treating the soil with corrosive sublimate, kainit, and copper sulfate, found that these chemicals added in sufficient quantity to reduce the amount of scab, also decreased the crop yield. In recent years more attention has given to treatment of soil by chemicals for the control of infectious soil parasites, and at the present time work is being carried on for the control of scab by soil treatment. Martin (44) using calomel, yellow oxide of mercury, formaldehyde and various organic mercury compounds, found them to be highly effective in New Jersey soils for controlling scab and Rhizoctonia. Work of a similar nature is also in progress at the Nebraska and Wisconsin Experiment Stations.

Soil treatments

In the experiments carried on both in the greenhouse and in the field, four chemicals were used, namely, calomel, yellow oxide of mercury, DuBay 7965H, an organic mercury compound, and aluminum sulfate. The chemicals were applied at the rate of 20, 15, 10 and 20 pounds per acre respectively in the rows at the time of planting. The field experiments were made in six plots in five different sections of the state; two plots were at the college. They were all treated in a like manner and the amount of scabby and clean tubers either counted or weighed when they were dug. The percentage of scabby tubers was listed in each case. The aluminum sulfate treatment was included only at the college.

Before being applied to the rows the chemicals were thoroughly mixed with fine quartz sand to afford easier application and more uniform distribution. Taking a given number of pounds per acre of a chemical, the number of grams of chemical to be added in a row 100 feet long was calculated. Allowing 300 square feet for each row of that length, there would be 145.2 rows 100 feet long per acre. The amount of chemical needed for each row would be the number of pounds of chemical per acre divided by 145.2. Accordingly calomel was applied at the rate of 61.8 grams per row 100 feet long, yellow oxide of mercury at the rate of 30.9 grams, DuBay 7965H at the rate of 30.9 grams, and aluminum sulfate at the rate of 61.8 grams per row. The results of soil treatments for the control of potato scab are presented in Tables I - VI inclusive.

TABLE I. Results of Soil Treatments for Control of Potato Scab Plots I and II, Field 19 at the College

PLOT I

Treatments

	Calomel	Yellow oxide	965W	$Al_2(SO_4)_3$	Check
No. of Rows	4	4	4	4	9
Clean					
Number	144	309	698	858	1827
Per cent	7.19	21.68	70.61	67.74	77.35
Scabby					
Light					
Number	144	185	198	282	358
Per cent	9.68	13.04	17.68	17.05	14.20
Medium					
Number	176	291	114	153	188
Per cent	13.07	20.36	9.62	11.75	7.38
Heavy					
Number	1004	644	37	71	50
Per cent	68.38	45.13	4.71	5.45	2.17
Total number of tubers	1478	1429	1384	1363	2581
Total per cent scab	92.81	78.78	29.19	34.26	21.75

PLOT II

No. of Rows	4	4	4	4	9
Clean					
Number	220	466	909	905	2339
Per cent	10.07	31.31	54.39	65.87	69.74
Scabby					
Light					
Number	253	244	365	315	769
Per cent	13.27	14.43	22.08	22.92	20.85
Medium					
Number	185	270	210	80	362
Per cent	8.97	13.37	12.7	5.82	10.03
Heavy					
Number	1404	791	169	74	378
Per cent	68.09	48.78	10.22	5.38	9.98
Total number of tubers	3062	1791	1653	1374	3798
Total per cent scab	89.33	77.39	45.01	34.13	40.76

Plots I and II were located in field W19 at the college. They were planted to Russet Rural potatoes May 25, 1931 and harvested October 29, 1931. They were planted by hand dropping in rows about 4 or 5 inches deep after the chemicals were added. The soil in Plot I was a heavy sandy loam and that of Plot II was more sandy and lighter. Checks were planted on the outer rows of each plot and in between the chemical rows, there being four rows of each chemical in these plots. It can be seen from the results in Table I that calomel and yellow oxide were the least effective treatments and that all of the treatments seemed to increase the amount of scab as well as the degree of scabbiness of the tubers. The two inorganic mercuries favored the production of scab in both plots, and the difference between them and the check rows as shown by the data was very evident at the time of digging. The tubers were all sorted into clean, and light, medium, and heavy scab, each group of which was then counted, and the tubers in each division recorded in Table I.

TABLE II. Results of Soil Treatments for Control of Scab in Potatoes on the Monroe County Farm

PLOT III

Treatments

	Colonel	Yellow Cobbler	903H	Check
No. of Rows	2	2	2	4
Clean				
Weight	80.25	117.75	137.0	287.0
Per cent	45.79	60.0	78.31	80.79
Scabby				
Weight	95.0	78.5	40.0	68.50
Per cent	54.21	40.0	21.69	19.21
Total weight	175.25	196.25	185.0	355.5
Total per cent scab	54.21	40.0	21.69	19.21

This plot was located in Monroe county on the Monroe County farm near Monroe, Michigan. This plot was planted to Burpee Rural potatoes June 6, 1931 and harvested October 12, 1931. The soil was of a heavy black type. It consisted of 10 rows each 100 feet long, two rows each of the three chemicals and four rows of check which were planted in groups of two rows so as to fit suits with the treated rows and so that no two treatments were adjacent to each other. The potatoes were dug, sorted into clean and scabby tubers and the results taken in which weights with the percentages were recorded. There are not the striking results in this plot that there were in the first two. The check rows still gave the greatest amount of clean tubers and Colonel gave the smallest yield of clean tubers and the greatest yield of scabby ones. As was true previously the chemically treated rows yielded fewer clean potatoes than did the untreated rows, although the rows treated with

905H varied but very little from the check rows in the amount of scabby tubers.

TABLE III. Results of Soil Treatment for Control of Potato Scab on Pedpath Farm, Kalamazoo County

PLOT IV

Treatments

	Calomel	Yellow oxide	905H	Check
No. of Rows	3	3	3	18
Clean				
Weight	19.5	32.0	75.5	591.5
Per cent	10.57	20.25	51.33	68.83
Scabby				
Light				
Weight	54.0	50.0	58.0	204.25
Per cent	29.27	31.65	39.59	24.57
Heavy				
Weight	111.0	76.0	13.0	34.5
Per cent	60.16	48.1	8.87	4.15
Total weight of tubers	184.5	158.0	146.5	830.25
Total per cent scab	89.43	79.75	48.47	31.17

This plot was located in Kalamazoo county on J. C. Pedpath's farm near Kalamazoo, Michigan. The plot was planted June 12, 1931 to Russet Rural potatoes, and they were harvested October 19, 1931. A sandy loam type of soil which had previously grown potatoes and which had been heavily limed for a cover crop was used. There were nine rows 300 feet long, the three center rows were divided transversely into nine rows 100 feet long, three laterally adjacent rows being treated

with one chemical. In this manner the treated rows were end to end there being three groups of three rows each with each group treated with a different chemical, all three treatments making three rows 300 feet long. Check rows were placed three rows on each side of the treated rows. The potatoes were sorted into clean, light scab, and heavy scab, and the results were taken in weight of each division of potatoes.

TABLE IV. Results of Soil Treatments for Control of Potato Scab on Tyler farm, Branch County

PLOT V

Treatments

	Calomel	Yellow oxide	965H	Check
No. of Rows	3	3	4	10
Clean				
Weight	71.75	53.75	133.75	338.75
Per cent	37.13	28.33	52.29	52.21
Scabby				
Light				
Weight	73.75	82.25	106.5	264.55
Per cent	38.16	43.33	40.47	40.77
Heavy				
Weight	47.75	53.75	18.5	45.55
Per cent	24.71	28.33	7.23	7.02
Total weight of tubers	193.25	189.75	255.75	648.85
Total per cent scab	62.87	71.67	47.71	47.79

This plot was located in Branch county on W. L. Tyler's farm near Coldwater, Michigan. The plot was planted to Russet Rural potatoes May 25, 1931 and harvested October 28, 1931. The soil was of a sandy loam type to which a fertilizer had been added. The plot consisted of

ten rows 200 feet long, five checks and five treated. The calomel and yellow oxide of mercury consisted of three rows each and each row 100 feet long. These two treatments were placed end to end making three rows 200 feet long. The checks were each 200 feet long as were the DuBay 965H treatment. Calomel and yellow oxide gave the least control in this plot although the percentages of scab were all quite high. The per cent of heavy scab showed a greater difference and was comparatively low for the 965H treatment and the check.

TABLE V. Results of Soil Treatments for Control of Potato Scab on Williams' farm, Oakland County

PLOT VI
Treatments

	Calomel	Yellow oxide	965H	Check
No. of Rows	3	3	3	12
Clean				
Weight	29.9	29.5	36.25	55.25
Per cent	28.43	26.16	33.72	14.23
Scabby				
Light				
Weight	34.0	40.5	36.75	122.5
Per cent	32.33	35.92	34.18	31.55
Heavy				
Weight	41.25	42.75	34.5	210.5
Per cent	39.23	37.91	32.09	53.9
Total weight of tubers	105.15	112.75	107.5	388.25
Total per cent scab	71.57	73.84	66.28	85.77

This plot was located in Oakland county on Frank Williams' farm near Milford, Michigan. The plot was planted to Russet Rurals June 5, 1931 and harvested October 14, 1931. The soil was of a sandy

loam type and a fertilizer had been added before planting. There were 21 rows 100 feet long, 12 rows of checks and three rows of each of the three treatments. It will be noticed that the results in this plot do not conform to those of any of the other plots. The chemical treatment here all gave more control than did the check rows. However, the percentages of scabby tubers are all so high that few if any conclusions can be drawn as to the effect of the chemical on the scab organism when considering this one individual plot. It is true that this section of the state received more rain than did any of those where the other plots were located, but comparing with the controlled moisture experiments this would not explain the results.

Summary of results of soil treatments

In considering the results of the field plots, it can be readily seen that the chemicals were not effective in the control of potato scab in any of the plots. In only one case, plot #6, was there a greater percentage of clean tubers in the treated rows than in the checks. The chemicals did not all produce identical results but seemed to produce proportionally the same results in all plots. DuBay #965H and aluminum sulfate did not seem to vary from the check rows a great deal. The aluminum sulfate on the two plots at the college gave slightly more scab on the average than did the untreated rows. DuBay #965H produced nearly the same results as did the aluminum sulfate, having slightly more scab on the average than did the checks. Calomel and yellow oxide of mercury in all but one plot gave considerably more scab than the untreated rows and the average percentage of

scab is much higher than for the checks.

TABLE VI. Percentages of the averages of scabby tubers accompanying each chemical treatment

Treatment	Average Total % Scab	Average % Heavy Scab
Calomel	73.27	32.51
Yellow oxide	69.34	41.35
DuPay #965H	40.23	13.84
Aluminum sulfate	34.19	5.42
Untreated rows	41.49	17.33

There was a wide variation in the different chemicals in the percentage of scab produced on a single plot and in a single chemical on several plots, but the proportions of percentage of scab for each chemical did not vary extremely. For instance if Plot #6 were eliminated the effectiveness of the treatments could be listed as follows: Calomel and yellow oxide treatments occupied the highest percentages of scab with calomel showing the highest in four plots and having an average of 6.09% greater than the average for yellow oxide in the five plots. Yellow oxide ranked highest in percentage of scabby tubers, with the DuPay #965H treatment considerably lower and only slightly higher than the check rows. In Plot #6 the data show the percentage of scab to be slightly less for the treated rows than for the checks, a point which would have been of much interest had not all the percentages of scab in this plot been very high, the lowest being 63.20% and the highest being 85.77%, a difference too small in such high percentages for

significance.

Greenhouse soil treatment

A section of greenhouse 17 feet by 21 feet was available for growing potatoes in a bed where pots were not desired. The soil was treated in exactly the same manner as in the field, except that the amount of the chemicals to be added was calculated for a row 17 feet long instead of 100 feet. Fifteen rows could be planted quite easily and this allowed three rows of each treatment, the fourth chemical, aluminum sulfate, being added to the list of treatments. There were three rows of checks. In the beginning this plot was limed heavily to produce a neutral if not alkaline reaction and the soil was heavily inoculated both with bulk cultures of Actinomyces, presumably scab producing types, and then with soil taken from a field where potato scab was found in great abundance during the summer of 1931. Three crops were grown, the first planted February 28, 1931 and harvested July 14, 1931; the second was planted October 15, 1931 and harvested January 20, 1932; the third was planted January 23, 1932 and harvested April 30, 1932. The first two crops were planted to the variety Russet Rural and the third was planted to Irish Cobbler. The soil moisture was held at what was thought to be optimum for growing conditions, about 20%, and the temperature of the air varied from 18° - 25° C., the average being about 23° C., while that of the soil was 3° or 4° C. less. After the first crop was dug the soil was changed, but when the second crop was grown there was not an opportunity to change the soil. The rows of potatoes were therefore planted alternately for the third crop in an effort to eliminate as much as possible

the effects of the first application of chemicals. Chemicals were applied for each crop of potatoes planted. The soil was reinoculated and relimed for the second crop but not for the third crop as the pH ranged from 7.0 to 7.2 and the previous crop of potatoes was quite heavily scabbed. At the time of harvesting the scabby as well as the clean tubers were counted, and the percentages calculated and recorded.

Summary of greenhouse soil treatment results

It can be seen from Table VII that the results obtained in the greenhouse are not the same as for the field and that the chemicals in this case did not alter the amount of scabby tubers one way or the other. The results obtained from the first planting of potatoes were not recorded because practically no scab developed and there was a possibility that the soil was not properly infested with the scab organism. The first and second crops of tubers were of the variety Russet Rural and the third crop was planted to Irish Cobbler a fact that probably explains why the third crop was so much more scabby than was the second crop. On the average the second crop showed slightly more scabbing in those rows that were treated with the mercury compounds than did the check rows. From a positive viewpoint the results obtained supplement those from the field plots and indicate that none of the chemicals were effective in reducing soil borne scab under the conditions of the experiment. The moisture in this case was controlled and held at an optimum growing condition, a fact that will disprove any idea which might arise, that the chemicals were ineffective in the field due to low moisture content of the soil.

TABLE VII. Results of soil treatments for the control of potato scab in the greenhouse

Second Planting*

Treatment

	Calomel	Yellow oxide	965M	$Al_2(SO_4)_3$	Check
No. of Rows	3	3	3	3	3
Clean					
Number	75	79	68	85	100
Per cent	43.6	59.4	53.54	63.91	60.61
Scabby					
Number	97	54	59	48	65
Per cent	56.40	40.6	46.46	36.09	39.39
Total number of tubers	172	133	127	133	165
Total per cent scab	56.4	40.6	46.46	36.09	39.39

Third Planting

No. of Rows	3	3	3	3	3
Clean					
Number	6	1	1	0	9
Per cent	0.032	0.005	0.006	0.0	0.409
Scabby					
Number	180	193	156	184	211
Per cent	99.968	99.995	99.994	100.0	99.591
Total number of tubers	186	194	157	184	220
Total per cent scab	99.968	99.995	99.994	100	99.591

*The results of the first planting are not recorded. (See page 28)

Reaction of Actinomyces to the mercury compounds

The results of the soil treatment experiments indicated that an increase in scab accompanied the use of inorganic mercury compounds, and that further study was needed before conclusions could be drawn. In view of this fact experiments were conducted dealing with the toxicity of the two inorganic mercurials used in the field upon the various strains of Actinomyces that had been isolated. Tyrosinate liquid medium was used for this work and at the beginning it was planned to run different concentrations of the mercury chemicals in the medium to determine in what strength of solution the strains of Actinomyces would produce growth. The formula for tyrosinate medium is as follows: Tyrosin 1 gram; glucose 10 grams; dipotassium acid phosphate (K_2HPO_4); 0.5 grams; ammonium sulfate ($(NH_4)_2SO_4$) 0.5 grams; agar 15 grams; and 1000 cc. distilled water, made neutral with NaOH. Due to the first results of this experiment and a shortage of flasks for carrying cultures in a limited length of time the dilutions were limited to three for each of the two chemicals, calomel and mercuric oxide. The dilutions were as follows: (1) concentrated solution with an excess of the undissolved chemical, (2) concentrated solution with the excess mercury filtered off, and (3) a solution with one-tenth the strength of the concentrated solution.

The same formula for tyrosine liquid medium was used. The full strength concentrations were made by first making a saturated solution of the chemicals in cold water and then adding the nutrient salts of medium; the dilution of one-tenth full strength concentration was made by adding nine parts of water to one part of the saturated solution. The

saturated with an excess of mercury was made by adding 0.5 grams of the compound to each flask of liquid medium. Checks of liquid medium without chemicals other than the base nutrient salts were kept as controls throughout these tests.

Erlenmeyer flasks with a capacity of 250 cc. were used and 100 cc. of liquid medium placed in each. These were stoppered with cotton, sterilized, and then inoculated in a steamed transfer room with the organisms that had been isolated and grown in test tubes. The inoculated flasks were allowed to incubate two weeks in most cases when comparisons were made in regard to the amount of growth.

When the first phase of the experiment had been completed, it was learned that all of the strains available were capable of producing growth in the concentrated solutions of both the calomel and the yellow oxide of mercury. In these cases the excess mercury compound had been filtered off the saturated solutions. A good growth was made in nearly all flasks and the first thought was that these chemicals were toxic to but very few of the organisms. However, on closer observations it could be seen that there was at first a larger amount of growth in the check flasks than in those with the saturated solutions, but that in time no difference could be observed. Accordingly the concentrated solutions with an excess of mercury were added to the experiment. When time had elapsed for the e flasks to be examined, it was noticed that a great many of the flasks had no growth at all, and that only on occasional organism produced a marked degree of growth. In those flasks containing only one-tenth of the concentrated solution growth was abundant, exceeding that in the check

flasks in most cases. Particular attention was given to this condition, the occurrence of which seemed to be consistantly the same. A marked increased growth would occur for a short time when the organisms were subjected to the weaker mercurial solutions but later on little or no difference could be observed.

The fact noticed in the above cases will be discussed in detail later in the chapter on discussion of results. It should be mentioned here that the two mercurials, calomel and yellow oxide of mercury are both highly insoluble. The solubility of calomel is 0.00031, and yellow oxide of mercury 0.00515 in 100 parts of water at 25° C.

TABLE VIII. Growth of strains of Actinomyces in tyrosinate liquid medium containing different concentrations of mercuric oxide and calomel.

	Concentrations of chemicals				
	HgO		HgCl		Check
Strain	1-20,000	1-200,000	1-333,333	1-3,333,333	
1	***	***	***	***	***
2	***	***	***	***	***
3	**	***	***	***	***
4	**	**	**	***	***
5	***	***	***	***	***
6	**	***	***	***	***
7	***	***	***	***	***
8	**	***	***	***	**
9	***	***	***	***	***
10	**	**	**	***	***
11	***	***	***	***	***
12	***	***	***	***	***
13	***	***	***	***	***
14	***	***	***	***	***
15	***	***	***	***	***
16	***	***	***	***	***
17	***	***	***	***	***
18	No growth in any of the flasks				
19	**	**	**	***	***
20	***	***	***	***	***
21	**	**	**	**	**
22	**	**	**	***	***
23	**	***	**	***	***
24	***	***	***	***	***
25	***	***	***	***	***
26	***	***	***	***	***
27	***	***	***	***	***
28	***	***	***	***	***
29	***	***	***	***	***
30	***	***	***	***	***
31	**	***	***	***	***
32	*	**	*	**	**
33	***	***	***	***	***
34	***	***	***	***	***
35	**	***	***	***	***
36	**	***	***	***	***
37	No growth in any of the flasks				
38	***	***	***	***	***
39	***	***	***	***	***

Note: The above solutions of HgO and HgCl were saturated and 1/10 saturated. The signs above are as follows: *** equals heavy growth, ** medium growth, * light growth, and - no growth.

TABLE VIII (continued)

	H_2O		$HgCl$		Check
Strain	1-50,000	1-100,000	1-50, 1-100,	1-500, 1-1,000,	
40	No growth in area of the filter				
41	**	***	***	***	***
42	***	***	***	***	***
43	No growth in area of the filter				
44	**	***	***	***	***
45	***	***	***	***	***
46	**	-	***	***	***
47	**	***	***	***	***
48	**	***	***	***	***
49	***	***	***	***	***
50	-	-	-	*	***
51	-	-	*	**	***
52	**	***	***	***	***
53	***	***	***	***	***
54	*	*	**	***	***
55	**	***	**	***	***
56	***	***	***	***	***
57	***	***	***	***	***

TABLE III. Growth of strains of Actinomyces in tyrosinate liquid medium to which mercuric chloride and calomel were added in excess of saturation.

Strain	HgO	Check	$HgCl$
1	*	***	**
2	-	***	very slight
3	-	**	-
4	*	***	very slight
5	-	***	*
6	-	***	very slight
7	-	**	*
8	*	***	**
9	-	*	very slight
10	-	*	very slight
11	-	***	*
12	-	***	very slight
13	very slight	***	**
14	-	*	*
15	*	***	**
16	*	***	very slight
17	-	***	**
18	This strain died in culture		
19	**	***	very slight
20	*	***	-
21	*	***	-
22	*	***	*
23	-	***	very slight
24	*	***	*
25	-	*	*
26	-	***	very slight
27	-	***	-
28	-	*	*
29	-	***	*
30	-	***	very slight
31	-	***	very slight
32	-	-	-
33	-	**	*
34	very slight	***	*
35	*	***	**
36	*	***	*
37	This strain died in culture		
38	-	*	*
39	-	***	very slight
40	This strain died in culture		
41	very slight	***	*
42	very slight	***	*
43	This strain died in culture		
44	very slight	***	very slight
45	-	***	very slight
46	*	***	*

TABLE IA. (continued)

Strain	H_2O	Check	H_2Cl
47	-	***	*
48	-	*	*
49	*	***	**
50	-	-	-
51	-	-	-
52	-	***	*
53	*	***	**
54	-	***	[illegible]
55	-	***	*
56	-	**	*
57	*	***	**

Effect of chemicals on the soil microflora under different moisture conditions.

As the chemicals used in the soil treatment for the control of scab gave no noticeable positive results, and in many cases seemed to give negative results, it was decided to study the effect of the chemicals under controlled conditions, especially in relation to the moisture content of the soil as most of the results were taken in the fall of 1931 after a very dry summer. The number of organisms per gram of soil was counted at the beginning and at the end of the experiment and it was hoped that in this manner it would be possible to find the actual effect of the chemicals on the microflora of the soil. The chemicals were the same as those used for the soil treatments, and they were to be used as nearly as could be calculated in the same proportional treatment as applied in the field. This was very difficult to estimate with any degree of accuracy since the applications in the field were more from the surface standpoint, with little regard given to the volume of soil treated. The field treatment was to use a given number of pounds of chemical per acre distributed as uniformly as possible in the rows, the rows not being much more if any than four inches wide and approximately four inches deep in a V shaped furrow. Upon this basis it was calculated that the actual volume of soil treated was four inches by two inches by 100 feet. From these data the amount of chemical for treating a one-pound box of soil was calculated.

The soil was first air-dried, weighed out in one pound samples and placed in 12 ounce paraffined cheese boxes which were provided with lids. By then oven-drying a similar sample, the per cent

of moisture in the air-dry soil was determined or the amount of water that was present when all of the samples were weighed. The soil was taken from Field #19 at the college where potatoes were grown just a few months before, and where negative results in scab control were obtained by chemical treatment. The samples were weighed, not including the box, as 453.5 grams (one pound) of air-dry soil, and then enough water added to produce the desired moisture percentages, which were 5, 8, 12, 15, and 20 per cent. These percentages should be explained to avoid confusion. They represent the per cent of the total weight, soil and water included, and do not represent the per cent of the maximum amount of water the soil would hold, which is the normal way of stating soil moistures, and which would have been a far less confusing way of giving percentages.

One hundred boxes of soil were used in the experiment each holding one pound of air dry soil, and these were separated into groups of 20 each. One group was left as checks, and the other groups were treated with one chemical each. Every group was then separated into smaller groups, the soil of which was held at the following percentages of moisture: three boxes at 5%, three at 8%, three at 12%, three at 15%, and five at 15 moisture. These were held at 15% for two months after which they were dried to 8% for one month, and then brought back to 15% moisture for one month when the experiment ended. Three boxes were held continuously at 20% moisture. The moisture content of five boxes was varied from 15% down to 8% and back to 15% to determine if variation in moisture content would in any way alter the effect of the chemicals on the soil microflora. Each of the

groups of 20 boxes was treated in a like manner. Each group included the entire range of moisture percentages, but differed only in the chemical treatment.

Based upon the dimensions of a field treated row, the amount of calomel and aluminum sulfate added per box at the rate of 20 pounds per acre, was .134 grams and in the case of yellow oxide and 9.5% the amount was .007 grams per box. To avoid technical weighings and errors, enough chemical was weighed out to treat 20 pounds of soil. Sand was then mixed with the chemical to make the total weight 100 grams. This was then separated into 5 gram lots, one being added to each box in the group to receive that chemical; this process made distribution much easier and more uniform. The chemicals were thoroughly mixed with the soil and then the moisture content brought up to the desired percentage.

The different percentages of moisture were calculated from the data given below.

Weight of oven dry soil	433.5 grams
Weight of air dry soil	453.5 grams
Per cent of water in air dry soil	3.03%
Maximum water holding capacity	27.5%
Weight of soil containers	84.5 grams
Weight of chemical plus sand added per box	5.0 grams
Weight of soil, chemical, water and container at 5%	481.5 grams
Weight of soil, chemical, water and container at 8%	506.5 grams
Weight of soil, chemical, water and container at 12%	533.5 grams
Weight of soil, chemical, water and container at 15%	545.5 grams
Weight of soil, chemical, water and container at 20%	573.5 grams

When the soil was brought in from the field January 2, 1932 and dilutions were made and plates poured, the following counts were obtained: 310.55 million bacteria, 46.5 million actinomyces, and

13.8 million fungi per gram of soil. The counts were taken from an average of 15 plates, five plates poured from each dilution of three and the dilutions made 1 to 2,500,000. Czam's glycerin asparaginate agar was used; it has the following formula: Dextrose 1 gram, glycerin 10 grams, sodium asparaginate 1 gram, $KH_4H_2PO_4$ 1.5 grams, $CaCl_2$ 0.1 gram, $MgSO_4$ 0.2 gram, KCL 0.1 gram, $FeCl_3$ trace, Agar 12 grams and Water 1000 cc. neutralized with NaOH.

Like dilutions, using the same medium, were made at the end of the experiment. Three dilutions were made from each box of soil and three plates were poured from each dilution. Nine plates were thus poured from each box of soil and as there were three boxes of each treatment, the final count was taken from an average of 27 plates. To assure as much accuracy as possible all of the organisms occurring on a plate were counted.

Note. All weighing s were made without the lids and for the checks where no chemical was applied, 5 grams were substracted from the total weight to make allowance for the sand and chemical.

TABLE X. Results of counts of bacteria, actinomyces and fungi in treated and untreated soil at different percentages of moisture.

Moisture	Treatment				
	Calomel	Yellow Oxide	965H	$Al_2(SO_4)_3$	Check
5% moisture					
Bacteria	114.15	417.5	505.15	370.2	153.15
Actinomyces	66.15	107.55	31.35	52.5	13.9
Fungi	14.9	16.0	5.3	14.9	5.5
8% moisture					
Bacteria	288.9	401.8	334.5	368.6	223.3
Actinomyces	147.5	154.5	19.45	44.9	39.0
Fungi	17.2	15.3	3.35	38.7	5.35
12% moisture					
Bacteria	354.05	431.35	348.35	782.5	181.4
Actinomyces	9.1	37.55	24.9	31.75	23.7
Fungi	12.65	13.8	5.0	11.75	8.2
15% moisture					
Bacteria	396.35	691.55	458.9	488.2	249.65
Actinomyces	13.6	10.0	18.35	31.05	10.05
Fungi	24.85	13.0	6.85	8.55	8.75
15% V 8% moisture					
Bacteria	420.85	475.05	566.3	508.35	248.125
Actinomyces	38.15	10.3	18.8	31.4	38.75
Fungi	12.6	10.55	8.05	12.8	8.1
20% moisture					
Bacteria	360.65	567.3	398.35	513.05	231.525
Actinomyces	37.8	7.36	7.2	25.65	32.9
Fungi	10.15	6.95	4.2	7.4	7.4

Note: Numbers represent millions of organisms per gram of soil.

TABLE XI. The ratio of actinomyces in relation to the bacteria of the soil which were treated with chemicals and held at different moisture percentages.

Moisture and Ratio	Treatments				
	Calomel	Yellow oxide	963H	Al_2 30 4_3	Check
5% moisture Ratio	1:1.08	1:2.61	1:3.11	1:7.05	1:11.23
8% moisture Ratio	1:1.93	1:3.20	1:13.08	1:8.21	1:8.98
12% moisture Ratio	1:38.90	1:16.51	1:15.42	1:14.15	1:8.08
15% moisture Ratio	1:25.65	1:89.13	1:37.16	1:14.98	1:14.15
15% V 8% Moisture Ratio	1:11.03	1:46.12	1:30.12	1:33.15	1:7.58
20% moisture Ratio	1:10.07	1:78.14	1:40.00	1:20.24	1:10.11
Average ratio	1:5.99	1:8.25	1:14.23	1:13.22	1:9.13

When the soil was brought in from the field January, 1932, the original ratio of Actinomyces to bacteria in the soil equalled 1:11.75, or nearly 12 times as many bacteria per gram of soil as Actinomyces. In Table XII it will be seen how this ratio varied according to the treatment. The soil treated with calomel and yellow oxide of mercury and held at moistures of 5% and 8% showed the greatest change in favor of the Actinomyces. The left hand number of the ratio figure represents Actinomyces and the right hand the number of bacteria. The nearer the ratio value equals the unit 1. the greater the number of Actinomyces in proportion to the bacteria. These results coincide perfectly with the increase in scab of potatoes in soil that was

treated with calomel and yellow oxide of mercury.

Morphology and pathogenicity

Very little work was done on microscopic morphology of the organisms dealt with in these experiments. Consequently very little can be mentioned here in that regard. Of the several hundred organisms isolated, 57 strains of Actinomyces were cultured and tested for pathogenicity to potato tubers and their ability to resist the toxicity of the inorganic mercury compounds. All of the culture work, after isolation, was done on tyrosinate medium, the formula of which has been given on page 33. By consistantly growing the organisms on this medium, a key for the separation of the organisms was formulated. Tap water was used in all cases for making up the medium and if the same conditions were to be used, the key would probably hold for those organisms at hand, however, if the medium were altered in any manner, or other strains of Actinomyces were added the key would not be of value for identification or separation of the organisms.

The chief merit of this key and the pathogenicity tests recorded here is to show that generalized morphological and pathological work will not be of value in studying the scab producing organisms in different regions and in different habitats. By close observation of the key formulated and the pathogenicity table, it will be seen that there is no correlation between the pathogenic properties of a strain and the morphological and physiological characteristics. Some of the parasitic strains produced pigment, others did not, often a saprophytic strain is separated from a parasitic one, by a slight characteristic only, and whether or not a strain is parasitic can not

be determined by either morphological or cultural characters.

Wakeman in his article (27) formulated a key showing Actinomyces scabies and another form of actinomyces to produce brown pigment in tyrosinate agar. Such may have been the case in the region and in the soils with which Wakeman worked. However, it does not hold true for those organisms studied by the writer. Scab producing organisms were found to fall in many different positions in the key, regardless of pigment production, aerial mycelium or several other characteristics possible of mention.

The absence of correlation between the morphology and pathogenicity of the scab organisms is shown here with a view to pointing out the desirability of further work in this direction, and also to show that specific application of generalized work or general application of specific work will not meet with any degree of success. The organisms of the Actinomyces group both pathogenic and non-pathogenic are so highly pleomorphic, that probably only specific work in a specific instance will be of value. Each worker in his own region must study the problem and develop a system of classification for his own immediate purpose.

TABLE XII. Pathogenicity tests of 57 strains of Actinomyces on potato tubers in the greenhouse

Strains	Degree of scabbiness
1	Heavy scab on all tubers
2	No scab present
3	Many small scabby spots
4	Tuber failed to germinate, no plant produced
5	Heavy scab on tubers
6	Many small scabby spots
7	Few medium sized scabby spots
8	Heavy scab on all tubers

TABLE XII (continued)

Strains	Degree of scabbiness
9	Plant died after it came up
10	Plant died after it came up
11	Plant died after it came up
12	A large number of small scabby spots
13	Few small spots produced
14	Heavy scab on all tubers
15	No scab produced
16	Plant did not produce pot toes
17	A few small spots produced
18	Organism died, no record of pathogenicity
19	Heavy scab produced on all tubers
20	Plant produced no potatoes
21	Few small spots on one potato
22	No scab produced
23	Many small scab spots
24	Many small scab spots
25	Many small scab spots
26	Two medium scab spots on one tuber
27	No scab produced
28	Heavy scab produced on all tubers
29	No scab produced
30	A few medium sized spots produced
31	A few small spots produced
32	Many small spots produced
33	Few medium sized spots produced
34	No scab produced
35	Heavy scab produced on all tubers
36	A few scattered scab spots
37	No scab produced
38	A few small spots produced
39	No scab produced
40	Heavy scab produced on one tuber
41	No scab produced
42	A few scattered spots produced
43	Plant died, tuber rotted
44	A few small scabby spots
45	Heavy scab, very virulent
46	A few small scabby spots
47	Slight scab produced, few scattered spots
48	No scab produced
49	No scab produced
50	Heavy scab produced on all tubers.
51	A few small scabby spots
52	No scab produced
53	Many small scabby spots produced
54	Many small scabby spots produced
55	No scab produced
56	Strain died in culture
57	One tuber was badly scabbed

Summing up these results it will be seen that 37 of the 57 strains were pathogenic on potato tubers to a certain degree, some more than others. This work was carried on with a great deal of care, and the soil was well sterilized before planting the tubers and inoculating with the cultures. The pots were watered with tap water due to the fact that it was almost impossible to keep enough sterile water on hand to water this large number of pots. Tests were run on the tap water and in no case did any forms of Actinomyces occur in the plate cultures. Care was used to avoid contamination of the pots of soil during the addition of cultures. A pure culture of each strain was used to inoculate each pot at the time the tubers were planted.

The important part of these tests was the fact that there are many highly variable forms of Actinomyces capable of producing scab on potato tubers. Doubtless many more strains could be isolated which would be capable of producing scab of tubers. Some of the strains that appear to be saprophytes in these tests might under different environmental conditions produce scab, and some of the strains that exhibited pathogenicity, might under conditions not so favorable, fail to produce scabby tubers. Each strain of the group probably has its own optimum growing conditions and its parasitic properties undoubtedly are governed by existing environmental factors.

Though occurring in another part of the work, it might be well to mention the connection of the toxic and pathogenic tests of the strains of Actinomyces studied. Several of the strains that were parasitic on potato tubers were able to grow moderately in constantly saturated solutions of calomel and yellow oxide of mercury. Others

were able to produce moderate growth in the same solutions but failed to cause scab of potatoes. And still others, some causing heavy scab and others not causing scab, grew only in the weaker mercury solutions. Again it is shown that there is a lack of correlation between pathogenic properties and other characters. Strains number 50 and 35 are the clearest examples of this variation. Strain 50 grew but slightly in the weakest solution of calomel, failing to grow in any of the others. Strain 35 was able to grow quite well in the saturated solution of both chemicals, and also produced heavy scabbing of all the tubers.

Key to strains of Actinomyces in Tyrosinate medium

It was the purpose in formulating this key to separate the strains of Actinomyces isolated by means of morphological and cultural characteristics. Much work of this kind has been done and in most cases is of definite value in the region where it occurred. It was thought in this experiment that a key could be formed for the separation of the present isolated strains and later as more strains are isolated and studied, the key could be enlarged to include them.

Key to Strains of Actinomyces on Tyrosinate Medium

	Strain
A. Production of pigment	
B. Black pigment	
C. Fluffy aerial mycelium	
D. Aerial growth white ----------	36
D. Aerial growth dark grey	
E. Growth one continuous colony ----------	28
E. Growth in many colonies ----------	53
C. Growth leathery, aerial mycelium slight	
D. Colony continuous	
E. Growth very rough, folded ----------	38
E. Growth quite smooth ----------	42

D. Growth many small colonies
E. Edge of colony irregular and rough --------- 41
E. Edge of colony smooth and circular --------- 27

B. Red pigment
C. Growth leathery
D. Growth rough and dark ---------- 4
D. Growth smooth and light gray ---------- 24
D. Growth smooth and dark ---------- 12
C. Growth delicate
D. Fluffy white growth ---------- 19
D. Dark gray growth ---------- 2

B. Brown pigment
C. Leathery growth
D. Growth very dark
E. Granular rough growth ---------- 54
E. Folded with aerial mycelium
F. Fold white with aerial mycelium --------- 23
F. Folds black with aerial mycelium --------- 17
E. Folds black with no aerial mycelium --------- 22
E. Smooth with aerial mycelium ---------- 1
D. Growth speckled with white and black wrinkles -- 45
D. Growth light gray
E. Fluffy aerial mycelium ---------- 6
E. Aerial growth short and powdery
F. Growth smooth ---------- 16
F. Growth folded
G. Growth with wavy folds ---------- 8
G. Growth with short wrinkled folds ------ 5
F. Concentric rings in colony ---------- 21
C. Growth delicate
D. Aerial growth fluffy
E. Dark brown pigment
F. Growth slightly wrinkled irregular ----- 11
F. Growth in concentric rings ---------- 25
F. Center of colony light gray ---------- 38
E. Light brown pigment
F. Aerial mycelium dark gray ---------- 51
F. Aerial mycelium dark, concentric bands - 34
F. Aerial mycelium light gray ---------- 50
D. Aerial growth short powdery
E. Colony continuous
F. Dark brown pigment
G. Dark gray mycelium ---------- 55
G. Light gray mycelium ---------- 29
F. Light brown pigment ---------- 39

G. Aerial mycelium nearly black ---------- 18

G. Aerial mycelium not dark

H. Aerial mycelium crusty ---------- 32

H. Aerial mycelium fine ---------- 7

F. Colony not continuous

F. Pigment dark brown ---------- 15

F. Pigment light brown

G. Colony edges very irregular ---------- 31

G. Colony edges smooth ---------- 30

A. Pigment not produced

B. Growth leathery

C. No aerial mycelium

D. Growth brown color ---------- 3

D. Growth white color ---------- 46

C. Growth with aerial mycelium

D. Colony continuous

E. Growth granular ---------- 38

E. Growth smooth

F. Growth white or gray ---------- 20

F. Growth brown ---------- 33

D. Colony not continuous ---------- 14

B. Growth delicate

C. Growth brown ---------- 13

C. Growth white

D. Colony continuous

E. Growth fluffy and gray

F. Growth granular ---------- 48

F. Growth concentric ---------- 50

E. Powdery growth white ---------- 9

D. Colony not continuous

E. Growth granular

F. Colony large concentric ---------- 35

F. Colony small concentric ---------- 37

F. Colony large not concentric ---------- 49

E. Growth not granular

F. Growth rough wrinkled ---------- 47

F. Growth not rough or wrinkled

G. Colony small white ---------- 10

G. Colony small dark gray ---------- 44

DISCUSSION OF RESULTS

Several facts brought out in these investigations are fundamental and should receive consideration before drawing any conclusions. They may be enumerated as follows: (1) none of the chemicals used for soil treatments in the field and greenhouse gave definite control for potato scab; (2) there was an increase in the amount of scab in comparison to the checks when two of the chemicals, calomel and yellow oxide of mercury were applied to the soil; (3) the field results were all taken in the fall after a very dry summer; (4) the number of Actinomyces increased in proportion to the number of bacteria in soils treated with calomel and yellow oxide of mercury when held at the lower moisture percentages which were 5% and 8%; (5) some of the Actinomyces organisms isolated from the soil and potatoes were able to make some growth in the concentrated solutions of these two chemicals; (6) nearly all of the organisms were capable of making good growth in these concentrated mercurials unless an excess of the compound was added to keep the concentration up to saturation; (7) some of these organisms seemed to be stimulated for a short time by the weaker solutions of the chemicals; (8) 35 of the 57 strains were capable of producing scab on tubers; and (9) several of the strains that produced heavy scab on tubers made a limited growth in the concentrated solutions of calomel and yellow oxide of mercury.

It could not be said that any of the chemicals controlled the scab in any degree when applied as soil treatments. The rows which were treated with aluminum sulfate showed a smaller percentage of scabby tubers than did the check rows and this was noticeably true

of the number severely scabbed. However, this treatment was applied to only two plots which were both at the college and consequently the number was insufficient to yield conclusive results. The rows treated with Du Pay #213H, an organic mercury compound, also gave a smaller percentage of scab than did the untreated, but the difference was not so great as for the aluminum sulfate and not large enough for any definite conclusions. The rows treated with calomel and yellow oxide of mercury yielded the greatest amount of scabby tubers in all but one plot, and in this plot the percentages of scab were all very high. Calomel treatments on the average accompanied the largest amount and the greatest degree of scab, a fact that to a lesser extent was true of the yellow oxide of mercury. The two treatments almost constantly were associated with a much higher percentage and a much greater degree of scab than were the untreated rows.

The potatoes were dug in October, 1931, after a very dry and hot summer. The soil temperature had been unusually high and the moisture content was exceptionally low, two factors which are favorable for heavy scab development. This fact might explain why the chemicals had exerted no control on scab. The two inorganic mercuries are highly insoluble and in a soil with low moisture content one might assume that very little of the chemical would go into solution. At least the condition was probably very unfavorable for the chemicals to exert any disinfecting power. A sample of the soil in field #19 at the college was brought into the greenhouse and one pound samples were treated with the different chemicals at different moisture percentages as given on page 37. There appeared to be a definite increase

in the number of Actinomyces in proportion to the number of bacteria where the treatments were made with calomel and yellow oxide of mercury and where the moisture contents were the lowest used in the experiment. The same is true to a lesser degree of the other two chemicals in the soil of 5% moisture content, though not in that at 8% moisture content. It is noticeable that the count decreased considerably in the boxes of untreated soil, and that the ratio of Actinomyces and bacteria changed, however, the ratio change was not so marked in any other case as it is in the soil at 5% and 8% moistures that was treated with calomel and yellow oxide of mercury.

Some of the Actinomyces strains isolated were able to grow in the saturated solutions of calomel and yellow oxide of mercury. This was true of nearly all of the organisms unless an excess of the chemical was added to the solution. Apparently the mercury was taken out of the solution when the organisms were grown upon it. How this was accomplished is not known but several reasons may be advanced. The base nutrient chemicals may have taken the mercury out of solution by adsorption or by chemical reaction, and there is a possibility that the organisms may be capable of adsorbing mercury. The weaker solution of one-tenth saturation appeared to stimulate the growth of the organisms for a short time, the growth exceeding that in the check flasks. This was not evident after a week or more had elapsed.

The pathogenicity tests conducted in sterilized potted soil showed plainly that there are several strains of the organism capable of producing scab on potato tubers. The scabbiness of the tubers varied from very slight to very severe. The type of scab also varied

considerably, but this could be noticed on a single tuber which had been subjected to only one strain of the organism and probably depends as much on the host relationship as on the type of organism. A great deal of work could be done with profit on the relationship of types of scab and types of organism.

One of the most outstanding facts in relation to the other phases of the work is that several strains of the organism that produced heavy scabbing of tubers were also capable of producing moderate growth in the saturated mercury solutions. Strains number 1, 8, 19, 35 and 57 produced moderate growth in the concentrated solutions. Several strains namely, 5, 14, 28, 43 and 50 produced varying degrees of scab but were able to make little or no growth in the saturated solutions. Strains number 24, 36, 46 and 53 were able to produce growth in the concentrate mercurials and also were pathogenic to a moderate degree. It was also true that several strains produced no scab but were able to show growth in the concentrated solutions. These facts tend to show that some forms are highly resistant to the toxicity of the two mercury compounds in question.

CONCLUSION

The first and most obvious conclusion, considering the amount of work done, is that the chemicals used for soil treatments were not capable of controlling scab. Secondly that two of these chemicals calomel and yellow oxide of mercury will cause either directly or indirectly an increase in the amount and degree of scabbing. Why the amount and degree of scabbing is increased, can be attributed to one or all

of three assumptions, which are as follows: (1) stimulation of the scab organisms by the weaker solutions which would be produced in the soil; (2) reduced competition, the bacteria being killed by the chemical allowing the scab organisms to multiply rapidly for a period of time; and (3) the presence of a mercury resistant scab organism which would be more virulent because the other organisms were inhibited.

Reasoning from the facts obtained in the results, it is logical to believe that a combination of assumptions number 2 and 3 may be responsible for the increase in scab by the chemicals. It will be remembered that several highly pathogenic strains were also highly resistant to both calomel and yellow oxide of mercury solutions, also that these two chemicals in the soil with a moderate moisture content increased the number of actinomyces in proportion to the bacteria quite appreciably. The reason this did not occur in the higher moisture percentages is probably due to the fact that actinomyces in general are not favored by high moisture content in the soil.

Therefore it is the belief of the writer that some of the forms of the scab organisms are more resistant to mercury compounds than are the soil bacteria in general, and that as the bacteria are inhibited, the scab organisms increase in number until the point is reached where the scab organisms are themselves competing for food. At this point a greater amount and degree of scab will be produced due to the massing action of increased numbers.

BIBLIOGRAPHY

(1) Dolley, W. L. — Potato scab: A bacterial disease. Agr. Science 4: 243 - 258, 277 - 287. Pl. 3 - 4. 1930.

(2) _____ — Potato scab and possibilities of prevention. N. D. Agr. Exp. Sta. Bul. 4: 1 - 14, 31 - 41. Pl. I. Fig. 1 - 4. 1891.

(3) Beijerinck, M. W. — Ueber Chinonbildung durch Streptothrix chromogena und Lebensweise dieses Microben. Centbl. f. Bakt. Etc. Par. II. Abt. 6: 2 - 13. 1900.

(4) Buchanan, R. E. — Studies in the classification and nomenclature of the bacteria VIII. The suborders and genera of the Actinomycetales. Jour. Bact. 3: 403 - 406. 1918.

(5) Conn, H. J. — A possible function of Actinomyces in soil. Jour. Bact. 1: 197 - 207. 1916.

(6) _____ — Distribution of bacteria in various soil types. Jour. Amer. Soc. Agron. 5: 218 - 221. 1913.

(7) _____ — Soil flora studies. V. Actinomyces in soil. N. Y. Geneva Exp. Sta. Tech. Bul. 60: 3 - 25. 1917.

(8) _____ — The use of various culture media in characterizing Actinomyces. N. Y. Agr. Exp. Sta. Tech. Bul. 83. 3 - 26. 1921.

(9) Claypole, E. J. — On the classification of the Streptothrices particularly in their relation to the bacteria. Jour. Expt. Med. 27: 99. 1913.

(10) Cunningham, G. C. — The relationship of Cospora scabies to the higher bacteria. Phytopath. Vol. 2: 97. 1912. (Abt.)

(11) Dreschler, Charles — Morphology of the genus Actinomyces. Bot. Gaz. 67: 65 - 83, 147 - 168. Pl. 8 - 9. Fig. 1 - 113. 1918.

(12) Fellows, Hurley — Relation of growth in the potato tuber to the potato-scab disease. Jour. Agr. Res. 32: 757 - 781. Pl. 1 - 5. Fig. 1 - 7. 1926.

(13) Fousek, A. — Über die Rolle der Streptotrichen in Boden. In Mitt. Landw. Lehrkan, K. K. Hochschule Bodenk. Wien. Bd. 1: 217 - 244. 1913 - 13.

(14) Frank, A. B. and Krüger, Fr. Untersuchungen über den Schorf der Kartoffeln. Zeitsch. f. Spiritus Industrie 1: 37 - 39. 1896.

(15) Galloway, B. T. Potato scab. U. S. D. A. Div. Bot. Bul. 8: 4 - 51, 1889.

(16) Orman, W. Corrosive sublimate and flour of sulfur for potato scab; experiments made in 1896. Ky. Agr. Exp. Sta. Bul. 72: 9 - 16. 1896.

(17) Gillespie, L. J., and Hurst, L. L. Hydrogen ion concentration - soil type - common potato scab. Soil Sc. 6: 219 - 233. Fig. 1 - 3. 1918.

(18) _____ The growth of the potato scab organism at various H^+ conc. as related to the comparative freedom of acid soils from the potato scab. Phytopath. Vol. 8: 257 - 269. Fig. 1. 1918.

(19) Güssow, H. T. The systematic position of the organism of the common potato scab. Science n. s. 39: 431 - 432. 1914.

(20) Halsted, B. D. Field experiments with potatoes. N. J. Agr. Exp. Sta. Bul. 115: Fig. 1 - 2. 1895.

(21) Hiltner, L., und Störmer, K. Studien über die Bakterien flora des Ackerbodens, etc., Arb. K. Gesundheitsamte, Biol. Abt. Bd. 3: 445 - 445. 1903.

(22) Humphrey, J. E. The potato scab. Mass. Agr. Exp. Sta. Rpt. 6: 131 - 132. 1888.

(23) _____ The potato scab. Mass. Agr. Exp. Sta. Rpt. 7: 214 - 233. 1890.

(24) Jensen, H. L. Contributions to our knowledge of the Actinomycetales I. A case of hereditary variation in the genus Actinomyces. Proc. Linn. Soc. N. S. W. 56: 79 - 98. Pl. 3. 1931.

(25) Jones, A. P. The history of potato scab. Jour. Appl. Biol. 18: 313 - 333. Pl. 28 - 29. Fig. 1 - 3. 1931.

(26) Jones, L. R., McKinney, H. H., and Fellows, H. Influence of soil temperature on potato scab. Wis. Agr. Exp. Sta. Res. Bul. 53: 1 - 35. Pl. 1 - 5. Fig. 1 - 9. 1922.

(27) Jones, L. R., and McKinney, H. H. The influence of soil temperature on potato scab. Phytopath. 9: 301 - 302. 1919.

(28) Jones, L. R., and Edson, A. W. Potato scab experiments of 1901. Vt. Agr. Exp. Sta. Ann Rpt. 14: 231 - 235. 1900 - 01.

(29) Jubainville, K. and Vesque, J. Variole de la Pomme de Terre. Les Maladies des Plantes Cultivees. 287. 1878.

(30) Kinney, L. F. The potato scab, etc. R. I. Exp. Sta. Bul. 14: Fig. 1 - 4. 1891.

(31) Krainsky, A. Die Aktinomyceten und ihre Bedeutung in der Natur. Centbl. f. Bakt. Etc. Par. II. Abt. 41: 649 - 688. Fig. 1 - 4. 1914.

(32) Kühn, Julius Schorf der Kartoffel. Frankheiten der Kulturgewachse. 222 - 228. 1858.

(33) Lint, H. C. Report of potato scab experiments.
N. J. Agr. Exp. Sta. Ann. 35: 477 - 488. 1914.
N. J. Agr. Exp. Sta. Ann. 36: 375 - 381. 1915.
N. J. Agr. Exp. Sta. An. 37: 618 - 625. 1916.

(34) _____ The influence of sulfur on soil acidity. Jour. Indus. Engin. Chem. 6: 747. 1914.

(35) Lipman, J. G. Lime and fertilizer for potatoes. Am. Fert. 72: 21 - 25. 1930.

(36) Lutman, B. F. Potato scab in new land. Phytopath. 13: 241 - 244. 1923.

(37) _____ The pathological anatomy of potato scab. Phytopath. 3: 255 - 264. Fig. 1 - 10. 1913.

(38) _____ Value of scabby potatoes. Bibliog. Vt. Agr. Exp. Sta. Bul. 291: 1 - 16. 1928.

(39) Lutman, B. F., and Cunningham, G. C. Potato scab. Vt. Agr. Exp. Sta. Bul. 184: Pl. 1 - 12. Fig. 1 - 7. 1914.

(40) Martin, W. H. Influence of soil moisture and acidity on the development of the potato scab. Soil Sci. 16: 69 - 73. 1923.

(41) _____ The relation of sulfur to soil acidity and to the control of potato scab. Soil Sci. 9: 393 - 408. Fig. 1 - 5. 1919.

(42) _____ Relation of soil conditions to the development of potato scab. Ann. Meeting of the Potato Assoc. of Amer. Cleveland, Ohio. 17: 62 - 73. Dec. 30 - 31, 1930.

(48) _____ The use of disinfectants in fertilizers for the control of potato scab and Rhizoctonia. (Abt.) Phytopath. 21: 194. 1931.

(49) McKinney, H. H. Morphology of the potato scab organism. Phytopath. 3: 257. 1913.

(45) Millard, W. A., and Burr, G. A. A study of 34 strains of actinomyces and their relation to types of common potato scab. Ann. Appl. Biol. 13: 580 - 644. Pl. 17 - 20. Fig. 1 and 2. 1926.

(46) Millard, W. A. Common scab of potatoes. Ann. Appl. Biol. 10: 70 - 90. Pl. 3 - 4. 1923.

(47) Monroe, T. J. Does the potato scab organism survive passage through the digestive tract of domestic animals? Phytopath. 9: 148. 1919.

(44) Munter, F. Vier Aktinomyceten des Bodens. Centbl. Bakt. Etc. Par. II. Bd. 38: 337 - 371. Fig. 1 - 3. 1913.

(43) Newton, J. D. Sulfur oxidation in Alberta soils and related experiments. Bibliog. il. Sci. Agr. 11: 613 - 632. 1931.

(50) Sanford, G. B. Some factors affecting the pathogenicity of Actinomyces species. Phytopath. 18: 833-849. Fig. 1 - 3. 1928.

(51) _____ The relation of soil moisture to the development of common scab of potato. Phytopath. 13: 421 - 433. Fig. 1 - 3. 1923.

(52) _____ Some factors influencing the development of potato scab. Phytopath. 14: 38. (Abt.) 1924.

(53) Shapovalov, Michael. Effect of temperature on germination and growth of the common scab of potato organism. Jour. Agr. Res. 4: 129 - 134. Fig. 1. 1915.

(54) _____ Is the common potato scab controllable by a mere reduction of spores? Phytopath. 9: 493. Fig. 1. 1919.

(55) Sherbakoff, C. D. Potato scab and sulfur disinfection. N. Y. Cornell Agr. Exp. Sta. Bul. 330: 709 - 743. Fig. 103. 1914.

(56) _____ The after effect of sulfur treatment on soil. Phytopath. 5: 313 - 333. Fig. 1 - 3. 1915.

(67) Smith, W. G. Field and garden crops. 37 - 38. 1884.

(68) Sorauer, P. Pflanzenkrankheiten. 1: 322. 1892.

(69) Sturgis, W. C. Experiments on the prevention of potato scab.
Conn. State Exp. Sta. Rpt. 16: 116 - 123. 1893.
Conn. State Exp. Sta. Rpt. 18: 153 - 170. 1895.
Conn. State Exp. Sta. Bul. 99: 248 - 260. 1897.

(60) Thaxter, Roland The potato scab.
Conn. Agr. Exp. Sta. Bul. 108: 3 -4. 1890
Conn. Agr. Exp. Sta. Rpt. 61 - 95. Fig. 1. 1890
Conn. Agr. Exp. Sta. Rpt. 130 - 150. 1891

(61) Waksman, S. A., and Curtis, R. E. The actinomycetes of the soil. Soil Sci. 1: 99 - 134. Pl. 1 - 3. 1916.

(62) _____ The occurrence of actinomyces in the soil. Soil Sci. 6: 309 - 316. 1918.

(63) Waksman, S. A., and Joffe, J. S. Thiobacillus thiooxidans, a new sulfur oxidizing organism isolated from the soil. Jour. Bact. 7: 239 - 256. Fig. 1 -3. 1922.

(64) Waksman, S. A. Bacteria, Actinomyces, and Fungi of the soil. (Ist.) Jour. Bact. 1: 101. 1916.

(65) _____ Cultural studies of species of Actinomyces. Soil Sci. 8: 71-215. Pl. 1-4. 1919.

(66) _____ The influence of reaction upon the growth of Actinomyces causing potato scab. Soil Sci. 14: 61 - 79. Fig. 1. 1922.

(67) _____ Soil fungi and their activities. Soil Sci. 2: 103 - 155. Pl. 1 -4. 1916.

(68) Wallroth, J. A. Der Knollenbrand der Kartoffel. Linnaea. 332. 1842.

(70) Welsemorth, H. H. Investigation on the relation of sulfur to the Actinomyces scab of potato. Thesis, Michigan State College. 1924.

(71) Wheeler, H. J., and Adams, C. T. On the use of flowers of sulfur and sulfate of ammonia as preventive of potato scab in contaminated soils. R. I. Agr. Exp. Sta. Ann. Rpt. 10: 254 - 260. 1897.

(72) Wheeler, H. J., Hartwell, B. L., and Moore, W. L. C. Upon the after effect of sulfur when applied to soils for the purpose of preventing potato scab. R. I. Agr. Exp. Sta. Rpt. 12: 133 - 167. 1899.