STUDIES ON SOIL ACTINOMYCETES IN RELATION TO POTATO SCAB AND ITS CONTROL

by

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Thesis

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INTRODUCTION

Measures which have been recommended for the control of scab include the use of resistant varieties, clean or disinfected seed, crop rotation, green-manures, potentially acid fertilizers, sulphur soil treatments, and soil disinfection by chemical means. Apparently any one of these control measures may be effective under certain conditions, but none has general application.

It seems probable that the scab problem will eventually be solved by the production of resistant varities, but until varieties are developed which are suitable to Michigan and which show a higher degree of resistance than those now grown, other control measures must be employed.

The main purpose of this investigation was to determine, if possible, the reason for failure of practices, which have been effective elsewhere, to control scab in infested soils under Michigan conditions. Special emphasis was placed on the cause of conflicting results from mercurial soil treatments. This problem was approached from several angles: (a) the results of numerous soil treatments were compared, (b) the effect of mercurials

on scabbing of potatoes and other hosts of Actinomyces was compared, (c) investigations were made on the influence of different soil actinomycete populations on the effect of mercurials on scabbing, (d) on the effect of mercurials on predisposition of various hosts plants to scabbing, and (e) on the effect of mercurials on the soil flora. Attempts also were made to obtain control of potato scab through associative action of various fungi and bacteria.

THE USE OF ANTISEPTICS AS SOIL TREATMENTS FOR SCAB CONTROL

Review of the literature

The chemicals which have previously been tried by various investigators as soil treatments for the control of potato scab are aluminum sulphate, benzene, bleaching powder, Bordeaux mixture, carbon disulphide, copper sulphate, creosote, formaldehyde, hexamethylenetetramine, tetrachloroethane, and various mercury compounds. Of these aluminum sulphate was partially effective in western New York but reduced yield (137), and was of no value even at 1080 lbs. per acre in Wisconsin (32) and reduced scabbing at low rates in one instance (158) but not in others (42,43) in Michigan. Copper sulphate was fairly effective in one trial in New Jersey (54), and in Michigan (158), but gave no control in Vermont (77). Bordeaux mixture applied to the soil was of ~~no~~ value in New Jersey in some instances only (54), while frequent heavy applications of Bordeaux mixture to the foliage also tended to reduce scabbing. As a foliage spray Bordeaux mixture also reduced scabbing under New York conditions (86,87). Formadehyde as a soil treatment has been tried in New Jersey (54),

England (63), Holland (126), and in Czecholovakia (112), and has been reported ineffective in controlling scab except in Czechoslovakia where it gave partial control at high rates of application. Carbon disulphide, benzene, and kerosene were ineffective in New Jersey (54). Cresote and bleaching powder were of no value in England (96). In limited trials tetrachloroethane and hexamethylene-tetramine gave no promise of scab control in western New York (139). Various mercury compounds have given partial to complete control of scab in New Jersey (52,53,89,91-93), Long Island, New York (26), Maine (93), Canada (83), England (63) Holland (126), Germany (138), and Hawaii (104). Conversely, mercurial soil treatments have failed to control scab in western New York (137,138,139), Michigan (42,43,68), Ohio (106), and in some soils in New Jersey (27,54,93).

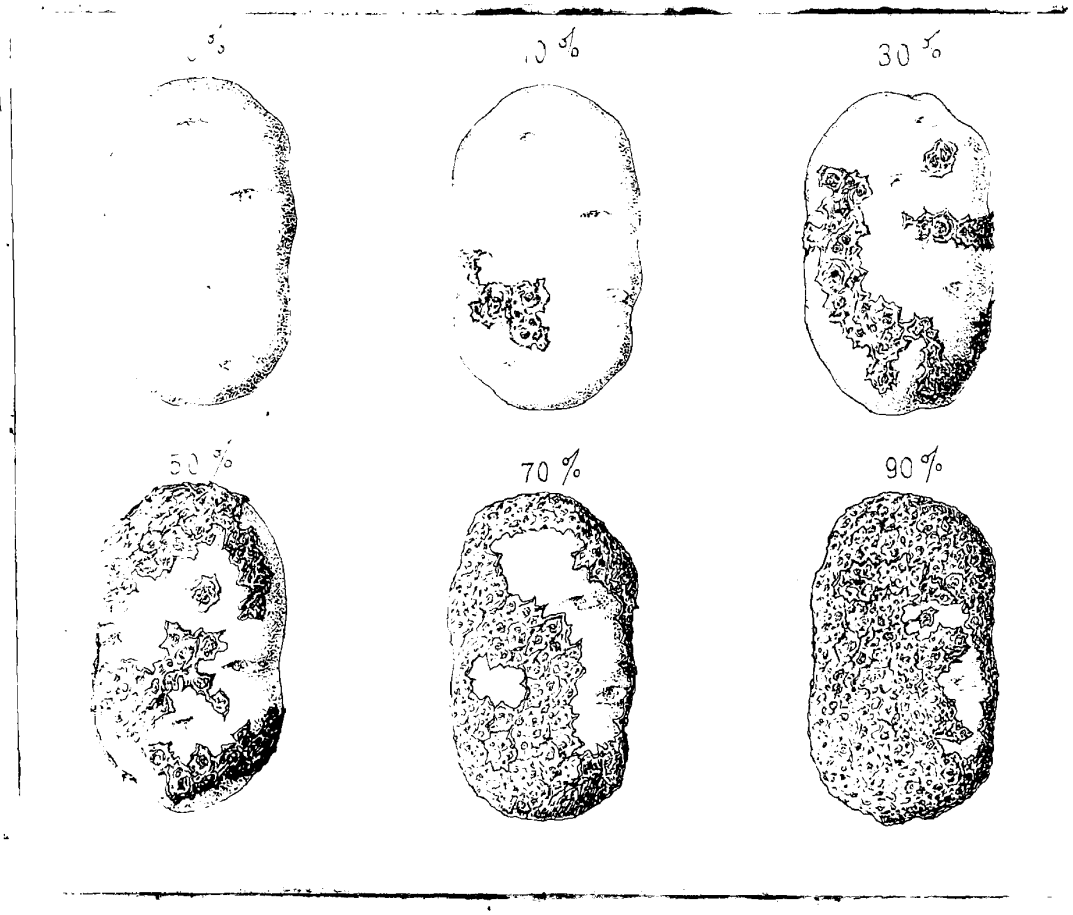
In a previous study (68) the author failed to control potato scab in Michigan with various antiseptics applied as soil treatment. These are here listed with the highest rate per acre of each employed: aluminum chloride, 500 lbs.; borax, 50 lbs., cerium oxalate, 500 lbs.; cobalt chloride, 250 lbs.; copper sulphate,

250 lbs.; cuprous oxide, 50 lbs.; lead acetate, 250 lbs.; mecuric oxide, 20 lbs.; mercurous chloride, 150 lb.; "New Improved Ceresan (5% ethyl mercury phosphate), 150 lbs.; nickel nitrate, 250 lbs.; potassium permanganate, 500 lbs.; potassium dichromate, 250 lbs.; sodium fluoride, 500 lbs.; zinc oxide, 50 lbs.; zinc sulphate, 250 lbs.; "Formacide", 650 lbs.; gentian violet 10 lbs.; malachite green, 150 lbs.; coal tar creosote, 20 gallons,; and wood creosote, 20 gallons. In a greenhouse trial, nickel cyanide controlled scab at 500 lbs. per acre, but not at 150. It therefore would be of little value because of the quantity required.

Materials and methods

Except where otherwise designated, all soil treatments were made in randomized, replicated, field plots. To facilitate application, the chemicals were diluted with air-dried soil from the field in which they were to be tried, and were applied in the planting furrow. The plots at East Lansing consisted of 10 hills each; those at Lake City of 40 hills each in 1937 and 80 hills in 1938. In all cases each treatment was repeated three times. The plots at East Lansing were planted to formadehyde-treated Katahdin tubers, and those at Lake City to untreated certified seed of the same variety.

Figure 1



United States Department of Agriculture chart for estimation of the percent of the surface area of tubers scabbed.

In applying statistical analysis to the data, the percent of the tubers scabbed was not a satisfactory figure, since at East Lansing this was often 100%. The crop was sorted into grades according to the percent of the surface area of the tubers scabbed, using the photographs supplied by the United States Department of Agriculture as a basis of estimation (figure 1). The range for each grade was: clean, 0-2% of the surface area scabbed; light, 3-20%; medium, 21-50%; heavy, 51-100%. The percent of potatoes by weight or by number falling into each class was then determined, and an arbitrary figure was taken as the average percent of the surface area of the tubers scabbed in each class. For example, in 1937 at East Lansing these figures were: light, 10%, medium, 30%; and heavy 70%. In other cases different figures were taken, an attempt being made to closely approximate the actual percent of the tuber surface area scabbed in each class in that particular trial. The same arbitrary figures were used for all treatments in an experiment, and in this manner the scabbiness of the tubers for each plot is expressed in a single figure. Thus the severity of scabbing in the various plots is readily compared.

Results

In 1937 at East Lansing in soil of pH 6.9-7.3 (Table 1), mercury compounds aggravated scabbing, in some cases causing a four-fold increase. A similar increase in scabbing occurred with combinations of calomel (6 and 20 lbs. per acre) with zinc (50 lbs.), with sodium nitrite (100 and 300 lbs.), and with potassium permanganate (100 lbs.). Sulphur alone at 300 lbs. and combinations of sulphur with calomel and with sodium nitrite had no significant effect on scabbing. Likewise zinc (50 lbs.), lead sulphide (50 lbs.), lead acetate (100 lbs.), and aluminum sulphate (50 lbs.) had no significant effect on scabbing.

In another experiment in the same year and place (Table II), hydrochloric acid and sulphuric acid, each at 1500 lbs. per acre, and the same treatments in combination with mercuric chloride (15 lbs.) and copper sulphate (30 lbs.) respectively, as well as the two latter compounds applied separately, showed no tendency to reduce scabbing. The combination of mercuric chloride with hydrochloric acid caused an apparent increase in scabbing.

In a third experiment at East Lansing in 1937, sodium chlorophenylphenate, sodium orthophenylphenate, sodium tetrachlorophenate, sodium 2,4,5, trichlo-

rophenate, and tribromophenol each at 310 and 930 lbs. per acre of the 10% preparation on both diatomaceous earth and bentonite, and 10% tetrachloroethane on diatomaceous earth at 310 lbs. all had no effect on scabbing. The scabbing in these plots was unusually uniform, running about 20% of the surface area of the tubers in control and treated plots alike.

Fairly acid soil (pH 5.2-5.8) at the Lake City Potato Experimental farm was artificially infested with scab in the spring of 1937 by sprinkling in the planting furrows fresh horse manure mixed with macerated peelings (2 bus.) from scabby potatoes and manure-cultures of half a dozen isolates of Actinomyces from potatoes (a two-quart jar of each). In this artifically infested acid soil, mercury compounds (calomel, corrosive sublimate, yellow oxide of mercury, and ethyl mercury iodide) tended to aggravate scabbing, although only yellow oxide of mercury gave an increase in scabbing that reached statistical significance. Powdered Zinc at 75 lbs. per acre, and sulphur, both as seed and soil treatments, apparently reduced scabbing almost to nothing, although the reductions in scabbing were not statistically significant. The mixture of mercury compounds with sulphur in seed and soil treatments was in no case better than sulphur alone. ~~Red copper oxide at all three rates of application (50, 100, and 150 lbs.) gave con-~~

~~ments was in no case better than sulphur alone~~. Red copper oxide at all three rates of application (50, 100, and 150 lbs.) gave considerably less scab than the controls but not significantly less. Lead arsenate was of no value as were also combinations of zinc (50 lbs.) with yellow oxide of mercury (6, 20, and 50 lbs.) and with calomel at the same rates, and combinations of both calomel and corrosive sublimate with red copper oxide, and corrosive sublimate with oxalic acid (Table III).

In the fall of 1937 scabby potatoes were broadcast over this field and the soil was limed at 600 lbs. per acre. Ammonium thiocyanate and the soil treatments that had given some reduction in scabbing in 1937 were tried in 1938 on the same land that had been used for soil treatments in 1937, the rows running at right angles to those of the previous year. The soil reaction at harvest was pH 6.6-6.9. If zinc, red copper oxide, and sulphur were of value in the more acid soil, they certainly were not after the soil had been limed. Ammonium thiocyanate depressed yield without affecting scabbing. At 500 lbs. per acre only a few plants emerged, and these yielded poorly (Table V).

In 1938 at East Lansing ammonium thiocyanate was tried at 500, 1000, and 2000 lbs. per acre in the planting furrow and the potatoes were planted both two and four weeks later. Not a plant in the treated plots

emerged. Dowicides A,B,C,F,G,1,2,4, and 6 were tried each at 25, 100, and 250 lbs. per acre, but it was so obvious that none of them had any appreciable effect on scabbing that the crop was not graded except for the controls which gave 21% of the surface area of the tubers scabbed.

In these field trials, the percent of the surface area of the tubers scabbed was calculated (as described under "Materials and methods") on the basis of the weight of the tubers falling into each class at Lake City, and on the small plots at East Lansing. No yield data were taken on the small plots at East Lansing, but very obvious reduction in yield was caused by "DuBay #1155HH" at high rates of application. Except in the case of ammonium thiocyanate, there were no significant reductions in yield at Lake City with the treatment tried in 1938, but in 1937 "DuBay #1155HH" at 75 and 100 lbs. per acre, red copper oxide at 150 lbs., combinations of both calomel (20 lbs.) and corrosive sublimate (20 lbs.) with red copper oxide (50 lbs.), and some of the seed treatments with mixtures of mercury compounds with sulphur caused significant reductions in yield. If the plots had been larger, it is possible that some of the other treatments also would have shown significantly reduced yields. (Tables IV & V).

Conclusion

Of all the chemicals and combinations of chemicals that have been tried in Michigan at various rates of application, in this and previous experiments, none have shown any appreciable value under field conditions, except sulphur in some instances. Of all these chemicals, none have shown a consistent tendency to aggravate scabbing except mercury compounds.

TABLE 1

Effect of Soil Treatments on Scabbing of Potatoes at East Lansing, 1937

Planted May 18; harvested Sept. 7, 37 & 38

Treatment	lbs. per acre	1	2	3	4	T	X
		% of surface area scabbed					
None		15	10	7	21	53	13.25
Sulphur roll		39	20	23	18	100	25.00
Sulphur	300	8	12	10	45	75	18.75
$KMnO_4$	100	9	18	14	33	74	18.50
Zinc	50	11	10	12	7	40	10.00
$Al_2(SO_4)_2$	50	10	10	12	15	47	11.75
PbS	50	29	23	19	31	102	25.50
$Pb(C_2H_3O_2)_3$	100	28	22	17	24	91	22.75
$Pb(AsO_3)_2$	50	17	24	13	27	81	20.25
$NaNO_2$ & sulphur	300 & 300	18	10	20	22	70	17.50
HgCl	6	21	32	38	44	135	33.75
HgCl	20	29	28	41	41	139	34.75
HgCl & sulphur	6 & 300	4	3	2	17	26	6.50
HgCl & sulphur	20 & 300	4	5	21	34	64	16.00
HgCl & zinc	6 & 50	45	45	32	33	155	38.75
HgCl & zinc	20 & 50	25	40	48	47	160	40.00
HgCl & $NaNO_2$	6 & 100	36	65	35	63	199	49.75
HgCl & $NaNO_2$	20 & 300	53	36	58	34	181	45.25
HgCl & $KMnO_4$	6 & 100	43	50	23	8	124	31.00
DuBay #1155HH	50	68	51	59	59	237	59.25
DuBay #1155HH	100	70	33	40	32	175	43.75
B		582,	547,	544,	655,	2328	

$ST^2 = 324,340$ $SB^2 = 1,362,894$ $Sx^2 = 88,262$

$C = (Sx)^2 \div n = (2328)^2 \div 84 = 64,518.857$

$Sx^2 - C + (ST^2 \div 4) - C + (SB^2 \div 21) - C +$ sum of squares due to error.

TABLE 1 (continued)

Analysis of variance

Variation due to	Degrees of freedom	Sum of Squares	Mean Square	Error
Total	83	23,743.143		
Replication	3	380.857		
Treatments	20	16,466.143	823.307	
Error	60	6,896.143	114.936	10.7208
	F = 5.51**		t = 2.000	

Standard error of difference between means $\pm$

$10.7208\sqrt{2} \div \sqrt{4} \pm 7.580.$

Difference between means required for statistical signifiance - 2.000 x 7.580 $\pm$ 15.160

TABLE II

Effect of Soil Treatments on Scabbing of Potatoes at East Lansing 1937

Planted May 22; harvested Sept. 10, 1937

Treatment	lbs. per acre	1	2	3	4	T	$\bar{X}$
		% of surface area					
None		34	49	39	32	154	38.50
$HgCl_2$	15	38	32	66	44	180	45.00
$CuSO_4$	30	37	49	36	27	149	37.25
HCl	1500	28	35	42	49	154	38.50
H_2SO_4	1500	34	32	47	33	146	36.50
$HgCl_2$ & HCl	15 & 1500	59	57	70	15	201	50.25
$CuSO_4$ & H_2SO_4	30 & 1500	31	45	48	33	157	39.25
B		261	299	348	233	1141	
$\bar{X}$		37.3	42.7	49.7	33.3		40.75

$ST^2 = 188,399$ $SB^2 = 332,915$ $Sx^2 = 50,543$

$C = (Sx)^2 \div n = (1141)^2 \div 28 = 46,495.7500$

$Sx^2 - C =$ $(ST^2 \div 4) - C +$ $(SB^2 \div 7) - C +$ sum squares due to error.

Analysis of variance

Variation due to	Degrees of Freedom	Sum of squares	Mean square	error
Total	27	4047.2500		
Replication	3	1063.5357	354.5119	
Treatments	6	604.00	100.6667	
Error	18	2379.7143	132.2064	11.4931

$F = 1.3133$ (not significant)

TABLE III

Soil effect of Treatments on Scabbing of Potatoes at Lake City Potato Experiment Station, 1937

Planted May 27-28; harvested Sept. 21, 1937

Treatment	Lbs. per acre	1	2	3	4	Sum T	Mean $\bar{X}$
		% of surface area scabbed					
None	-	3	5	4	2	14	3.50
DuBay #1155HH	50	8	3	2	1	14	3.50
DuBay #1155HH	75	5	4	11	1	21	5.25
DuBay #1155HH	100	11	19	0	1	31	7.75
HgO	6	16	8	6	9	39	9.75
HgO	20	39	13	6	10	68	17.00
HgO	50	5	14	10	21	50	12.50
HgCl	6	4	2	7	2	15	3.75
HgCl	20	11	2	3	6	22	5.50
HgCl	50	15	1	4	3	23	5.75
$HgCl_2$	20	11	7	9	7	34	8.50
HgO & zinc	6 & 50	4	0	0	2	6	1.50
HgO & zinc	20 & 50	13	5	8	5	31	7.75
HgO & zinc	50 & 50	25	1	3	2	31	7.75
HgCl & zinc	6 & 50	3	0	4	1	8	2.00
HgCl & zinc	20 & 50	4	0	7	7	18	4.50
HgCl & zinc	50 & 50	7	4	1	7	19	4.75
HgCl & Cu_2O	20 & 50	10	3	1	7	21	5.25
$HgCl_2$ & Cu_2O	20 & 50	1	2	5	0	8	2.00
$HgCl_2$ & $H_2C_2O_4$	20 & 20	11	12	1	5	29	7.25
$HgCl_2$ & $H_2C_2O_4$	20 & 50	14	16	6	2	38	9.50
$HgCl_2$ & sulphur	20 & 100	1	2	1	0	4	1.00
$HgCl_2$ & sulphur	20 & 300	2	0	0	0	2	.50
$HgCl_2$ & sulphur	20&600	8	0	1	0	9	1.75
$HgCl_2$ & sulphur*	1/9	3	2	3	3	11	2.75
HgCl & sulphur*	1/9	1	1	0	1	3	.75
HgCl & sulphur*	1/4	6	3	1	1	11	2.75
Sulphur*	-	3	0	0	2	5	1.25
Sulphur	100	1	2	2	1	6	1.50
Sulphur	300	1	0	0	1	2	.50
Sulphur	600	0	7	0	0	7	1.75
Zinc	25	4	0	25	1	30	7.50
Zinc	50	8	2	0	2	12	3.00
Zinc	75	1	0	0	0	1	.25
Cu_2O	50	0	1	0	1	2	.50
Cu_2O	100	0	5	0	6	5	1.25
Cu_2O	150	0	0	1	1	11	.50
$Pb(AsO_3)_2$	50	2	4	1	4	11	2.75
$Pb(AsO_3)_2$	100	3	2	2	4	11	2.75
$Pb(AsO_3)_2$	150	6	5	5	2	18	4.50
Sum B		270	157	140	125	692	
Mean X		6.75	3.93	3.50	3.12		

* Chemicl applied as dust seed treatment only. 1/9 and 1/4 indicate 1 part of mercural (by weight) to 9 parts of sulphur and one part of mercurial to 4 parts of sulphur respectively.

ST^2 = 20440 SB^2 = 132774 Sx^2 = 7900

C = $(Sx)^2 \div n = (692)^2 \div 160$ = 478864 ÷ 160 = 2992.900

Sx^2 - C = $(ST^2 \div 4)$-C +$(SB^2 \div 40)$-C +sum of squares due to error.

7900-C = (20440÷4)-C + (132774÷40)-C + s.s. due to error.

4907.1 = 2117.1 + 326.45 + 2463.55

Analysis of variance

Variation due to	Degrees of freedom	Sum of squares	Mean square	Error
Total	159	4907.1	30.8623	
Replication	3	326.45	108.8167	
Treatments	39	2117.1	54.2846	
Error	117	2463.55	21.0560	4.5886

F = 54.2846÷21.0560 = 2.578** t = 1.980

Standard error of difference between means = 4.5886/2÷

$\sqrt{4}$ = 6.4883÷2 = 3.2441

Difference between means required for significance =

3.2441 x 1.980 = 6.4233

TABLE IV

Effect of Soil Treatments on Yield of Potatoes at Lake City, 1937

Planted May 27-28; harvested Sept. 21, 1937

Treatment	Lbs. per acre	1	2	3	4	T	$\bar{X}$
		Yield in lbs. per 35-ft. row					
None		21.6	24.4	24.4	18.8	89.2	22.30
DuBay #1155HH	50	24.9	18.1	22.7	24.9	90.6	22.65
DuBay # "	75	17.1	8.2	12.6	22.1	60.0	15.00
DuBay # "	100	9.1	8.7	1.1	1.3	20.2	5.05
HgO	6	19.9	28.1	21.1	23.0	92.1	23.03
HgO	20	9.0	29.5	15.2	24.1	77.8	19.45
HgO	50	13.2	16.3	13.6	21.8	64.9	16.23
HgCl	6	17.6	25.3	18.8	30.5	92.2	23.05
HgCl	20	20.0	25.1	28.1	16.1	89.3	22.33
HgCl	50	24.4	27.1	16.4	16.6	84.5	21.13
$HgCl_2$	20	23.8	25.1	18.1	15.7	82.7	20.68
HgO & zinc	6 & 50	21.0	16.8	17.8	34.8	90.4	22.60
HgO & zinc	20 & 50	27.0	22.6	25.0	18.9	93.5	23.38
HgO & zinc	50 & 50	20.7	13.2	15.5	20.9	70.3	17.58
HgCl & zinc	6 & 50	26.0	14.3	23.2	20.8	84.3	21.08
HgCl & zinc	20 & 50	21.5	13.7	17.2	16.6	69.0	17.25
HgCl & zinc	50 & 50	17.5	22.2	11.5	18.6	69.8	17.45
HgCl & Cu_2O	20 & 50	12.0	8.4	11.7	23.2	55.3	13.33
$HgCl_2$ & Cu_2O	20 & 50	11.4	10.5	22.2	13.4	57.5	14.38
$HgCl_2$ & $H_2C_2O_4$	20 & 20	18.1	22.3	21.1	22.4	83.9	20.98
$HgCl_2$ & $H_2C_2O_4$	20 & 50	13.1	21.3	21.5	15.9	71.8	17.95
$HgCl_2$ & sulphur	20 & 100	18.0	27.3	25.8	31.3	102.4	25.60
$HgCl_2$ & sulphur	20 & 300	28.8	20.9	17.0	33.3	100.0	25.00
$HgCl_2$ & sulphur	20 & 600	26.9	25.9	27.5	31.3	111.6	27.90
$HgCl_2$ & sulphur*	1/9	7.4	7.6	9.1	8.1	32.2	8.05
$HgCl_2$ & sulphur*	1/9	15.2	15.7	12.0	6.3	49.2	12.30
HgCl & sulphur*	1/4	16.7	19.7	15.5	12.8	64.7	16.18
Sulphur*		16.0	29.7	13.7	10.5	69.9	17.48
Sulphur	100	27.9	22.0	24.6	31.5	106.0	26.50
Sulphur	300	22.8	19.0	23.5	33.6	98.9	24.73
Sulphur	600	13.3	20.8	18.7	30.9	83.7	20.93
Zinc	25	28.0	17.6	17.8	28.2	91.6	22.90
Zinc	50	27.5	21.7	15.3	31.0	95.5	23.88
Zinc	75	24.3	16.8	19.0	24.5	84.6	21.15
Cu_2O	50	20.2	20.4	26.8	24.5	91.9	22.98
Cu_2O	100	20.2	13.3	16.9	18.1	68.5	17.13
Cu_2O	150	11.5	13.7	14.6	16.4	46.2	14.05
$Pb(AsO_3)2$	50	24.1	22.7	17.8	27.2	91.8	22.95
$Pb(AsO_3)2$	100	25.2	17.0	25.1	24.4	91.7	22.93
$Pb(AsO_3)2$	150	24.5	22.1	22.6	24.5	93.7	23.43
		787.4	775.1	742.1	868.8	3173.4	

* Used as a seed treatment: seed-pieces rolled in the material.

$Sx = 3173.4$

$ST^2 = 266,602.88$ $SB^2 = 2,526,304.62$ $Sx^2 = 69,640.64$

$C = (Sx)^2 \div n = (3173.4)^2 \div 160 = 10,070,467.56 \div 160$

$= 62,940.4223$

$Sx - C = (ST^2 \div 4) - C + (SB^2 \div 40) - C +$ sum of squares due to error.

$69,640 - C = 66,650.72 - C + 63,157.62 - C +$ ss. due to error.

$6,700.21 = 3,710.29 + 217.23 + 2772.69$

Analysis of variance

Variation due to	Degrees of freedom	Sum of squares	Mean square	Error
Total	159	6700.22		
Replication	3	217.20	72.40	
Treatments	39	3710.30	95.1359	
Error	117	2772.72	23.6985	4.8681

$F = 95.1359 \div 23.6985 = 4.0144$ $t = 1.980$

Standard error of difference between means -

$4.8681 \sqrt{2} \div \sqrt{4} = 3.4417$

Difference required for significance $= 3.44 \times 1.980 =$ 6.811

TABLE V

Effect of Soil Treatments on Scabbing and Yield of Potatoes at the Lake City Experiment Station, 1938**

(a) Effect of scabbing

Treatment	lbs. per acre	1	2	3	4	Mean
		% of surface area scabbed				
None	-	4.3	10.9	6.8	2.6	6.15
Zinc	50	1.8	8.1	11.0	-	6.97
Zinc	100	1.3	.0	14.8	10.3	6.60
Cu_2O	25	5.0	13.5	6.0	10.3	8.70
Cu_2O	150	5.3	10.5	7.7	23.7	11.80
NH_4CNS	25	5.7	6.1	.7	16.2	7.18
NH_4CNS	100	6.4	4.8	12.0	3.9	6.78
NH_4CNS	500	10.8	-	12.8	6.9	10.17
Sulphur	600	10.4	16.7	7.0	4.5	9.65
Sulphur*	-	2.4	9.2	.4	18.5	7.63

By inspection: no significant differences.

(b) Effect on yield

Treatment	lbs. per acre	1	2	3	4	Mean
		yield in lbs.				
None	-	60.0	53.5	37.0	71.5	55.50
Zinc	50	54.5	33.0	37.5	-	41.67
Zinc	100	48.5	55.5	66.5	74.0	61.13
Cu_2O	25	55.5	53.5	53.0	51.0	53.25
Cu_2O	150	54.0	54.0	55.0	63.5	56.63
NH_4CNS	25	55.5	80.0	53.0	33.5	55.50
NH_4CNS	100	50.0	55.5	51.0	67.5	56.00
NH_4CNS	500	5.5	.0	1.3	8.0	3.70
Sulphur	600	53.5	78.5	68.0	55.0	63.75
Sulphur*	-	82.0	65.0	61.0	72.5	70.13

* Seed pieces rolled in sulphur.

By inspection: no significant differences except a marked reduction in yield from 500 lbs. per acre of ammonium thiocyanate.

** Planted May 24; harvested Oct. 3-4, 1938

THE REASON FOR FAILURE OF MERCURIALS TO CONTROL SCAB

Review of the literature

In certain areas in the eastern part of the United States and Canada and in Europe mercury compounds as soil treatments have given partial to complete control of potato scab in sub-infested soil. In Germany (133) and in New Jersey (93(mercurial soil treatments have controlled scab only in certain soils. In one test in Ohio (106) mercurials had no appreciable effect on scabbing. In western New York and in Michigan (42,43,68, 137-139) the application of mercury compounds to scab-infested soil generally results in a marked increase in scabbing.

In a paper presented before the Potato Association of America in 1936, Daines and Martin (27) presented data showing that zinc in combination with calomel controlled scab in a New Jersey soil where calomel alone failed. They also found that mercuric nitrate leached more readily from soil in which mercurials were effective in controlling scab than it did from soil in which they were not effective. They concluded that mercurials are effective in controlling scab only in soils where they are permitted to migrate sufficiently to afford protection, but that in certain soils mercury is ren-

dered ineffective. They considered this inactivation of the mercury related to the oxidation-reduction potential of the soil.

More recently Störmer (133) obtained adequate control of potato scab and Rhizoctonia with applications of superphosphate containing 1% corrosive sublimate at 357 lbs. per acre. She concluded that an acid medium (represented in this case by superphosphate in sandy soil of pH 5) is essential for the release of the fungicidal properties of the mercury, which is immobilized in the presence of alkaline fertilizer, such as calcium cyanamide.

MacLeod and Howatt (83) found that mercuric and mercurous chloride applied in the dry form at the rate of 10-15 lbs. per acre could be depended upon to control scab and black scurf in heavily infested soils at Fredericton, New Brunswick, Canada. From repeated tests they concluded that the efficiency of this type of treatment depends largely upon the uniformity with which the space to be occupied by the tubers is impregnated with the fungicidal agent.

In field trials in Michigan mercury compounds have been applied in the planting furrow. In view of the findings of other investigators, the failure to control scab there might be due to not mixing the fungicide

through the soil, or to the soil having a hydrogen-ion concentration or an oxidation-reduction potential which is unfavorable for the release of the fungicidal properties of the mercury.

Soil Reaction

Mercurial soil treatments are not ineffective in Michigan; they generally cause a marked increase in scabbing. As previously reported (68) 329 individual hill records of soil reaction and severity of scabbing in soil that had been limed and sulphured in strips at East Lansing showed that mercury compounds aggravated scabbing over the entire pH range of 6.0-8.4. The results of this experiment are summarized in Table V1. The apparent trend towards increased scabbiness with rise in pH was not statistically significant. At Lake City in 1937 (Table 111) yellow oxide of mercury caused a significant increase in scabbing in soil of pH 5.2-5.8. No evidence was obtained that the effectiveness of mercurials in our soils is in any way related to the soil reaction.

Oxidizing and Reducing Agents

In the field trials of calomel and yellow oxide of mercury in combination with oxidizing and reducing agents and sulphur which were carried out at East Lansing (Table 1) and at Lake City (Table 111) in 1937, neither oxidizing nor reducing agents at the

TABLE V1

Influence of Soil Reaction on the Effect of Mercurial Soil Treatments on Scabbing of Potatoes

329 observations, East Lansing, 1936

Soil pH	% of surface area of tubers scabbed		
	control	HgCl	$HgCl_2$
6.0-6.4	8.2	19.9	21.5
6.5-6.9	9.1	26.9	24.8
7.0-7.4	10.7	21.6	29.9
7.5-7.9	11.6	19.6	23.4
8.0-8.4	11.7	38.0	12.0
Mean:	10.1	23.5	25.3

at the rates employed had any marked effect on the tendency of mercurials to increase scabbing.

In pot experiments described under the next heading (Table VIII), calomel (340 parts per million) in combination with powdered zinc (8500 p.p.m.) caused a marked reduction in scabbing in both Long Island and local soils that had been infested with scab from Michigan potatoes. Potassium permanganate (8500 p.p.m.) in combination with calomel had no effect on scabbing in either soil. Lime (18,000 p.p.m.) with calomel caused a marked increase in scabbing in Long Island soil but none in local soil where the controls were too scabby to expect any increase from soil treatments. In local soil zinc alone at high rates of application caused a reduction in scabbing, and potassium permanganate apparently caused a slight reduction.

In cases where combinations of zinc with calomel caused a reduction in scabbing while calomel alone did not, it seems apparent that it was the effect of the zinc itself rather than the combined effect of the zinc and calomel that caused the reduction.

Mercury placement tests

If prevention of scabbing and aggravation of scabbing as a result of mercurial soil treatments are

both due to the effect of the mercurials on the soil flora, depending upon local conditions, then in mercury placement tests, the degree of control on the one hand and the amount of increase in scabbing on the other may both be taken as measurements of the thoroughness with which the area in which the tubers develop became impregnated with the antiseptic. MacLeod and Howatt in eastern Canada (83) and Daines and Martin in New Jersey (27) found that mercury compounds were most effective in controlling scab when they were mixed thoroughly through all the soil in the space later to be occupied by the tubers. The latter authors concluded that mercury compounds applied with the fertilizer are effective in controlling scab only in soils where they are permitted to migrate sufficiently to afford protection throughout the area in which the tubers develop.

In placement tests with yellow oxide of mercury at Lake City, Michigan, in 1938, the mercurial mixed thoroughly with the soil in the area in which the tubers later developed was no more effective in aggravating scab than where other methods of application were employed. IN 80-hill plots with three replications, yellow oxide of mercury caused a marked increase in scabbing when (a) mixed thoroughly through the soil along the planting row to a depth of 5 to 6 inches, (b) applied on the surface of the soil in a

band 6 inches wide after the tubers were planted and covered, (c) banded two inches from and on either side of the seed pieces and on a level with them, (d) applied in the planting furrow, and (e) applied in a band 2 inches directly below the seed pieces. However, when the mercurial was placed 4 inches below the seed pieces, the increase in scabbing was small and insignificant. (Table VII).

If the cause of increased scabbing from yellow oxide of mercury soil treatments at Lake City were due to an effect of the mercurial on the soil flora, resulting in an increase in number of parasitic Actinomycetes, it must be concluded that a sufficient amount of the antiseptic migrated from the position of placement through the area in which the tubers developed to cause a significant increase in scabbing. From this view point it appears that the yellow oxide of mercury showed greater tendency to migrate down in the soil than upward, since all of the placements gave approximately equal increases in scabbing except that the placement 4 inches below the seed pieces gave a smaller increase in scabbing.

It should be noted here that two plots in this experiment (represented by x and y in Table VII) were lost through an error in harvesting. For the purpose of statistical analysis, estimates for these missing plots were supplied by Baten's method (8).

TABLE VII

Placement test with HgO as a Soil Treatment for Potato Scab at Lake City, 1938

Planted May 24, harvested Oct. 3-4, 1938

Placement	1	2	3	4	T sum	$\bar{X}$ mean
	% of surface area scabbed					
0. no treatment	11.9	11.7	14.0	12.8	50.4	12.60
1. mixed	38.0	30.3	35.4	37.5	141.2	35.30
2. surface	50.5	43.3	40.9	14.0	148.7	37.18
3. banded	12.6	41.3	31.5	37.8	123.2	30.80
4. furrow y =	29.8	25.2	34.6	38.9	98.7 + y	32.13 128.5
5. 2 in. below	27.6	28.4	41.7	28.1	125.8	31.45
6. 4 in. below	10.7	18.1	z = 20.8	21.2	50.0 + z 70.8	17.70
sum B	151.3 + y 181.1	198.3	98.1 + z 218.9	190.3	738.0 + y + z 788.8	

Estimates for missing plots:

Where r is the number of replications, s is the number of treatments, n is the total number of plots, y is the value of the missing plot in the first replication and P_i and B_h are respectively the sums of the row and the column in which y falls, z is the missing plot in the third replication and P_j and B_k are respectively the sum of the column and the row in which z falls, and T is the sum of all the plots exclusive of y and x:

$$y = \frac{[(r-1)(s-1)(sP_i + rB_h - T) - (sP_j + rB_k - T)]}{[(r-1)(s-1)]^2 - 1} =$$

$$\frac{18[7(98.7) + 4(151.3) - 738] - [7(50) + 4(198.1) - 783]}{(3 \times 6)^2 - 1}$$

$$= 298.$$

$$z = \frac{[(r-1)(s-1)(sP_j + rB_k - T) - (sP_i + rB_h - T)]}{[(r-1)(s-1)]^2 - 1} =$$

$$\frac{18[7(50) - 4(198.1) - 738] - [7(98.7) + 4(151.3) - 738]}{(3 \times 6)^2 - 1} =$$

20.8

Using these estimates the fundamental summations are:

$ST_2 = 97,118.06$, $SB^2 = 156,251.40$, $Sx^2 = 25,893.78$,

$C = (Sx)^2 \div n = (788.6)^2 \div 28 = 22,103.04$

Analysis of variance

Variation due to	Degrees of freedom	Sum of squares	Mean square	Error
Total	23*	3790.74		
Treatments	6	2176.48	362.747	
Blocks	3	218.58	72.860	
Error	16*	1395.68	87.230	9.340

* 2 degrees of freedom lost by fixing the values of x and y. F = 5.88, which is significant.

Omitting the values of x and y from the fundamental summations: $Sx = 738.0$, $C = (Sx)^2 \div 26 = 20,947.846$, $ST^2 \div k = S(T_5^2 + T_1^2) \div 3 - S(T_1^2 + T_2^2 + T_3^2 - T_4^2 + T_5^2) / 4 = 12,241.69/3 - 75,593.17/4 = 22,978.855$. $SB^2 / s = S(B_2^2 + B_4^2) / 7 - S(B_1^2 + B_3^2)/ 6 = 75,536.98/7 - 62,135.30/6 = 209.034$.

Corrected analysis of variance

Variation due to	Degrees of freedom	Sum of squares	Mean square	Error
Total	25	3625.25		
Error	16	1395.68	87.230	9.340
	9	2229.57		
Blocks	3	199.03	69.678	
Treatments		2030.54	338.423	

F (treatments) = 3.88

Tabular value of t = 2.12. The standard error of differences between means = $(9.34 \times \sqrt{2} \div \sqrt{4} = 6.603$

The difference between treatment means required for statistical difference = $6.603 \times 2.12 = 14.00$

Experiments with Long Island and New Jersey Soils in 1937

In 1937 about 1200 lbs. of scab-infested New York (Long Island) soil were obtained from H. S. Cunningham, and a smaller quantity of New Jersey soil from R. H. Daines. Both soils had been fumigated with carbon disulphide before shipment because of the Japanese beetle quarantine. The soils were stored in a dusty greenhouse until the odor of carbon disulphide had entirely dissipated. It being feared that fumigation and desiccation of the soils might have eliminated most of the pathogenic Actinomycetes, a portion of each soil was reinfested by adding to it one gram of macerated potato scabs for each ten lbs. of soil. Calomel was applied at four rates which were intended to be 6, 15, 50, and 150 lbs. per acre, but since there might be some disagreement as to how much chemical in a pot is equivalent to a given rate of application in the field, the rates of application are expressed in parts per million. The chemicals for soil treatments were mixed thoroughly through the soil. The pots were planted to clean, formaldehyde-treated Katahdin potatoes and placed out of doors.

In Table VIII each figure under "% scab" represents the percent of the surface are of the tubers scabbed in ten 8-inch pots. In the case of Long Island soil that had not been artificially infested, the controls gave 5.8% scab. Calomel at 340 p.p.m. had little, if any, effect on scabbing. In Long Island soil that had been infested with scab from Michigan potatoes, the percent of scab increased in proportion to the amount of calomel applied, from 7.6% for the controls to 38.7% for 2850 p.p.m. In local soil calomel had no appreciable effect on scabbing, a phenomen that frequently occurs at East Lansing when the controls are very scabby.

In the case of New Jersey soil (Table VIII) there were only five 6-inch pots for each treatment and the potatoes were planted six weeks later, i.e., in mid-July. Because of weak seed and hot weather only a 50% stand was secured. Nevertheless, the results are very similar to those for Long Island soil, calomel causing an increase in scabbing in soil that was infested with scab from Michigan potatoes, but having no effect on scabbing in soil that was not artificially infested.

In brief, In 1937 the effect of calomel on scabbing in Long Island and New Jersey soils that had been infested with scab from Michigan potatoes was exactly what generally occurs under field conditions in Michigan, - calomel aggravated scabbing. In view of this it ~~seemed likely that in~~

it seemed likely that in Long Island and New Jersey where calomel controls scab the soil must be infested with strains of Actinomyces that react differently to calomel from those that cause scab in Michigan. The failure to control scab in Long Island and New Jersey soils that had not been artifically infested might be explained by their having become contaminated with local strains of Actinomyces during their storage in the greenhouse, either from dissemination of the organism in dust in the air or by Arthropods such as sow bugs which were abundant in the greenhouse.

That the Long Island and New Jersey soils that had not been artifically infested were contaminated with local Actinomycetes seems probable, not only because of the failure of calomel to control scab in those soils, but because natural contamination of sterilized soil has been of constant occurrence in the greenhouse. For example, in an attempt to test the pathogenicity of Actinomyces-isolates 114 6-inch pots of soil were saturated with water and steamed for 15 hrs. Sixty of these were placed out of doors on a lawn and the others in the greenhouse. Duplicate pots were infested with actinomycete-isolates; planted with formaldehyde-treated Katahdin potato tubers. At harvest, every tuber in the pots in the greenhouse was heavily scabbed while only 3 out of 33 control pots out of doors showed any trace of scab.

In the case of Long Island and New Jersey soils

that calomel did not cause an increase in scabbing in the soil that was not artificially infested, may have been due to its not having become so thoroughly contaminated with local strains of Actinomycetes as was the soil that had not been artificially infested.

Evidence that soil would become contaminated with Actinomycetes (spores of bits of mycelium) settling down out of the air in the greenhouse in question is furnished in Table X. 24 Petri dishes, (each containing 30 cc. of beef-peptone agar of pH 7.0) were placed on a bench in the greenhouse and the covers removed for varying lengths of time. The plates were then incubated for one week, one-half of them at room temperature and the rest at 37° C. Actinomyces colonies developed on most of the plates. The failure of the Actinomycetes to appear in proportion to the length of time of exposure, and the complete absence of fungi may have been due partially to the large numbers of bacteria (these acting as antibiotics at least by crowding, and possibly also through the production of toxins), and partially to the fact that the agar in plates exposed longer than 15 minutes became very dry.

It is not known whether or not the Actinomycetes that developed on the plates were pathogenic, but it seems probable that, if sparophytic Actinomycetes are present in considerable numbers in the air of a greenhouse (with

ventilators closed), parasitic species could be disseminated in that manner also. It will be noted in Table X that more than one-half (6 out of 11) of the actinomycete colonies on plates exposed only 5 minutes turned the medium blue. This is a characteristic that is commonly associated with actinomycete-isolates from scabby potatoes from the Lake City Experiment Station.

TABLE VIII

Effect of Soil Treatments with Calomel, Zinc and Potassium Permanganate on Scabbing and Yield of Potatoes in Michigan and Long Island Soils

Treatment	parts per million	local soil		Long Island soil infested with Michigan scab		Long Island soil not artificially infested	
		% scab	yield* in grams	% scab	yield* in grams	% scab	yield* in grams
Check	-	63.0	120	7.6	80	5.8	53
HgCl	340	54.1	64	15.8	70	4.6	62
"	850	60.0	56	28.0	45	-	-
"	2850	51.0	34	38.7	23	-	-
"	8500	70.0	13	-	-	-	-
HgCl & CaO	340 18500	58.0	66	30.1	67	-	-
HgCl & Zn	340 8500	26.4	81	3.8	26	-	-
HgCl & $KMnO_4$	340 8500	65.8	118	8.4	27	-	-
Zn	8500	41.7	100	-	-	-	-
"	17000	28.0	93	-	-	-	-
"	51000	24.2	70	-	-	-	-
$KMnO_4$	8500	47.3	74	-	-	-	-
"	17000	57.6	68	-	-	-	-
"	51000	51.1	52	-	-	-	-

* per pot.

TABLE IX

Effect of Soil Treatments with Calomel in New Jersey Soil.

soil	# pots	p.p.m. HgCl	% surface area of tubers scabbed
artificially	4	0	55
infested	3	340	60
	3	850	63
	0	2800	-
not artificially	3	0	23
infested	2	340	24

TABLE X

Organisms on Plates Exposed to contamination in a Greenhouse in which the Soil was Infested with Potato Scab

Jan. 3, 1938

Time of exposure, minutes	Temp. of incubation of plates	Plate counts bacteria	actinomycetes "blue"	actinomycetes total	fungi
5	room	215	2	2	0
		260	2	7	0
15		560	0	5	0
		80	1	5	0
		200	0	0	0
30		560	0	27	0
		*	-	-	-
80		1400	2	5	0
		912	0	10	0
140		1200	6	12	0
		1760	0	21	0
	37 C.				
5		120	2	2	0
		*	-	-	-
15		78	0	1	0
30		128	0	9	0
		840	0	0	0
80		1088	0	2	0
		1000	0	1	0
140		2700	0	2	0
		560	0	48	0
24 hours		#	0	15	0
		#	2	36	0
		#	3	19	0

* count ruined by "spreader bacteria". #too many to count

TABLE X continued

Beef-peptone Agar, pH 7.0. The agar became very dry in plates from which the covers were removed for more than 15 minutes. Counts were made on the 7th day; bacterial counts of more than 200 are estimates arrived at by counting the colonies on a fraction of the plate.

Experiments with Long Island Soil and "Long Island Scab" in 1938

In 1938 scabby potatoes as well as scab-infested soil was obtained from Dr. Cunningham of Long Island, N. Y. As in the preceeding year, the soil was aired in the greenhouse until no odor of carbon disulphide could be discerned. It was then divided into three lots. One lot was not artificially infested. The other two lots were saturated with water and steamed for 15 hours. One half of the steamed soil was infested with Long Island strains of Actinomyces by adding to it cultures of Actinomyces isolated from Long Island potatoes and 1% of unsterilized Long Island soil that had been protected from contamination from the time of its arrival by storing it in clean, sealed fruit jars. The other half of the steamed soil was infested with Michigan strains of Actinomyces by adding to it cultures of Actinomyces isolated from potatoes from Michigan and 1% of unsterilized scab-infested local soil. In like manner a lot of local soil was sterilized and infested with Long Island strains of Actinomyces; another lot was fumigated with carbon disulphide (one lb. per cubic yard and sealed in garbage cans for 48 hrs.) and desiccated to make it more comparable with the Long Island con-

trol set; and a third lot was neither sterilized nor fumigated.

Each lot was divided into four parts and treated with calomel as in 1937 except that the rates employed were 50, 350, and 1000 p.p.m. The pots were planted to clean, washed, formaldehyde-treated Katahdin tubers and placed on sod in an orchard, care being taken not to contaminate them.

In order to make possible the application of statistical analysis, the pots were harvested separately. The percent of the surface area scabbed and the weight of each tuber was recorded, and the percent of the surface area scabbed of all the tubers in a pot was calculated on the basis of the weights of the tubers. It is obvious that the surface areas of tubers do not vary in proportion to their weights, but this objection was counterbalanced by the fact that in these experiments the smaller tubers were less scabby than the larger ones.

In local, naturally infested soil the control gave 56.6% of the surface area of the tubers scabbed, while 350 p.p.m. of calomel gave 82.2%, an increase in scabbing that closely approaches significance by analysis of variance (Table IX).

In local soil that had been fumigated with carbon disulphide and desiccated to make it more comparable

with the Long Island soil, calomel caused a marked increase in scabbing, giving significant differences from the control at 50 and 350 p.p.m. (Table X).

In Long Island soil that had been steamed and infested with Michigan strains of Actinomyces, 50 and 350 p.p.m. had no appreciable effect on scabbing, while with 1000 p.p.m. there was a highly significant increase (Table XI).

In Long Island soil that had been steamed to remove contamination and then reinfested with strains of Actinomyces from Long Island, calomel at 1000 p.p.m. gave complete control of seat (Table XII).

In local soil that had been sterilized and infested with Long Island strains of Actinomyces, calomel caused a significant reduction in scabbing at all three rates of treatment, practically eliminating scab with 1000 p.p.m. (Table XIII).

In Long Island soil that had not been artificially infested, but that had probably picked up some contamination with local strains of Actinomyces, there was very little scab, but calomel seemed to aggravate rather than control scab (Table XIV).

Rhizoctonia scurf occurred only in pots of local soil that had not been steamed. Although the tubers from these lots were too scabby to leave much space for

Rhizoctonia sclerotia to develop, 71 % of the tubers were more or less scurfy from the control pots of the lot of soil that had been fumigated with carbon disulphide. From the control pots of the other lot of local soil 12% of the tubers were scurfy, whereas there was no trace of scurf on any of the tubers from soil that had been treated with calomel at any of the three rates of treatment. Although partial to complete control of scurf with mercurials has been reported by several other investigators (15, 26, 83, 91, 92, 104), neither calomel nor yellow oxide of mercury controlled scurf in 1937 under field conditions at Lake City, Michigan, when applied at 6, 20, and 50 lbs. per acre in the planting furrow.

Effect on Yield

The effects of these soil treatments on yield in 1937 are given in Table VIII, for Michigan soil in 1938 in Table XV, and Long Island soil in Table XVI. In the summarizing table (Table XVII) are given the yields of treatments within each set of pots.

In 1937 calomel depressed yield in proportion to the amount applied in Michigan soil, but only at high rates in Long Island soil. The Michigan soil controls averaged almost double the yield of the Long Island soil controls, but whereas 340 p.p.m. of calomel cut in half

the yield in Michigan soil, it caused only a slight reduction in yield in Long Island soil.

The results for 1938 are sharply in contrast with those for 1937. Long Island soil yielded better, but calomel apparently depressed yield even at 50 p.p.m. while with 350 p.p.m. the decrease was statistically significant. Michigan soil yielded less, but calomel caused an increase in yield reaching statistical significance at 350 p.p.m. At 1000 p.p.m. there was a highly significant reduction in yield. The data on individual sets (Table XVII) show that all of the increase in yield with calomel in Michigan soil came in soil that had been fumigated with carbon disulphide, whereas in the other sets 50 and 350 p.p.m. did not effect the yield.

There was no recognized difference in the handling of the pots during the two years except in the moisture supply. In 1937 the pots were watered very sparingly. It was expected that this would insure heavy scabbing of the controls since there are many statements in the literature that potatoes scab most severely in dry soil (32, 88, 116). Since the potatoes in Long Island soil did so poorly under drouth conditions, the following year the pots were watered heavily early in the season. Later in the season heavy precipitation kept the soil wet.

A decrease in yield was to be expected in treated Long Island soil since Cunningham (26) obtained a slight decrease in yield with low rates of application of calomel under field conditions. Under field conditions in Michigan mercury compounds have no effect on yield up to 50lbs. per acre. Increases in yield from mercurial soil treatments which have been reported from western New York (138), have never been observed under field conditions in Michigan.

It is puzzling why there should be an increase in yield from calomel soil treatments in local soil that had been treated with carbon disulphide, and also why fumigation with carbon disulphide should cause an increase in Rhizoctonia scurf as noted above.

Conclusions Regarding the Effect of Calomel on Scabbing

In pot experiments, calomel mixed thoroughly through the soil controlled scab in both Long Island and Michigan soils that had been steam-sterilized and infested with strains of Actinomyces from Long Island. Conversely, calomel aggravated scabbing in both Long Island and Michigan soils, infested with Michigan strains of Actinomyces, at the same time controlling Rhizoctonia scurf.

In view of this it is concluded that calomel is not rendered ineffective as an antiseptic when used as

a soil treatment in Michigan soil, and that differences in the effect of calomel on scabbing of potatoes are due to differences in the strains or species of paroaitic Actinomyces infesting the soil.

TABLE XI

Effect of Calomel Soil Treatemtns on Scabbing in Michigan Soil, not Fumigated, Sterilized, nor Desiccated

	% of surface area of tubers scabbed	
Calomel p.p.m.	0	350
Pot#1.	93.1	84.3
2.	85.0	91.1
3.	.7	68.0
4.	90.0	97.0
5.	95.1	94.0
6.	78.3	89.9
7.	5.5	78.8
8.	56.3	90.3
9.	5.0	94.8
10.	57.4	40.0
Sum T	566.4	828.2
Mean $\bar{X}$	56.64	82.82

$Sx = 1394.6$ $S\bar{x}^2 = 116,994.98$ $ST^2 = 1,006,724.2$

$C = (Sx)^2 \div n = (1394.6)^2 \div 20 = 97,245.46$

$Sx^2 - C = (ST^2 \div 10) - C$ + sum of squares due to error.

Variation due to	Degrees of freedom	Sum of squares	Mean squares	Error
Total	19	19,749.52		
Treatments	1	3,426.96	3426.96	
Error	18	16,322.56	906.81	30.11

$F = 3426.96 \div 906.81 = 3.779$, which is not significant.

The standard error of differences between means $=$

$30.11 \times \sqrt{2} - 10 = 13.46$ From Fischer's table, t-2.101

The difference between means required for statistical significance $= 13.46 \times 2.101 = 27.607$

TABLE XII

Effect of Calomel Soil Treatments on Scabbing in Michigan Soil, Fumigated with Carbon Bisulfide and Desiccated.

% of surface are of tubers scabbed

p.p.m.	0	50	350	1000
Pot # 1.	10.0	30.0	88.5	13.0
2.	5.0	90.2	75.4	62.4
3.	47.8	54.0	13.8	65.0
4.	17.0	70.3	68.4	3.0
5.	8.1	60.0	95.0	50.0
6.	41.6	26.0	68.2	50.0
7.	93.2	91.7	65.3	7.6
8.	10.0	46.3	68.9	50.0
9.	5.0	20.0	82.6	65.0
Sum T	237.7	488.5	626.1	366.0
Mean $\bar{X}$	26.42	54.28	69.57	40.67

$Sx = 1718.3$ $Sx^2 = 113,405.19$ $ST^2 = 821,090.75$

$C = (Sx)^2 \div n = (1718)^2 \div 36 = 82,015.4136$

$Sx^2 - C = (ST^2 \div 9) - C$ = sum of squares due to error.

Analysis of variance

Variation due to	Degrees of freedom	Sum of squares	Mean square	Error
Total	35	31,389.78		
Treatments	3	9,216.89	3072.296	
Error	32	22,172.89	692.902	26.323

$F = 3072.296 \div 692.902 = 4.434$, which is significant.

The standard error of differences between means =

$26.323 \times \sqrt{2} \div \sqrt{9} = 12.407$

From Fischer's table, $t = 2.036$

The difference between means required for statistical significance $= 12.4 \times 2.036 = 25.261$

TABLE XIII

Effect of Calomel Soil Treatments on Scabbing in Long Island Soil, Fumigated with Carbon Disulfide, Steam-sterilized, and Infested with Michigan Scab Organisms.

	% of surface area of tubers scabbed			
Calomel p.p.m.	0	50	350	1000
Pot #1.	9.2	7.7	2.5	60.0
2.	4.5	4.4	6.7	65.0
3.	14.2	13.5	10.0	5.0
4.	4.5	10.0	17.2	3.0
5.	8.3	8.6	10.3	75.0
Sum T	40.7	44.2	46.7	208.0
Mean $\overline{X}$	8.14	8.84	9.34	41.60

$Sx = 339.6$ $Sx^2 = 14,867.6$ $St^2 = 49,055.02$

$C = (Sx)^2 \div 20 = 5,766.41$?

$Sx^2 - C = (ST^2 \div 5) - C$ + sum of squares due to error.

Analysis of variance

Variation due to	Degrees of freedom	Sum of squares	Mean square	Error
Total	19	9,101.19		
Treatments	3	4,044.59	1348.20	
Error	16	5,056.60	316.038	17.777

$F = 1348.20 \div 316.038 = 4.27$, which is significant.

The standard error of differences between means = $17.777 \times \sqrt{2} \div \sqrt{5} = 11.24181$ From Fischer's table, t= 12

The difference between means required for statistical significance = $11.2418 \times 2.12 = 28.833$

TABLE XIV

Effect of Calomel Soil Treatments on Scabbing in Long Island Soil, Fumigated with Carbon Disulfide, Steam-sterilized, and Infested with Long Island Scab Organisms.

	% of surface area of tubers scabbed			
Calomel p.p.m.	0	50	350	1000
Pot #1.	.5	.0	1.0	.0
2.0	.5	.0	.0	.0
3.0	5.5	.0	.0	.0
4.0	12.0	traces	.0	.0
5.0	.5	.9	.0	.0
Sum T	19.0	.9	1.0	.0
Mean $\bar{X}$	3.8	.18	.20	.0

$Sx = 20.9$, $Sx^2 - 176.81$, $ST^2 - 362.81$, $C = (Sx)^2 \div 20 - 21.84$

$Sx^2 - C - (ST^2 \div 5) - C +$ sum of squares due to error.

Analysis of variance

Variation due to	Degrees of freedom	Sum of squares	Mean square	Error
Total	19	154.97		
Treatments	3	50.72	16.907	
Error	16	104.25	6.516	2.5526

$F = 16.907 \div 6.516 = 2.59$, which is not significant.

The standard error of differences between means = $2.5526 \times \sqrt{2} \div \sqrt{5} = 1.61$ From Fischer's table, $t = 2.12$

The difference between means required for statistical significance - $1.61 \times 2.12 = 3.41$

TABLE XV

Effect of Calomel Soil Treatments on Scabbing in Michigan Soil, Steam-sterilized and Infested with Long Island Scab Organisims.

	% of surface area of tubers scabbed			
Calomel p.p.m.	0	50	350	1000
Pot #1.	85.0	1.6	.0	.0
2.	7.8	4.0	.0	.0
3.	2.6	.0	trace	trace
4.	16.1	.5	2.8	.0
5.	60.8	2.4	27.1	.0
6.	10.8	2.9	.0	.0
7.	60.4	1.8	14.5	trace
8.	51.9	.0	.0	.0
9.	25.1	.0	3.7	.0
10.	61.4	2.5	.0	.0
Sum T	381.9	15.7	48.1	trace
Mean $\bar{X}$	38.19	1.57	4.81	trace

$Sx = 445.7$ $Sx^2 = 23,115.49$ $St^2 = 148,407.71$

$C = (Sx)^2 \div n = (445.7)^2 \div 40 = 4966.212$

$Sx^2 - C = (ST^2 \div 10) - C$ + sum of squares due to error.

Analysis of variance

Variation due to	Degrees of freedom	Sum of squares	Mean square	Error
Total	39	18,149.28		
Treatments	3	9,874.56	3291.52	
Error	36	8,274.72	229.85	15.161

$F = 3291.52 \div 229.85 = 14.32$, which is significant.

The standard error of differences between means =

$15.161 \times \sqrt{2} \div \sqrt{10} = 6.780$.

From Fischer's table, $t = 2.030$.

The difference between means required for statistical significance = $6.780 \times 2.030 = 13.76$

TABLE XVI

Effect of Calomel Soil Treatments on Scabbing in Long Island Soil, Fumigated with Carbon Disulfide and Desiccated in a Dusty Greenhouse: probably Contaminated with Dust from Local Scab-infested Soil.

	% of surface area of tubers scabbed			
Calomel p.p.m.	0	50	350	1000
Pot #1.	.0	.0	.4	3.1
2.	.0	1.5	1.8	.0
3.	trace	trace	.0	.0
4.	trace	.3	.6	.0
5.	trace	.3	.0	.0
Sum T	trace	2.1	2.8	3.1
Mean $\overline{X}$	trace	.42	.56	.62

$Sx = 8.0$ $Sx^2 = 15.8$ $ST^2 = 21.86$ $C = (Sx)^2 \div 20 = 3.20$

$Sx^2 - C = (ST^2 \div 5) - C$ + sum of squares due to error.

Analysis of variance

Variation due to	Degrees of freedom	Sum of squares	Mean square	Error
Total	19	12.600		
Treatments	3	1.172	.3907	
Error	16	11.428	.714	.8850

$F = .301 \div .714 = .547$, which is not significant.

The standard error of differences between means =

$.8450 \times \sqrt{2} \div \sqrt{5} = .534$ From Fischer's table, $t = 2.12$

The difference between means required for statistical significance = $.534 \times 2.12 = 1.13$

TABLE XVII

Effect of Calomel Soil Treatments
on Yield of Potatoes in Michigan Soil

	Wt. in grams					
Calomel p.p.m.	0	50	350	1000	Sums Q	
Soil fumigated but not steamed						
Pot #1.	85	36	91	13		
2.	21	88	113	102		$\bar{X}$ = 71.0278
3.	93	60	65	11		
4.	90	64	55	28		
5.	133	80	111	38		Sx^2 = 238,309
6.	38	139	66	21		
7.	95	122	92.	51		
8.	30	69	118	40		ST^2 = 1,792,109
9.	21	58	180	40		
Sums T	606	716	891	344	2557	
Soil steamed and infested with Long Island scab.						
Pot #1.	44	74	79	71		
2.	94	77	40	52		$\bar{X}$ = 90.3333
3.	82	101	67	42		
4.	94	66	157	30		
5.	123	65	120	71		Sx^2 = 344,712
6.	130	116	189	85		
7.	96	124	123	34		
8.	93	148	72	109		ST^2 = 2,755,770
9.	156	98	97	33		
Sums T	912	869	944	527	3252	
Sums P	1518	1585	1835	871	5809	
Means	84.3	88.1	101.9	48.4	80.7	

$C = (Sx)^2 \div n = (5809)^2 \div 72 = 468,673.3472$

Analysis of variance

Source of variation	Formulae	Degrees Freedom	Sum of squares	Mean square
Total	Sx^2-C	71	114,347.65	
Subclasses	$(ST^2 \div 9)-C$	7	36,646.54	
Within subclasses		64	77,701.11	1214.08
Between sets	$(SQ^2 \div 36)-C$	1	6,708.68	6708.68
Between treatments	$(SP^2 \div 18)-C$	3	28,127.49	9375.83
Subclass discrepency		3	1,788.94	596.31

F (treatments) = 9375.83 ÷ 1214.41 = 7.72, which is significant. From Fischer's table, t = 2.002.
The difference between treatment means required for statistical significance = $(\sqrt{1214.08} \times \sqrt{2} \times 2.002) \div \sqrt{18}$ = 23.2244 F (sets) = 6708.68 ÷ 1214.08 = 5.526, which is significant. The difference between set means required for statistical significance = $(\sqrt{12.1408} \times \sqrt{2} \times 2.002) \div \sqrt{2}$ = 6.9766

TABLE XVIII

Effect of Calomel Soil Treatments
on Yield of Potatoes in Long Island Soil

Wt. in grams

Calomel p.p.m.	0	50	350	1000	Sums Q	
Soil not steamed						
Pot #1.	46	25	60	35		$\bar{X}$ = 63.55
2.	105	42	39	62		
3.	98	50	101	35		Sx^2 = 97,523
4.	120	77	63	15		
5.	105	72	50	71		ST^2 = 440,925
Sums T	474	266	313	218	1271	
Soil steamed and infested with Long Island scab.						
Pot #1.	109	120	52	33		$\bar{X}$ = 82.05
2.	99	81	92	74		
3.	118	79	88	46		Sx^2 = 145,715
4.	80	180	41	88		
5.	81	92	80	80		ST^2 = 695,219
Sums T	487	480	353	321	1641	
Soil steamed and infested with Michigan scab.						
Pot #1.	141	65	48	28		X = 57.05
2.	92	82	41	29		
3.	84	91	34	28		Sx^2 = 88,445
4.	93	40	53	38		
5.	104	21	21	8		ST^2 = 409,567
Sums T	514	299	197	131	1141	
Sums P	1475	1045	863	670	4053	
Means	98.3	69.7	57.7	44.7		

$C - (Sx)^2 \div n - (4053)^2 \div 60 = 273,780.150$

Analysis of variance

Source of variation	Formulae	Deg. Free.	Sum of squares	Mean square	Error
Total	Sx^2-C	59	57902.85		
Subclasses	$(T^2 \div 5)-C$	11	35362.05		
Within subclasses		48	22540.80	469.60	21.67
Between sets	$(SQ^2 \div 20)-C$	2	6730.00	3365.00	
Between treatments	$(SP^2 \div 15)-C$	3	23641.11	7880.37	
Subclass discrepency		6	4990.94	831.82	

F (treatments) = 7880.37÷469.6=16.78, which is significant.
Standard error of difference between treatment means = $21.67 \times \sqrt{2} \div \sqrt{15} = 7.9116$ From Fischer's table, t= 2.01
The difference between treatment means required for statistical significance = 7.912 x 2.01 = 15.903
F (sets) = 3365 ÷ 469.6 = 7.17, which is significant.
The difference between set means required for statistical significance = $(21.67 \times \sqrt{2} \div \sqrt{3}) \times 2.01 = 35.559$

TABLE XIX

Summary of 1938 Results of Pot Experiments with Calomel as a Soil Treatment for Potato Scab Control in Michigan and Long Island Soils.

Soil	Calomel p.p.m.	% of tubers scabbed	% of surface area of tubers scabbed	Yield per pot grams
Michigan, fumigated but not steamed	0	100	26.42	67.3
	50	100	54.28	79.6
	350	100	69.57	99.0
	1000	100	40.67	44.3
Required difference for significance			25.26	33.5
Michigan, neither fumigated nor steamed	0	90	56.64	88.1
	350	100	82.82	90.6
Required difference for significance			27.61	
Long Island, steamed and infested with Michigan scab	0	100	8.14	102.8
	50	100	8.84	59.8
	350	100	9.34	39.4
	1000	100	41.60	26.2
Required difference for significance			23.83	18.2
Long Island, steamed and infested with Long Island scab	0	93	3.80	97.4
	50	13	.18	96.0
	350	20	.20	70.6
	1000	0	.00	64.2
Required difference for significance			3.41	27.4
Michigan, steamed & infested with Long Island scab	0	97	38.19	103.3
	50	48	1.57	91.7
	350	27	4.81	98.4
	1000	22	trace	58.6
Required difference for significance			13.76	32.4
Long Island, not steamed but contaminated with Mich. scab	0	10	trace	98.8
	50	37	.42	53.2
	350	37	.56	62.6
	1000	60	.62	43.6
Required difference for significance			1.13	36.3

THE CAUSE OF INCREASES IN SCABBING FROM MERCURIAL SOIL TREATMENTS

Except in cases where the controls are severly scabbed, mercurial soil treatments in Michigan nearly always give an increase in scabbing which is frequently statistically significant even when one deals with small numbers. Apparently the mercurial either stimulates the activity of Actinomycetes in the soil, or it in some way predisposes the host to infection. By dilution plate counts, Frutchey and Muncie (43) showed that under low soil moisture conditions the presence of mercurials in a soil from East Lansing, Mich., increased the ratio of Actinomycetes to other soil organisms. This increase in number of Actinomycetes took place in soil samples treated with calomel and yellow oxide of mercury held at 20% and at 35% of the moisture holding capacity while there was a decrease in the ratio of Actinomycetes to bacteria and fungi in soil samples held at 55, 70 and 100% of the moisture holding capacity. Under low soil moisture conditions the mercurials apparently either stimulated the development of soil Actinomycetes or inhibited the growth of other soil organisms, so that the Actinomycetes had less competition. These data lend weight to the theory that mercurials aggravate scabbing through their effect on the soil flora. However, seed

treatment with corrosive sublimate sometimes causes a significant increase in scabbing (15). It is difficult to understand how so small an amount of mercury introduced into the soil could affect the soil flora through a sufficient volume of soil to cause a significant increase in scabbing. In this connection, it is of interest to note that Botjes (16) obtained an increase in blackleg (Erwinia carotovora) of potatoes from seed treatment with corrosive sublimate, and Tucker (17) reported an increase in Rhizoctonia infection from soil treatment with yellow oxide of mercury.

It has been shown that calomel at 50 p.p.m. controlled Rhizoctonia scurf and also scab caused by Long Island strains of Actinomyces in Michigan soil. Although this is proof that mercury compounds can be effective in controlling scab in a soil in which an increase in scabbing would have resulted with normal soil infestation, it does not necessarily indicate that the mercurial migrated through the soil since the calomel was mixed thoroughly through the soil. The failure to control scurf with mercurials under field conditions at Lake City, while it was controlled in a pot experiment, might be taken to indicate that the mercurials, which were applied in the planting furrow, did not migrate through the soil sufficiently to afford protection. However, as shown in Table Vll,

yellow oxide of mercury at Lake City had the same effect on scabbing no matter whether it was applied in the planting furrow, or banded on either side of the seed pieces, or placed two inches below the seed pieces, or applied on the surface of the soil, or mixed through the soil in the area in which the tubers would later be formed. There was a much smaller and statistically insignificant increase in scabbing where the mercurial was placed four inches below the seed pieces. Therefore, if the increase in scabbing from mercurials is due to their effect on the soil flora the mercury compound did migrate sufficiently to affect the soil flora except when the mercurial was placed too deep. Since the potato roots would reach the mercurial readily in the case of every placement employed except the one in which only a small increase in scabbing was noted, these data do not detract from the plausibility of the hypothesis that the cause of increase in scabbing with mercurials is due to a predisposing effect on the host plant, and so do not prove that the mercurial migrated through the soil.

In an attempt to throw more light on this subject, several experiments were designed to test each of the two hypotheses as stated above.

Effect of mercurial soil treatments on the parasitism of Actinomyces spp. on beets, radishes, and other hosts.

In 1937, in a study of the host range of phytopathogenic Actinomycetes, the test plants were planted in 100-foot rows in soil that had produced several consecutive crops of heavily scabbed potatoes. In 20-foot strips at right angles to the rows, the soil was treated respectively with calomel at 20 lbs. per acre, lime at one ton, and a combination of 20 lbs. of calomel and a ton of lime. The chemicals were applied to the surface of the soil and raked in. The initial soil reaction of the plot was unusually variable; soil samples gave readings from pH 6.0 to pH 6.8.

The results of these trials are recorded in Table XX. Since there was no replication except for potatoes and the controls, it is not known how much of the variability was due to soil heterogeneity. However, the figures show certain consistent trends. It should be pointed out here that the failure of calomel to cause an increase in scabbing of potatoes in heavily infested soil is in no wise unusual. Calomel usually has no marked effect when the controls average over 50-55% of the surface area of the tubers scabbed, but, when the controls are only lightly to moderately scabbed, calomel nearly always causes a marked increase in both

incidence and severity of scabbing of potatoes under Michigan conditions. In this instance calomel did not greatly affect either the incidence or the severity of scabbing on the roots of radishes, turnips, and rutabagas, but apparently greatly aggravated scabbing on the roots of table beets and eggplants. Lime caused no marked increase in scabbing on any of the hosts except table beets, and the increase in that instance was no greater than occasionally occurs between replications in a randomized system. The combination of calomel with lime aggravated scabbing on all of the hosts except White Icicle radishes, and caused a greater increase in scabbing than did either calomel or lime applied separately except in the case of White Icicle radishes and eggplants.

In 1938, the same hosts were again planted in scab-infested soil using yellow oxide of mercury at 20 lbs. per acre in replicated 20-foot rows. Although eggplants, and the crucifers were scabbed, as noted at mid-season, after heavy rainfall in August the roots were so damaged by fungus rots and maggots that it was not possible to determine the incidence of scab with an adequate degree of accuracy for comparison of the treatments. As shown in Table XXI, yellow oxide of mercury caused an increase in scabbing of beets that

fell just short of statistical significance for percent of the surface area of the roots scabbed, but that was significant when calculations were based on the ~~surface area~~ percent of the roots scabbed.

It is concluded that mercurials not only generally aggravate scabbing of potatoes in field trials at East Lansing, but that they also cause an increase in scabbing on the roots of table beets and eggplants.

TABLE XX

The Effect of Calomel and Lime on the Incidence and Severity of Plant Actinomycosis

Field trial, East Lansing, 1937

Host	Control		Calomel 20 lbs. per acre		Lime 1 ton per acre		Calomel & Lime	
	% scabbed							
	Heavy	Total	Heavy	Total	Heavy	Total	Heavy	Total
Radishes								
Early Scarlet Turnip	7	87	0	80	14	100	63	100
Crimson Giant Globe	15	100	13	100	10	100	56	100
White Icicle	9	89	0	90	0	79	0	89
Turnips								
Early Purple Top	0	22	0	20	0	27	0	52
Rutabagas								
Am. Purple Top	0	67	0	47	4	50	29	71
Beets								
Burpee's Extra Early	0	27	59	75	0	50	67	100
Eggplants								
Burpee's Black Beauty	0	5	85	100	0	18	3	45
Potatoes								
Katahdin	62	100	59	100	63	100	72	100

TABLE XXI

The Effect of Yellow Oxide of Mercury on Scabbing of Table Beets,

Field Trial, East Lansing, 1938

	Control		Mercurial Treatment		Totals	
	% of roots scabbed	% surface area of roots scabbed	% of roots scabbed	% surface area of roots scabbed	% roots	% area
1	34	1.8	99	19.6	133	21.4
2	7	0.4	30	6.8	37	7.2
3	27	1.7	92	16.4	119	18.1
Sum	68	3.9	221	42.8	289	46.7
Mean	22.7	1.3	73.7	14.3	48.64*	17.38*

* Difference between means required for significance.

Variation due to	Deg. of freedom	Sum of squares	Mean square	Error	F
% of roots scabbed					
Total	5	7178.89			
Treatments	1	2689.33	2689.33		9.15
Blocks	2	3901.52			
Error	2	588.04	294.02	13.998	
% of surface area scabbed					
Total	5	342.17			
Treatments	1	237.94	237.94		9.72
Blocks	2	55.25			
Error	2	48.98	24.49	4.949	

The value of "F" required for significance is 4999.

Effect of mercurials on the host plant

Several attempts were made to determine whether or not mercurials predispose potatoes to scabbing. In one instance potato tubers were sprouted, and, when the sprouts were 10-14 inches long, they were detached and planted with each sprout having one end in one pot and the other end in another. Twenty pairs of pots were used for each of three treatments in scab-infested soil: (a) one end of the sprout in soil treated with yellow oxide of mercury (350 p.p.m.) and the other in untreated soil, (b) each end in a pot of treated soil, and (c) each end in a pot of untreated soil. It was intended that each pair of pots should produce a single plant with two sets of roots, but one end of the sprout generally produced no roots when only one top was permitted to develop. The pots were replanted and one plant was permitted to gorw at each end of each sprout. After several replantings in some of the pots, an almost complete stand of such pairs of connected plants was obtained, but the connecting sprout in every case died before the plants reached maturity. If the plants in untreated soil, but connected to plants in treated soil, had produced a scabbier crop than did the connected pairs of plants in untreated soil, it would have indicated that increased scabbing from mercurials is due to predisposition

of the host to disease. Unfortunately, in this trial the controls were too scabby for the mercurial to cause a large increase in scabbing. There were no significant differences in scabbing between the three sets of pots.

With the same motive, another experiment of similar nature was attempted. Seed-pieces with one long sprout each were planted in 8-inch pots and the sprouts threaded through the holes in the bottom of 6-inch pots. As the plants grew the upper pots were filled with soil. There were 40 such pairs of pots. Twenty of the lower pots were filled with soil containing yellow oxide of mercury at 350 p.p.m. The soil in the other pots was untreated. By means of wood blocks, a narrow space was left between the upper and lower pots so that the soil was not continuous. The upper pots were watered sparingly to discourage the production of roots there, and the lower pots were watered heavily. Out of 40 plants, 14 produced tubers only in the lower pot, 12 produced tubers only in the upper pot, and 7 produced tubers in both; consequently the numbers for comparison are small. Only a few of the tubers were severely scabbed; the majority had less than 10% of the surface area scabbed. In the lower pots the tubers in mercury-treated soil were considerably

more scabby than those in untreated soil.. The tubers produced in the upper pots ranged from moderately scabby to clean with no apparent difference between those above treated and untreated soil. Since none of the differences approached significance, the results are not tabulated.

In a third trial with the same aim in mind, mercuric chloride solutions were swabbed on the leaves of potato plants with cotton. No mercurial was applied to the soil. Alternate plants were left as controls. Three concentrations of mercuric chloride, 0.2%, 0.1%, and 0.01%, were applied each to 20 plants. The first application was made three weeks after planting at which time all of the plants had emerged. Two-tenths percent mercuric chloride caused such severe burning that it was not repeated, while the 0.1% solution was applied three times and the 0.01% solution four times at weekly intervals. At harvest all of the tubers were severely and rather uniformly scabbed with no differences between treated and control plants.

In these three trials no evidence was obtained that the increase in scabbing from mercurials is due to a predisposing effect on the host plant. However, the results were not at all conclusive since in two cases the controls were too scabby for mercurials to show

any marked effect on scabbing. Furthermore, the possibility that mercurials predispose potatoes to scabbing through injury to the lenticels, through which infection is said usually, if not always, to take place (29, 41, 78, 118), was not investigated. However, studies with this point of departure were discontinued because dilution plate counts of soil samples left little doubt in the mind of the author that the principal cause of increased scabbing from mercurials arises from the effect of the treatments on the soil flora.

Effect of mercurials and other antiseptics on the soil flora

During the summer of 1937 an attempt was made to determine the effect of various soil treatments on the soil flora by taking samples directly from the field. The results were unsatisfactory due to tremendous differences in plate counts from a small number of samples from plots treated in the same manner. These differences were probably due to soil heterogeneity as regards moisture content, reaction, aeration, and supply of readily decomposable organic matter. Part of the difficulty arose from an unfortunate choice of medium,- beef-peptone agar of pH 7.0. These platings indicated that mercurial soil treatments caused an increase in number of " pin point" and "lens-shaped" bacterial colonies and in the total number of bacterial colonies, while "spreader" bacteria were reduced in number. The Actinomycetal counts were too variable to draw any conclusions from them.

In later studies an effort was made to eliminate the factor of soil heterogeneity by treatment of aliquot samples of thoroughly mixed soil in pots, bottles, or test tubes. In the case of bottles and test tubes the effect of varying soil moisture was also greatly reduced by incubation of the samples in moist chambers.

Technique

In greenhouse experiments the soil was thoroughly mixed by shoveling it over several times before potting. After a crop of potatoes had been harvested, each pot was dumped onto a clean platform and its contents again thoroughly mixed. A soil sample was then taken from each pot, and soil samples from pots with the same treatment were poured together and mixed to give a composite sample. Pebbles and organic debris were removed from the samples with a forceps, but the soil was not screened, since in order to do so it would have been necessary to dry the soil, and unless the screens were cleaned after each sample and precautions were taken against raising dust, a considerable amount of contamination of one sample with another might have arisen from this procedure.

In laboratory studies, the soil for each experiment was air-dried and sifted through a 40-mesh screen, thoroughly mixed, treated, and aliquots placed in large moist chambers for incubation. Care was taken to adjust the volume of samples to equality since it has been shown that the density of soil samples incubated in this manner greatly influences the actinomycetal plate count. No water was added directly to these soil samples, but they absorbed water rapidly in a saturated atmosphere.

To offset the effect of air-drying the samples at the start, long incubation periods were employed in some of the studies.

For plating, 10-gram samples of soil were added to 90 cc. of sterile tap water in a bottle (or 11 grams to 100cc.) In the case of samples in test tubes it was unnecessary to reweigh the samples and thus contamination with laboratory organisms at time of plating was largely avoided. In the case of samples incubated in bottles, sterile water was added directly to the sample to give a 1-10 dilution. One cc. of this dilution was then pipetted into 100 cc. of sterile water to give a 1-1000 dilution. In like manner dilutions up to 1-1,000,000 or higher were prepared.

The 1-10 dilution was shaken 100 times, allowed to stand for 15-30 minutes and shaken 100 times more. After each step in dilution thereafter the dilution bottles were given 100 shakes each. In most cases two or more dilutions were plated, usually 1-100,000 and 1-1,000,000, but usually counts were made from only one of these. One cc. of dilution was pippetted into each Petri dish and roughly 20 cc. of agar (at about 50-55 C.) added to it and the plate rotated a dozen times. The medium employed and temperature of incubation varied from one experiment to another. Colony counts for fungi

in dilutions of 1-10,000 or less were generally made on the third day, and those for bacteria and Actinomycetes (regardless of degree of dilution) on the sixth day and the counts checked on the tenth day. All colonies not definitely recognized as not being Actinomycetes were examined under low power of the microscope. Counts recorded in parentheses were from plates obviously damaged by "spreader" bacteria. Since on some plates Actinomycetes come up apparently uninhibited through spreader bacteria that blot out the bacterial count, a few actinomycetal counts are recorded in the absence of parallel bacterial counts.

That this technique could be repeated closely enough to give comparable results from aliqouts of a single soil sample was demonstrated by plating three 10-gram portions of a thoroughly mixed, air-dried sample using Waksman's egg albumin agar. The results of the 1-100,000 dilution after 10 days incubation at 25 C. are given in Table XXll. Possibly even more comparable results could have been obtained by adjusting the volume of the sterile water blanks after autoclaving and by measuring with precision the volume of agar added to each plate, and by holding the agar for plating in a water-bath so that its temperature would have been exactly the same for each sample; but it is questionable whether the additional accuracy accruing from such a practice would compensate for the additional time and labor required.

TABLE XXII

Plate Counts from Three Aliquots of the Same Soil Sample.

at Dilution of 1-100,000*

Aliquot	Plate	Bacteria	Actinomycetes	Fungi
1	1	95	24	2
	2	109	19	1
	3	98	22	2
	4	120	28	3
	5	102	28	4
	6	124	23	1
	7	135	28	0
	8	168	15	6
	9	122	26	2
	10	(overrun by fungi)		
	Mean	119.33	23.67	2.33
2	1	115	20	1
	2	76	16	2
	3	117	28	3
	4	149	39	0
	5	128	23	3
	6	90	13	2
	7	152	15	2
	8	86	40	3
	9	101	16	2
	10	(overrun by fungi)		
	Mean	112.67	23.33	2.00
3	1	119	29	5
	2	86	38	4
	3	111	33	2
	4	148	41	1
	5	117	27	2
	6	73	12	1
	7	127	36	0
	8	110	27	1
	9	119	34	3
	10	92	16	3
	Mean	110.20	29.30	2.20

By inspection: no significant differences.

*Waksman's egg albumin agar, incubated 10 dyas at 25° C.

Mercuric chloride in local soil.

In a preliminary study of the action of antiseptics on the soil flora, duplicate 10-gram soil samples, untreated and treated with 10 and 100 parts per million of bichloride of mercury, were incubated in a moist chamber (placed in a 25 C. constant-temperature incubator) for two weeks and then plated with sodium asparaginate agar adjusted to pH 7.0. The results from the duplicate samples checked fairly well. The average number of Actinomycetes per plate for the 1-100,000 dilution increased from 5.12 for the controls to 7.13 and 8.78 for 10 p.p.m. and 100 p.p.m. of bichloride of mercury, respectively. The number of bacterial colonies increased from 7.4 for the control to 921 for 10 p.p.m. and 15.9 for 1000 p.p.m. The percent of plate counts ruined by spreading bacterial colonies decreased from 50% for the control to 20% and 10% for 10 and 100 p.p.m. respectfully (Table XXIII).

Calomel in Long Island soil (contaminated with local flora):

In like manner scab-infested Sassafras Loam from Riverhead, New York (from a pot employed as a "not artifically infested" control in the 1937 experiment on the effect of calomel on scabbing in Long Island soil) was treated with calomel at rates up to one part

by weight of calomel to 100 parts of soil. The treated samples were incubated in test tubes in a moist chamber at room temperature. Platings were made on beef-peptone agar (pH 7.0) after two, three, and four weeks. The plates were incubated at 25 C. except that for the third plating an entire set was also run at 16 C. The soil samples in the moist chamber contained about 12% moisture at each plating. A few untreated samples were also placed in a rack outside the moist chamber as "air-dried controls".

The results for the 1-1,000,000 counts for bacteria and Actinomycetes are given in Tables XXIV and XXV respectively. Since the order of recording of plate counts in the two tables is identical, one may compare the bacterial and actinomycetal counts for individual plates as well as for treatments.

In analyzing these data one must proceed with caution since duplicate samples were not plated as was done in the experiment with corrosive sublimate. All of the plates at one plating were poured from a single batch of agar, but a new batch was made up for each plating. The increase in numbers of both bacteria and Actinomycetes in nearly all treatments including air-dried soil in later platings might lead one to suspect that the medium was not duplicated and that this increase

was not real. With these short-comings in mind, it yet seems fair to conclude that:

Calomel at high rates of application, and even up to 1% of the weight of the soil, did not eliminate Actinomycetes from soil samples, and did not reduce the bacterial population as measured by the number of colonies on dilution plates. A small amount of calomel in the soil (10 p.p.m.) caused a striking increase in number of Actinomycetes which was consistent at every plating, and a smaller increase in the bacterial population. Air-drying soil samples up to four weeks did not reduce the number of actinomycetal colonies developing on plates but reduced the number of bacterial colonies to about one half that of similar samples incubated in a moist chamber. The number of bacterial colonies and the percent of total colonies that were bacterial was much greater in plates incubated at 16 C. than at 25 C.

There were few to no fungi on the plates at high dilutions. However, at the second plating, ten 1-5000 dilution-plates were poured from each sample. One half of these were incubated at 16 C. and the others at 25 C. The results (Table XXVI) seem to indicate that the calomel had very little effect on the fungal count. However, with high rates of treatment the fungi appeared to

develop much better at the lower temperature. Since practically all the colonies counted were Penicillia, no information was obtained on the effect of the treatments on other fungi.

Calomel in local soil:

In the preceding experiment it was shown that in unsterilized soil actinomycetes and bacteria increased in number with small applications of calomel. In the 1938 pot experiment 1% unsterilized soil was added to sterilized soil in some instances. Parallel to this a soil treatment experiment was carried on in bottles held in a garbage can converted into a moist chamber, for comparison of the effect of calomel on the soil flora in unsterilized soil and in sterilized soil to which 1% unsterilized, scab-infested soil was added. No Actinomycetes cultures were added to the sterilized soil in the bottles as had been done in the pot experiment. Water was added to the soil in the bottles to bring it up to 50% moisture holding capacity (14% of the oven-dry weight of the soil) before placing the bottles in the moist chamber. Samples were plated on glucose agar after one week and after three months of incubation. The plates were held at 25 C.

The results for the counts of both the 1-1000,000 and the 1-1,000,000 dilutions after one week of incubation

of treated and untreated soil are given in Tables XXV11 and XXV111. In the unsterilized soil calomel had a marked depressing effect on the number of Actinomycetes even at 10 p.p.m. (a rate at which there was an increase in Actinomycetes after two-weeks incubation in Long Island soil). However, calomel did not eliminate Actinomycetes even at the rate 1 part of calomel to 99 parts of soil. No Actinomycetes developed in plates from samples of 1 part of calomel to 9 parts of soil, but this does not necessarily prove that the Actinomycetes were killed in the soil, they may have been prevented from growing through the transfer of a considerable amount of calomel to the agar in the dilution process.

After one week's incubation extremely heavy applications (10,000 & 100,000 p.p.m.) of calomel caused enormous increases in number of bacterial colonies, while 100 & 1000 p.p.m. had little to no effect on the number of colonies although it was obvious that the predominant types of colonies in the treated soil were not the same as those in untreated soils.

After three month's incubation of unsterilized soil in the moist chamber (Table XX1X), the number of Actinomycetes in the control appeared to have decreased,

In all of the treatment samples the Actinomycetes had increased in numbers since the first plating, with more than a 1200% increase in soil containing 1% (10,000 p.p.m.) of calomel. The number of bacteria was again greatest in the mixture of 1 part of calomel to 9 of soil. In this case there were nearly as many Actinomycetes in the soil contain 10% of calomel as there were in the control.

At least theoretically there were only one percent as many of each of bacteria, fungi, and actinomycetes in the sterilized soil to which 1% of unsterilized soil had been added as there were in the unsterilized soil. However, after one week of incubation the bacteria had increased so enormously that there were 18 to 56 times as many bacterial colonies from the initially sterilized soil as from the unsterilized soil not only in the untreated soil, but with all rates of treatments except the two highest. Although with 10,000 p.p.m. (1 part calomel to 99 of soil) there were as many or more bacteria than there were in the control in initially sterilized soil, there was with that rate of treatment just as large an increase in the unsterilized soil.

At the first plating of initially sterilized soil there were no Actinomycetes on any of the plates at any dilution employed (including 1-10,000) except in the

case of the control where there were 5 actinomycetes on four plates. This is slightly less than 1% as many as found in the parallel unsterlized sample, so there appears to have been little or no change in the actinomycetal flora (as measured in this manner) at a time when the bacteria were increasing at a tremendous rate. After three months, however, the Actinomycetes had shown an increase, reaching 120,000 per gram in the control as compared with 400,000, 460,000 and 280,000 with calomel at 100, 1000, and 10,000 p.p.m. respectively. On the other hand, the number of bacteria in initially sterilized soil had decreased since the first plating in all samples. This was true for unsterilized soil also except in the case of the control where the difference was small and in the other direction.

TABLE XXIII

Effect of Mercurials on the Soil Flora as Determined by Dilution-plate Counts

$HgCl_2$ p.p.m.		I bact.	I act	II bact.	II act.
0		9	5	6	7
		3	5	11	5
		*	*	8	4
		*	*	*	*
		*	*	*	*
	$\bar{x}$	6.0	5.0	8.3	5.3
10#		670	11	850	12
		1000	3	800	10
		900	4	900	8
		1350	4	900	5
		*	*	*	*
	$\bar{x}$	980.	5.5	863	8.8
100		14	10	20	8
		16	16	16	6
		15	5	14	8
		19	7	18	7
		*	*	11	12
	$\bar{x}$	16.0	9.5	15.8	8.2

Corrosive sublimate treatments in local scab infested soil in test tubes, incubated 2 weeks at 25 C. in a moist chamber. Plated on sodium asparaginate agar of pH 7.0. Plates incubated at 32 C. and counts made on 6th day and checked on 10th. Dilution: 1-100,000.

1/16 of plate counted and number of bacterial colonies estimated from this, entire plate counted for actinomycetes.

* Count ruined by "spreader" bacteria.

TABLE XXIV

Effect of Mercurials on the Soil Flora as Determined by Dilution plate Counts

ACTINOMYCETES COUNTS

Soil incubated		parts per million of calomel						
		0*	0	10	100	500	1000	10000
2 weeks			15	15	6	6	6	2
			15	16	7	7	4	1
			10	17	6	3	4	3
			15	17	2	7	7	1
			-	22	4	3	-	4
	$\bar{x}$		13.8	17.4	5.0	5.2	5.3	2.2
3 weeks		25	34	122	44	18	39	4
		25	27	117	31	25	36	1
		15	39	101	22	-	27	1
		29	57	108	48	-	44	1
		28	-	120	-	-	6	1
	$\bar{x}$	24.4	39.3	113.6	36.5	21.5	36.5	1.6
4 weeks		50	64	65	39	24	57	13
		48	46	171	42	18	24	8
		41	30	133	50	20	40	4
		41	60	66	32	13	40	12
		50	62	176	-	-	33	9
	$\bar{x}$	46.0	52.4	122.2	40.8	18.8	38.8	9.2
4 weeks (plates incubated at 16C;) counted after 2 weeks.		59	56	248	46	36	44	8
		53	52	263	32	36	36	9
		29	51	269	32	40	56	11
		52	41	261	34	42	45	3
		-	-	279	-	37	47	6
	$\bar{x}$	48.3	50.0	267.0	36.0	38.2	45.6	7.4

* "Air-dried control", similar to the control except that the tubes were placed in a rack out side the moist chamber.

All missing counts are due to "spreader bacteria."

Calomel treatments in Long Island soil (contaminated with local soil flora) in test tubes incubated at room temperature in a moist chamber. Plated on beef-peptone agar (pH 7.0) agar; incubated at 25C; counts made on 6th day and checked on 10th.
Dilution: 1-1,000,000.

TABLE XXV

Effect of Mercurials on the Soil Flora as Determined by Dilution-plate counts

BACTERIA COUNTS
1-1,000,000

soil incubated		parts per million of calomel						
		0*	0	10	100	500	1000	10000
2 weeks			7	16	18	11	18	20
			10	18	58	23	21	27
			11	14	41	16	19	21
			8	8	21	39	26	19
			-	16	18	32	-	18
	$\bar{x}$		9	14.4	31.2	24.2	21.0	21.0
3 weeks		29	92	101	46	33	43	80
		40	67	105	50	38	49	36
		38	90	122	32	-	35	40
		35	97	108	49	-	37	113
		-	-	105	-	-	-	35
	$\bar{x}$	35.5	86.5	108.2	44.3	35.5	41.0	60.8
4 weeks		43	116	41	59	54	103	208
		43	106	85	47	24	39	90
		32	56	102	38	17	46	109
		35	103	-	47	-	84	115
		37	85	-	-	-	88	70
	$\bar{x}$	38.0	93.2	76.0	47.8	31.7	73.0	118.4
4 weeks (plates incubated at 16 C.)		82	114	160	186	91	131	180
		73	119	124	26	105	131	176
		69	117	134	22	101	119	165
		45	84	128	101	95	125	168
		-	-	112	-	107	121	172
	$\bar{x}$	67.8	108.5	131.6	83.8	99.8	125.4	173.0

* "Air-dried control", same as control except that the tubes were placed in a rack out side the moist chamber.

$\bar{x}$ = mean. All missing counts are due to "spreader bacteria."

(Soil and method the same as for Table XXIV)

TABLE XXVI

Effect of Mercurials on the Soil Flora as Determined by Dilution-plate Counts

FUNGI COUNTS
1-5,000

plates incubated at		parts per million of calomel					
		0*	0	10	100	500	1000	10000
25° C. (count on 3d day)		306	170	342	276	215	142	123
		274	166	347	256	209	119	117
		290	220	335	313	212	127	131
		294	188	344	289	224	110	111
		264	128	366	235	180	131	86
	$\bar{x}$	285.6	174.4	346.8	279.6	208.0	125.8	113.6
16° C. (count on 4th day)		56	58	103	91	82	78	118
		108	63	106	82	92	104	112
		90	64	110	97	89	106	115
		51	61	106	64	84	79	107
		57	59	106	82	87	98	114
	$\bar{x}$	72.4	61.0	106.2	83.2	86.8	93.0	113.2

* "Air- dried control", same as control except that the tubes were placed in a rack outside the moist chamber.

$\bar{x}$ = mean.

(Soil incubated 3 weeks, plated on Cook's agar, pH 4.8.)

TABLE XXVII

Effect of Mercurials on the Soil Flora as Determined by Dilution-plate Counts

Unsterilized Soil; Incubated one Week

p.p.m. HgCl	1-100,000* bact.	act.	fungi	1-1,000,000* bact.	act.	fungi
0	28	28	0	1	0	0
	17	13	2	3	0	1
	16	18	2	1	2	0
	24	22	2	2	3	0
	16	15	2	1	0	1
x̄	20.2	18.8	1.6	1.6	1.0	0.4
10	13	2	0	1	0	1
	11	3	1	2	0	0
	6	1	1	1	1	0
	5	3	0	1	1	0
	8	2	1	0	0	0
x̄	8.6	2.2	0.6	1.0	0.4	0.2
100	23	4	3	0	0	0
	24	3	2	0	0	0
	25	1	0	1	1	0
	25	1	3	5	1	1
	21	0	1	1	0	0
x̄	23.6	1.8	2.2	1.4	0.4	0.2
1000	17	3	2	3	1	0
	19	3	1	2	1	0
	18	3	4	2	0	1
	29	0	0	3	0	1
	37	4	1	2	1	1
x̄	24.0	2.6	1.6	2.6	0.6	0.6
10000	-	7	2	42	0	1
	-	8	2	44	0	2
	-	8	3	43	0	1
	-	11	0	40	1	0
	-	7	0	42	0	0
x̄	-	8.2	1.4	42.2	0.2	0.8
100000	107	0	0	15	0	0
	98	0	0	6	0	0
	105	0	2	9	0	0
	89	0	2	7	0	1
	85	0	3	5	0	2
x̄	96.8	0	1.4	8.4	0	0.6

TABLE XXVII
(continued)

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* Dilution counted.

Calomel treatments in local scab-infested soil incubated in dilution bottles in a moist chamber at 20-22 C. Plated on glucose agar; incubated at 25 C.; counts made on 6th day and checked on 10th.

TABLE XXVIII

Effect of Mercurials on the Soil Flora

Steam Sterilized Soil Mixed with 1% Unsterilized, Scab-infested Soil; incubated one Week

p.p.m. HgCl	1-1,000,000 bact.	act.	fungi	1-100,000 bact.	act.	fungi
0	38	0	0	-	0	0
	42	0	1	-	-	1
	32	0	0	-	1	0
	40	0	0	-	2	0
	31	0	0	-	0	0
	x 36.6	0	0.2	-	0.8	0.2
10	46	0	2	-	-	+
	50	0	2	-	-	+
	38	0	1	-	0	4
	48	0	2	-	0	2
	-	-	+	-	0	2
	x 45.5	0	1.8	-	0	2.7+
100	108	0	1	-	-	+
	106	0	1	-	-	+
	89	0	3	-	0	0
	96	0	0	-	0	8
	84	0	0	-	-	+
	x 96.6	0	1.0	-	0	8+
1000	142	0	0	-	0	6
	144	0	2	-	0	2
	136	0	1	-	0	2
	139	0	3	-	0	5
	117	0	1	-	0	6
	x 135.6	0	1.0	-	0	4.4
10000	40	0	1	-	0	1
	37	0	0	-	0	1
	45	0	2	-	0	2
	53	0	0	-	0	2
	34	0	2	-	0	2
	x 41.8	0	1.0	-	0	1.6
100000	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	1	0	0	0
	0	0	1	0	0	1
	x 0	0	0.4	0	0	0.2

TABLE XXVIII
(continued)

1-10,000 dilution: Actinomycetes from control: 2,1,1,1, and 5th plate overrun by fungi. No actimomyces at any dilution from treated soil.

[Soil and method the same as for Table XXVII]

TABLE XXIX

Effect of Mercurials on the Soil Flora as Determined by Dilution-plate Counts

Soil incubated three Months; Dilution 1-100,000

	Unsterilized soil			Soil sterilized and infested with 1% unsterilized soil.		
p.p.m. HgCl	bact.	act.	fungi	bact.	act.	fungi
0	27	4	1	91	1	1
	21	7	0	88	1	0
	31	5	1	107	1	3
	21	8	3	93	2	1
	26	8	1	-	1	+
$\bar{x}$	25.2	6.4	1.2	94.8	1.2	1.0
10	13	2	2	307	0	1
	10	6	2	315	0	4
	15	2	4	285	0	2
	12	8	1	300	0	4
	23	9	2.2	-	-	+
$\bar{x}$	14.6	5.4	2.2	301.8	0	2.8
100	9	9	4	47	4	2
	9	0	2	24	1	1
	5	12	1	32	3	5
	8	8	3	43	5	1
	4	12	2	65	7	1
$\bar{x}$	7.0	8.2	1.4	42.2	4.0	2.0
1000	2	4	0	98	5	5
	8	4	1	93	8	3
	4	3	1	96	4	6
	7	3	2	112	2	5
	2	5	0	106	4	4
$\bar{x}$	4.6	3.8	0.8	101.0	4.6	4.6
10000	12	81	5	226	4	2
	18	104	6	209	0	0
	24	99	5	238	3	1
	13	112	4	212	4	0
	6	114	3	233	3	0
$\bar{x}$	14.6	102.0	4.6	223.6	2.8	0.6
100000	90	4	7	0	0	3
	86	4	6	0	0	0
	84	5	6	0	0	0
	77	4	7	0	0	0
	81	3	12	0	0	0
$\bar{x}$	83.6	4.0	7.6	0	0	0.6

(Soil and method the same as for Table XXVII)

Calomel in the pot experiment of 1938:

On July 17, 1938, a soil sample was taken from each pot in the experiment with Long Island and local soil, using a cork borer. Samples from pots treated in the same manner were mixed to form composite samples so that only one composite sample was plated for each treatment. Five plates of each of two dilutions (1-200,000 and 1-20,000) were poured, but in all cases there were too many fungi to permit bacteria and Actinomycetes to be counted at the lower dilution. An attempt was again made to use beef-peptone agar, but with disastrous results. In Table XXX missing counts are due to plates having been spoiled by "spreader" bacteria. Counts recorded in parenthesis were from plates obviously damaged by "spreaders" and were not taken into consideration in deriving mean values.

The plate-counts are summarized in Table XXXl. Apparently there was little, if any, relationship between the number of soil Actinomycetes as determined in this manner and the severity of scabbing of the tubers at harvest. However, it will be noted that there was a tendency for fewer "spreader" colonies in treated soil. 26.7% of all plates (of the 1-200,000 dilution) from all control pots were free from spreaders, whereas 45.0%, 60.0%, and 58.0% of the plates from all pots treated with calomel at 50, 350, and 1000p.p.m., re-

spectively, were free from spreaders.

Sodium thiocarbonate in local soil:

Soil from an orchard and on which potatoes had not been grown in years, if at all, was thoroughly shoveled over to avoid soil heterogeneity and then potted in 12-inch pots in the greenhouse. Two pots were used for each treatment, but one of each pair was waxed and the basal hole plugged with a tight-fitting stopper while the other was neither waxed nor plugged: consequently, there was no real replication. All of the soil except enough for two pots was artificially infested with scab by adding to it one gram of macerated potato peels from scabby tubers for each pound of soil. The next day two pots each were treated with sodium thiocarbonate at rates of 0.001, 0.01, 0.02, 0.05, 0.10, 0.20, 0.30, and 0.50 lbs. of the 50% solution per square foot of soil surface. The soil was kept saturated for eight days and then planted with formaldehyde-treated Katahdin tubers (March 10, 1938).

The crop was harvested nine weeks after planting. The number of scab lesions were counted for each tuber, and the weight of the tuber taken. These results are summarized in Table XXll. It will be noted that there was no tendency for the chemical to control scab except at rates that caused a sharp reduction in yield. At

high rates of application there was a marked reduction in number of lesions per tuber. As usual the soil that was not artificially infested became contaminated in the greenhouse.

Two days after harvesting the tubers soil samples were taken from six pairs of the pots, the soil in each pair of pots being first thoroughly stirred. These were plated with sodium asparaginate agar of pH 6.0. The results of counts of the 1-1,000,000 dilution plates are given in Table XXXlll along with a statistical analysis. Such an analysis is an experiment of this sort is objectionable on the grounds that all of the readings for a given treatment are for a single (composite) soil sample and not from separate samples from individual pots.

In Table XXXll a comparison is made between the number of lesions per tuber and the number of Actinomycetes that developed on the dilution plates for pairs of pots. It will be noted that the correlation between these two sets of observations is fairly good, that is, reduction in scabbing was accompanied by a reduction in the total number of soil Actinomycetes as determined in this manner. Bacterial numbers were less affected by the treatments, but there was a "significant" reduction with the highest rate of treatment. Fungal numbers ran roughly parallel to the actinomycetal numbers.

TABLE XXX

Comparison of Severity of Scabbing with Dilution-plate Counts of Soil Microorganisms in the Pot Experiment with Long Island and Local Soils in 1938.

Plated July 18 on beef-peptone agar, pH 6.0; incubated at 25C.; counts made on 7th day; dilution: 1-200,000.

Soil	HgCl p.p.m.		Bacteria	Actino-mycetes	% scab. Severity	% scab. Incidence
Michigan; not fumigated, nor steamed, nor artifically infested.	0		56	13		
			(28)	(7)		
			(15)	(3)		
		Mean*	56	13	56.64	90
	350		38	8		
			42	18		
			41	12		
		Mean	40.3	12.7	82.82	100
Michigan; fumigated, not steamed, nor artifically infested	0		22	3		
			51	12		
			53	6		
			42	7	26.42	100
	50		25	12		
			15	9		
			14	17		
			18	12.7	54.28	100
	350		19	11		
			18	8		
			19	7		
			20	5		
			14	6		
			18	12.3	69.57	100
	1000		32	8		
			32	15		
			69	14		
			33	8		
			41.5	11.3	40.67	100
Michigan; steamed and infested with "Long Island Scab"	0		262	0		
			(164)	0		
			192	0		
		Mean*	227	0	38.19	97
	50		76	2		
			62	0		
			(42)	(0)		
		Mean*	69	1	1.57	48

TABLE XXX (continued)

Soil	HgCl p.p.m.	Bacteria	Actinomycetes	% scab Severity	% scab Incidence
Michigan; steamed and infested with "Long Island Scab"	350	54	0		
		38	0		
		62	0		
		52	0		
		Mean 51.5	0	4.81	27
	1000	68	0		
		82	0		
		48	0		
		Mean 66	0	trace	22
Michigan; air dried from planting date	0	27	6		
		38	8		
		Mean 32.5	7.0	--	--
Long Island; fumigated, not steamed, nor artifically infested; probably contaminated "Mich. scab".	0	all spreaders		trace	100
	50	51	15		
		(1)	(3)		
		(1)	(4)		
		(1)	(6)		
		Mean*51	15	0.42	100
	350	122	13		
		132	19		
		Mean127.5	16	0.56	100
	1000	88	19		
		79	17		
		(1)	(5)		
		(1)	(3)		
		(1)	(2)		
		Mean*83.5	18	062	100
Long Island; fumigated.	0	all spreaders		0.80	93
steamed, and infested with "Long Island " scab".	50	all spreaders		0.18	13
	350	80	6		
		72	4		
		(1)	(2)		
		Mean*76	5	0.20	20
	1000	130	2	0.00	0

TABLE XXX (continued)

Soil	HgCl		Bacteria	Actino-mycetes	% scab Incidence	% scab Severity
Long Island; fumigated, steamed, and infested with "Mich. scab".	0		48	4		
			46	11		
			(35)	(3)		
			-	(6)		
		Mean*	47	7.5	100	8.14
	50		150	4		
			138	4		
			157	3		
			140	8		
			125	10		
		Mean	142	5.8	100	8.88
	350		82	3		
			132	2		
		Mean	112	2.5	100	9.34
	1000		181	2		
			72	9		
			194	8		
			180	0		
			200	12		
		Mean	145.4	6.2	100	41.60
Long Island, air dried from date of shipment	0		2	3		
			5	1		
			7	3		
			-	(3)		
		Mean	4.7	(2.5)	--	--

TABLE XXXI

Summary of the Plate-Counts from the Pot Experiment with Long Island and Local Soils.

Soil	Calomel p.p.m.	Dilution 1-200,000 ave. count per plate bact.	act.	% plates free from "Spreaders"	% scab
Michigan: air-dried from planting date.	--	32.5	7.5	40	--
Mich.: not fumigated nor steamed	0	56.0	13.0	20	56.64
	350	40.0	12.7	60	82.82
Mich: fumigated, not steamed	0	42.0	7.0	60	26.42
	50	18.0	12.7	60	54.28
	350	18.0	12.3	100	69.57
	1000	41.5	11.5	80	40.67
Mich.: steamed and infested with "Long Island Scab"	0	227.0	0	40	38.19
	50	69.0	1	40	1.57
	350	51.5	0	80	4.81
	1000	66.0	60	60	trace
Long Island: not steamed or artificially infested but probably contaminated with local scab.	0	---	---	0	trace
	50	51.0	15	20	0.42
	350	127.5	16	40	0.56
	1000	83.5	18	40	0.62
Long Island: steamed, infested with "Long Island Scab"	0	---	---	0	3.80
	50	---	---	0	0.18
	350	76.0	5.0	40	0.20
	1000	130.0	2.0	10	0.00
Long Island: steamed infested with "Michigan scab"	0	47.0	7.5	40	8.14
	50	142.0	5.8	100	8.88
	350	112.0	2.5	40	9.34
	1000	145.4	6.2	100	41.60
Long Island: air dried from date of shipment	-	4.7	2.5	60	---

TABLE XXXII*

Effect of Sodium Thiocarbonate on Scabbing, Yield, and Dilution-plate Counts of soil Actinomycetes in a Pot Experiment.

$NaCS_3$, lbs. per sq. ft.	pot	Tubers no.	Tubers wt. grams	Scab: no. lesions total per tuber	Scab: no. lesions total per tuber	Actinomycetes 1,000,000's per gram
0*	u.	6	112	1030	136	18.8
	p.	2	59	57		
0	u.	11	97	387	76	17.0
	p.	3	124	674		
0.001	u.	5	52	385	58	-
	p.	6	48	253		
0.01	u.	8	124	418	70	16.4
	p.	4	44	422		
0.02	u.	4	55	143	27	*
	p.	7	46	158		
0.05	u.	2	16	123	61	17.0
	p.	4	70	245		
0.10	u.	4	28	26	26	-
	p.	7	80	260		
0.20	u.	2	8	11	3	5.2
	p.	2	0.8	0		
0.30	u.	7	42	29	5	-
	p.	4	19	24		
0.50	u.	2	0.8	0	0	3.8
	p.	0	0.0	0		

Pots: u. = hole in bottom of pot not plugged; p. = hole plugged with a cork. 0* = soil not artifically infested; soil from an orchard was used in this experiment and all the soil except that for this set of controls was infested by means of macerated potato scabs.

* in published data the means were derived in a different manner, e.g. number of lesions per Tuber for 0* = $\left(\frac{1030}{6} + \frac{57}{2}\right) \div 2 = 101$ instead of $\frac{1057}{8} = 136$

TABLE XXXIII

Effect of Sodium Thiocarbonate on the Soil Flora as Determined by Dilution-plate Count

(Plated with sodium asparaginate agar, pH 6.0; incubated at 25°C.; counted on 7th day; dilution 1:1,000,000)

Na_2CS_3, lbs. per sq. ft.	1	2	3	4	5	T	$\bar{X}$	
Bacteria								
None*	0	0	7	5	2	14	2.8	
None	2	2	12	3	4	23	4.6	Sx = 77
.01	5	1	0	2	6	14	2.8	Sx^2 = 391
.05	0	3	3	3	3	12	2.4	ST^2 = 1195
.20	2	1	2	2	4	11	2.2	C = 2.5667
.50	0	0	0	2	1	3	.6	
Fungi								
None*	3	4	4	3	3	17	3.4	
None	5	4	4	4	3	20	4.0	Sx = 66
.01	5	8	6	1	3	23	4.6	Sx^2 = 284
.05	1	2	0	1	1	5	1.0	ST^2 = 1244
.20	0	0	1	0	0	1	.2	C = 145.2
.50	0	0	0	0	0	0	.0	
Actinomycetes								
None*	20	20	20	14	20	94	18.8	
None	20	16	18	15	16	85	17.0	Sx = 391
.01	5	9	16	38	14	82	16.4	Sx^2 = 7197
.05	14	12	30	17	12	85	17.0	ST^2 = 31,047
.20	11	4	3	5	3	26	5.2	C 5096.0333
.50	5	2	6	4	2	19	3.8	

$Sx^2 - C = (ST^2 \div 5) - C$ + sum of squares due to error.

Analysis of variance

Variation due to	Degrees of freedom	Sum of squares	Mean square	Error
Bacteria				
Total	29	388.433		
Treatments	5	236.433	47.2866	
Error	24	152.000	6.3333	2.5166
Fungi				
Total	29	138.8		
Treatments	5	103.6	20.7200	
Error	24	35.2	1.4667	1.2111
Actinomycetes				
Total	29	2100.967		
Treatments	5	1113.367	222.6734	
Error	24	987.6000	41.1500	5.4113

	F	Standard error difference between means	Difference required for significance
Bacteria	7.47	1.5914	3.2847
Fungi	14.13	.9275	1.9143
Actinomycetes	5.41	4.0566	8.3728

* Soil from an orchard was used in the experiment and all the soil except for this set of controls was infested by means of macerated potato scabs.

Studies on the tolerance of Actinomycetes to mercuric chloride

It was concluded from pot experiments that the parasitic Actinomycetes in scab-infested Long Island (New York) and New Jersey soils must be less tolerant of mercury compounds as antiseptics than are those in scab-infested Michigan soils. To test this hypothesis numerous Actinomycetes were planted in duplicate tubes of glycerine synthetic solution (20 g. glycerine, 2 g. $NaNO_3$, 1 g. KH_2PO_4, $\frac{1}{2}$ g. each of KCl and $MgSO_4 \cdot 7HOH$ and a trace of $FeCl_2$ in a liter of distilled water; pH 6.6 after autoclaving) with four concentrations of mercuric chloride: 1 to 100,000 250,000 500,000, and 1,000,000. Isolates that did not grow well in the control tubes were discarded. Of the remainder, 5 (out of 14) were growing in one or more of the mercury dilutions after two weeks of incubation. After 4 weeks one more isolate had made appreciable growth in a mercury dilution. At that time 4 of the strains that had shown no growth on any of the dilutions were discarded, while some of the others were saved. It will be noted in Table XXXIV that Act. #20 which showed no growth in any of the dilutions in 4 weeks was growing even at 1-250,000 in 8 weeks, and that Act. #10 which had made no noticeable growth in

four weeks even at 1-1,000,000, was growing at 1-250,000 after 8 weeks, and at 1-100,000 after 12 weeks.

This preliminary experiment showed that the range of dilutions (1-100,000 to 1-1,000,000) was not sufficiently broad, that it was necessary to draw a time limit to observations, and that a medium more favorable to the growth of a large number of Actinomycetes was to be desired.

The medium was modified by substituting 10 grams of glucose for glycerine, leaving 10 g. glycerine and the same basic salt solution as employed in the first trial. The reaction was adjusted to pH 6.8 with NaOH. 23 Actinomycetes were planted in duplicate tubes of this solution using 9 dilutions of mercuric chloride beginning with 1-10,000,000 and doubling the concentration of mercuric chloride for each successive dilution. The organisms all grew fairly well in the control tubes. Readings were taken after 2 and 4 weeks of incubation at a constant temperature of 25° C.

The results of the two trials are given in Tables XXXIV and XXXV. In all 31 Actinomycetes were employed. These included two from England (Act. Setonii and Act. viridis from the National Type Culture Collection), two from Maine (C#23 and C#66 from L. A. Schaal), three from Long Island, New York (isolated by the writer

from scabby potatoes sent to him from Riverhead, N.Y. by H. S. Cunningham), and 24 others isolated from various plant hosts and from soil in Michigan. Two of the Long Island strains were very similar if not identical (Act. #40 and Act. #41). Possibly some of the others also should be considered as the same species.

The Actinomycetes from England and from Long Island were all less tolerant of mercuric chloride as an antiseptic than were 12 out of 13 Actinomycetes isolated from plant hosts in Michigan. Two of the Michigan Actinomycetes (Act. #43 isolated from a radish scab-lesion and Act. #38 isolated from a potato of the Warba variety) tolerated more than 100 times the concentration of mercuric chloride than did Act. viridis from England and Act #40 & 41 from Long Island.

Act. #6, isolated from a scabby eggplant root, was the only Actinomycete isolated from a plant host in Michigan that was less tolerant of mercuric chloride than any of the isolates from plant hosts from other areas. Since a mercurial soil treatment aggravated scabbing of eggplant at East Lansing (Table XX), this would appear to be evidence that either Act. #6 was not the organism causing the scab lesions on the eggplant root, or more than one strain or species of Actinomyces is capable of scabbing eggplant roots.

Conclusions

Not only was there an increase in numbers of pathogenic Actinomycetes in mercury-treated scab-infested Michigan soils as shown by the effect of the treatments on scabbing of potatoes and other hosts, but in some instances there was an increase also in the total actinomycetal count of treated soil as measured by dilution-plate counts on various media. Mercurial soil treatments apparently gave an initial reduction in number of Actinomycetes, but those that could tolerate the mercurial multiplied. Extremely high rates of application of mercuric chloride and mercurous chloride to the soil did not eliminate Actinomycetes. A test of the value of mercuric chloride as an antiseptic in a synthetic solution demonstrated that in general Actinomycetes isolated from plant hosts in Michigan were more tolerant of the mercurial than were Actinomycetes isolated from soil in Michigan or from plant host in England and Long Island. These findings bear out the belief that increased scabbing of potatoes in Michigan as the result of mercurial soil treatments is caused by the effect of the chemicals on the soil flora, resulting in an increase in number of parasitic Actinomycetes.

TABLE XXXIV

Tolerance of Actinomycetes to $HgCl_2$ in glycerine Synthetic Solution

Actinomyces ref. no. or name	habatat	source	time in weeks	growth: dilution (x 1000) 1000	500	250	100
A. viridis	potato	England	4	0	0	0	0
11	"	L.I.,N.Y.	4*	0	0	0	0
2	"	L.C.,Mich.	2	+	+	+	+
12	"	"	2	+	0	0	0
			8	+	0	0	0
18	soil	"	4	0	0	0	0
19	"	"	4	0	0	0	0
20	"	"	4	0	0	0	0
			8	+	+	+	0
21	"	"	2	+	+	+	0
			4	+	+	+	0
7	turnip	E.L.,Mich	2	+	+	+	+
10	air	"	4	0	0	0	0
			8				0
			12	+	+	+	
13	Solanum nigrum	E.L.,Mich.	4	+	0	0	0
			8	+	0	0	0
16	"	"	2	+	+	+	0
			8	+	+	+	0
48	Amaranthus graecizans	"	2	+	+	+	0
			4	+	+	+	+

Abbreviations: L.I.,NY. = Long Island, New York.
L.C.,Mich. = Lake City, Michigan.
E.L.,Mich. = East Lansing, Michigan

* At 4 weeks was growing at 1:5,000,000 but not at 1:2,000,000.

TABLE XXXV

Tolerance of Actinomycetes to $HgCl_2$ in Glycerine-glucose Synthetic Solution

Actinomyces ref. no. or name	habitat	source	time in weeks	lowest dil. giving growth: 1 to	highest dil. giving no growth: 1 to
Act. viridis	potato	Eng.	2	5,000,000	2,500,000
			4	"	"
Act. Setonii	potato	Eng.	2	2,500,000	1,250,000
			4	1,250,000	625,000
C#23	potato	Maine	2	312,500	156,250
			4	"	"
C#66	potato	Maine	2	625,000	312,500
			4	"	"
40	potato	L.I., N.Y.	2	5,000,000	2,500,000
			4	1,250,000	625,000
41	potato	L.I., N.Y.	2	5,000,000	2,500,000
			4	312,500	156,250
12	potato	L.C., Mich.	2	312,500	156,250
			4	"	"
35	potato	L.C., Mich.	2	78,125	39,063
			4	39,063	------
37	potato	L.C., Mich.	2	156,250	78,125
			4	39,063	------
38	potato	L.C., Mich.	2	39,063	------
			4	"	------
39	potato	L.C., Mich.	2	156,250	78,125
			4	"	"
5	beet	E.L., Mich.	2	625,000	312,500
			4	78,125	39,063
6	egg-plant	E.L., Mich.	2	---------	10,000,000
			4	5,000,000	2,500,000
7	turnip	E.L., Mich.	2	78,125	39,063
			4	"	"
13	*Solanum nigrum*	E.L., Mich.	2	625,000	312,500
			4	156,250	78,125
14	*Solanum nigrum*	E.L., Mich.	2	312,500	156,250
			4	"	"
15	*Solanum nigrum*	E.L., Mich.	2	78,125	39,063
			4	"	"
43	radish	E.L., Mich.	2	39,063	------
			4	"	------
48	*Amaranthus graecizans*	E.L., Mich.	2	78,125	39,063
			4	39,063	------
4	soil	E.L., Mich.	2	625,000	312,500
			4	78,125	39,063
9	soil	E.L., Mich.	2	1,250,000	625,000
			4	312,500	156,250
31	soil	E.L., Mich.	2	156,250	78,125
			4	78,125	39,063
10	air	E.L., Mich.	2	78,125	39,063
			4	39,063	------

Abbreviations: L.I. = Long Island; L.C. = Lake City; E.L. = East Lansing; Eng. = England.

STUDIES IN BIOLOGICAL CONTROL OF POTATO SCAB

It has been observed repeatedly by various investigators dealing with divers organisms that soil-borne plant pathogens in general, when inoculated into sterilized soil, usually produce much more prompt and severe disease symptoms on a susceptible host than when the same organism is inoculated into unsterilized soil. Presumably, the other organisms in the unsterilized soil inhibit the pathogen to a certain extent. This is known as biological control or antibiosis. The complexity of the soil population makes it difficult to study the interrelationships of the various groups of soil microorganisms as a whole. However, the interreactions of a great many isolates from the soil population have been studied by various investigators under labratory conditions. These workers have shown that the inhibiting effect of one organism upon another may be due to one or more of several factors: Production of toxins; change in the reaction or oxidation-reduction potential of the medium rendering it unsuited to the growth of the other organism; and competition for nutrients.

Review of the literature

Although the inhibiting effect of one organism upon another is readily demonstrated in the case of many

microorganisms on artificial media, relatively few attempts have been made to control plant diseases by antibiosis under field condition. In the case of potato scab, Millard (96, 97) in England obtained a reduction in scabbing by adding organic matter to the soil in the form of green grass or spent hops at the rate of 10 to 20 tons per acre. Later, Millard and Taylor (100) found that grass alone exerted no inhibitory action on scabbing, but that when Actinomyces praecox (a saprophytic species) and Act. scabies were both inoculated into soil mixed with grass, less scab occurred than when Act. scabies was employed alone. Goss (48), who tested the value of Actinomyces praecox for biological control of scab in Nebraska, failed to confirm the findings of Millard and Taylor.

Sanford (118, 120) considers the soil flora an important factor in determining the pathogenicity of Act. scabies. He suggested that when scab is controlled by green rye crops the antibiotic qualities of certain predominant soil microorganisms influence the development of the pathogen. However, he found that green rye plants, applied in the field at the rate of 50 tons per acre showed no tendency to reduce scab in thoroughly infested soil of pH 5.0-5.4 in Minnesota.

Although green manuring has been widely recommended

as a control measure for potato scab, there are relatively few experimental data on the subject. White (160) found that green manuring reduced the amount of scab in Kansas, and that green manure in combination with sulphur was more effective than alone. Dippenaar (32) got no control of scab in Wisconsin with green pea vines as a manure, now with sulphur in combination with the manure. Riha (112) reported negative results with green manure for scab control in Czechoslovakia. As pointed out by Huisman (59) it is well known that the disease is prevalent on plowed up grass lands although the contrary might be expected from the large quantity of plant remains in the soil. However, he reported that it had been noted that infection had been reduced by a green manure of oats. As intimated by Millard and Taylor (100) and Sanford (118), it may well be that green manuring is effective in reducing the scabbing of potatoes only where the green manure serves as a medium for the growth of organisms that are antibiotic to the pathogenic Actinomycetes.

Kiessling (70), in Germany, infested soil under field conditions with a mixed culture of bacteria antibiotic to Act. scabies and obtained a crop of tubers free from scab except for a small amount of a type of scab due to a different species than Act. scabies, where-

as the controls were heavily scabbed. He concluded that the bacteria were not antagonistic to all types of scab, but that biological control would be practical.

Wieringa and Wiebols (161) isolated a polyvalent bacteriophage for species of Actinomycetes, including Act. scabies, and they suggested the possibility of using bacteriophagy in controlling potato scab. However, as yet no bacteriophage for any plant pathogen has been utilized in controlling a disease under field conditions.

Weindling (151, 152, 155) and Weindling and Emerson (154) found that Trichoderma lignorum and Gliocladium produce a substance lethal to certain fungi. That Trichoderma lignorum and other species of Trichoderma markedly effect the growth of various other fungi has been frequently reported (6, 57, 66, 67, 102, 151). Butler (19) found that a species of Trichoderma caused a marked reduction in the amount of Texas root rot caused by Phymatotrichum omnivorum. Bisby, James, and Timonin (12) showed that T. lignorum suppressed the the virulence of Helminthosporium sativum and Fusarium culmorum on wheat. However, Christensen (23) found that the addition of Trichoderma lignorum and several other fungi and bacteria to naturally infested barley seed or to sterilized or nonsterilized soil did not

inhibit or delay the parasitic action of seed-bone parasites; but the addition of T. lignorum and certain other fungi and bacteria to seed or sterilized soil inoculated with Helminthosporium sativum increased the stand, decreased the number of deformed and stunted plants, and suppressed seddling injury. Bliss (13) failed to prevent the infection of seedling palms with Omphalia by means of infesting potted soil with T. lignorum. In laboratory, greenhouse, and field experiments, Weindling and Fawcett (153) successfully controlled Rhizoctonia damping-off of Citrus by acidifying the soil layers next to the seed to about pH 4 by application of aluminum sulphate or acid peat moss, but the treatment was effective only in the presence of Trichoderma spp., and the evidence obtained indicated that the decisive factor was a change in the soil microflora. Falck (39) stated that the well-known fact that structural timber that has become waterlogged by floating it in water during its transportation is less liable to decay from dry rot (Merulius lacrymans), Coniophora, and the like is due in part to heavy infection of such wood by T. lignorum and T. viridis, the enzymes of which are poisonous to the wood-destroying fungi while they themselves cause little injury. His experiments showed that such

Trichoderma-infected wood is resistant to the attack of C. cerebella. Allen and Haensler (4) reported that a species of Trichoderma was antagonistic towards the damping-off fungi, Rhizoctonia and Pythium, when the former was added to cucumber seed beds contaminated with the latter. However, Daines (28) obtained no reduction in potato scab on the resulting crop by dipping the potato seed pieces in a suspension of Trichoderma spores.

In addition to the claims for control of scab by soil infestation with bacteris by Kiessling, and with Act. praecox by Millard and Taylor, and the intimation that inhibition of scabbing by green-manuring is due to biological factors, there are other indications that biological control of scab does occur under field conditions. For example, Goss (48) noted that: "Soil sterilization before inoculation resulted in the most severe scab, This effect could be greatly reduced by the addition of filtrates of unsterilized soil or of organic matter in the form of manure and by delaying inoculation until soil saprophytes had become established in the soil". Again, the sptimum soil temperature for scabbing of potatoes has been reported as 17-21 C. (32, 65), whereas the optimum temperature for growth of most soil Actinomycetes on artificial media falls between

28 and 37 C. That this discrepancy is explainable by the inhibitory action of other soil microorganisms at the higher temperatures is indicated by the results of an experiment conducted by Goss (48) who sound that: "Sterilized soils inoculated with A. scabies and incubated at temperatures below 22° C. did not give rise to as much scab as those incubated at temperatures from 22° to 30°. (All were held at a temperature of 22 during the infection period). Unsterilized soils did not show this effect of temperature upon the development of the pathogen".

Sanford suggested that antibiosis may play an important role in natural control of potato scab. However, in the case of natural control sometimes brought about by a wet soil condition, he theorized (116-118) that the reduction in scabbing was due to the exclusion of air from the soil, thus inhibiting Act. scabies which is said to be strongly aerobic. Futhermore, Moore (101) observed that shallow planting and deep covering (ridging) tended to make scabbing worse. That soil aeration does have an effect on the soil Actinomycetal flora was shown by KenKnight and Muncie (69) who incubated aliquot soil samples in test tubes in a moist chamber and found a marked correlation between the ratio of soil volume to soil weight and the

numbers of Actinomycetes as determined by dilution-plate counts. Dippenaar (32), however, obtained no increase in scabbing of potatoes by artificially aerating soil, and, consequently, he doubted that exclusion of air was the cause of inhibition of scabbing in wet soils. Using the Cholodny slide methos (22), Conn (25) found that by increasing the moisture content of the soil, the natural flora of fungi and Actinomycetes quickly became changed to one in which bacteria predominated. Dippenaar (32) confirmed Conn's findings and came to the conclusion that "The inhibitive effect of a high soil moisture content on the potato scab organism appears to be indirect. Abundant soil moisture favours the activities of the bacterial flora in the soil and this seems to be chiefly responsible for the lack of spore germination and of development shown by the scab organism in wet soils".

Dippenaar's explanation that control of scab in wet soils is biological in nature, appears to be more probable than that of Sanford. The conflicting literature on the effect of soil moisture on scabbing of potatoes could be explained on the basis that in some soils there are microorganisms which are antibiotic to the parasitic Actinomycetes in that soil, whereas in other

wet soils either these antibiotic organisms are few or lacking, or are themselves inhibited by other organisms, or certain parasitic Actinomycetes in the soil are tolerant of them.

In regard to this conflict of opinions concerning the effect of soil moisture on scabbing, it will be noted that although Sanford (116-118), Martin (88), and Dippenaar (32) all demonstrated experimentally that scabbing decreased with increased soil moisture in their soil samples, Schacht (123), Caspri (33), Frank (41), Sorauer (129), Humphrey (60), and Lutman and Cunningham (77) all considered that a wet soil condition tended to increase scabbing, while Voekel and Klemm (142) recorded very severe scabbing during an unusually wet year. MacMillan (84) observed in potatoes under irrigation that steadily growing plants, maintained free from excess of drought or moisture, appeared to escape the disease a longer time than where improper application of water had occurred. Although severe scabbing of potatoes occurs in Michigan in rather wet as well as in dry years, the writer has noted, in three years of greenhouse studies, that when soil in pots is kept distinctly wet throughout the period of tuber development, little or no scabbing occurs. However, when the soil is permitted to dry out for a brief period at any time during the development of the tubers, scabbing is generally

severe. Further, it will be noted that in contrast with the results of Conn and of Dippenaar reviewed above, Lutman, Livingston, and Schmidt (80), from monthly plate counts over a period of eight years, found that both Actinomycetes and bacteria increased with increase in soil moisture with no antagonism observed between bacteria (rods and cocci) and Actinomycetes in the plate counts, the two running reasonably parallel. It may well be that in the absence of soil organisms antagonistic to Actinomycetes the latter increase in numbers with rise in soil moisture, as reported by Lutman et al, and in such a situation an increase in scabbing of potatoes might be expected from an increase in soil moisture. On the other hand, an increase in soil moisture in some soils may favor the development of organisms antagonistic to parasitic Actinomycetes to the detriment of the latter with the result that an increase in soil moisture would inhibit scabbing. These antagonistic organisms would not necessarily be bacteria, since species of Trichoderma (which are antibiotic to many organisms) abound in wet soils (143).

Other discrepancies in the literature on potato scab also possibly are due to the associative action of facultatively pathogenic soil Actinomycetes with

different soil populations. For example, although scabbing of potatoes in heavy soils is not at all unusual, in most regions light, well-aerated soils are said to be most conducive to scabbing, but there are a few diametrically opposite reports for potato scab (20, 37) and for beet scab (131). In spite of the frequent observation that old barnyard sites commonly produce extremely scabby potato crops and that manure (especially fresh manure) generally aggravates scabbing, application of well-rotted manure has been recommended as a control measure for scab in Germany (40, 58).

It should be emphasized here that the apparent discrepancies in the literature on potato scab that have been pointed out as possibly due to the associative action of different soil floras, could be explained as well by assuming the occurrence of divers parasitic species of Actinomycetes with different geographic distributions, these species differing in optimum soil moisture content, optimum soil reaction, and in other ways.

The effect of mercurial soil treatments on scabbing and on the soil flora in Michigan soils has been discussed in detail, and is another indication of biological control of scab. In all probability, the mercury

compounds remove from the soil certain organisms, resulting in some cases in a marked increase in the total number of Actinomycetes and nearly always in an increase in the pathogenic species as determined by planting a susceptible host.

Materials and methods

It is clear, from the survey of the literature, that organisms antibiotic to the causal organisms of potato scab might be expected among fungi, Actinomycetes, and bacteria; consequently, in the present study, representatives of all three groups were tested. The organisms tested included such well-known species in the literature on antibiosis as Weindling's Trichoderma lignorum, and Millard and Burr's Actinomycetes praecox, as well as Bacillus megatherium and Pseudomonas fluorescens. Bacteria 416, 484, 1401, 1402, and 1403 were obtained from F. E. Clark who found the last three strongly inhibitory towards Phymatotrichum omnivorum and certain other fungi, while the other two were common soil types of unknown value. The majority of the Actinomycetes tested were of unknown pathogenicity and it was hoped that if any were parasitic, this would be indicated by the test. Bacteria 1 to 6 were unidentified, common soil types, isolated from local scab-infested soils, and were tried without any partic-

ular reason for supposing them to be antibiotic to pathogenic Actinomycetes. Bacteria 7-14 were unidentified isolates from platings from mercury-soil-treatment experiments, and in their selection an attempt was made to choose those types that appeared to be more common in untreated than in treated soil, proceeding with the hypothesis that, if increased scabbing in mercury-treated soil is due to an effect on the soil flora, it must be some one or more of these organisms that are responsible for the lesser severity of scabbing in untreated soils.

Included in this study also were Bacillus megatherium and Pseudomonas fluorescens, the former of which was found by McCormick (82) to be antagonistic to certain species of Actinomycetes while it was itself antagonized by others. McCormick reported Ps. fluorescens to be antibiotic to Actinomycetes on solid media, the presence of the seeded Actinomycetes being beneficial to the growth of the Pseudomonas; and Lewis (74) found that Ps. fluorescens repressed the growth of ~~Bact~~ Bacillus mycoides and other spore-forming bacteria and micrococci.

The organisms were grown on various media including nutrient agar, liquid media, sterilized horse manure, and oat sprouts (6-12 inches long). The manure and

green manure were sterilized by autoclaving for three hours a day for three consecutive days. Only organisms which made considerable uncontaminated growth on the media were employed except in the case of 3 organisms which the writer was particularly desirous of trying because of their notoriety in the literature on antibiosis; thus Ps. fluorescens made poor growth on sprouted oats, while Act. praecox on oats was contaminated, and Weindling's Trichoderma made little or no growth on oats except when the latter were contaminated with bacteria, so the contaminated cultures were used as was also Ps. fluorescens (these all were tried in pure culture xx in 1938).

Actinomycetes Nos. 3,9, and 12 grew very well on the oat sprouts as did also Fusarium lycopersici and Mucor mucedo, while several strains of Actinomycetes made no perceptible growth and could not be used. All of the strains of Actinomycetes tried made more or less growth, some of them excellent growth, on sterile horse manure.

The cultures were distributed along the planting furrows at time of planting. In all cases precautions were taken against contaminating one culture with another. The sources of the organisms are given in Table XXXV.

Greenhouse trials in 1936:

In the greenhouse in 1936, six isolates of soil bacteria and two fungi, Fusarium lycopersici and Dendryphium sp., were inoculated into unsterilized soil in 8-inch pots. In the case of the bacteria, for each pot 100 cc. of three-day potato-broth culture was mixed through the top four inches of soil. In like manner, about 300 cc. of 4-week sterilized-oat cultures of the fungi were employed for each pot in those sets. Since it was known whether the soil in this experiment was scab-infested, one-half of it was artificially infested by adding to it 10 grams of macerated potato scabs per pot. Six pots were employed for each organism and these were randomized on a bed in the greenhouse. The tubers were harvested about 10 weeks after planting, at which time about one-fifth of the plants had died from Fusarium wilt. The artificially scab-infested soil produced a scabbier crop than did that which was not artificially infested. None of the organisms employed showed any tendency to reduce scabbing (Table XXXVI).

Field trials in 1936:

Twenty-two actinomycete-isolates from soil and one from a potato scab, a species of Pythium, and five of the organisms employed in the greenhouse experiment on biological control were tested under field conditions in 1936. No attempt was made to identify these organisms to species, and the majority of them were not maintained in culture after it was ascertained that they were of no value in combating scab. One liter of 5-day potato-broth culture per 10 hills was employed in the case of the bacteria 1 and 2, 500 cc. of 5-day sterile-oat culture for the other two bacteria, and a three-week culture from a liter flask containing 200 cc. of potato-glucose agar for each of the other organisms for 10 hills. In order to make use of all of the culture in a flask, about one-fourth of the agar was scraped out along with the mycelium. This was rinsed out with tap water and distributed along the planting furrow. Care was taken not to contaminate one culture with another, The hands of workers and all implements employed were rinsed thoroughly with 0.1% bichloride of mercury before and after infestation of a row with an organism. For each treatment there were two 10-hill randomized plots.

All of the organisms except bacterium 1 apparently

caused an increase in scabbing although the increase was not significant by analysis of variance except in the case of bact. 4 and Act. S17. However, it seems significant that the only organisms that did not give an apparent increase in scabbing (bact. 1&2) were also the only ones that were applied to the soil in broth cultures. All of the organisms applied on sterile oats or on agar gave an apparent increase in scabbing of 50 to 200 percent. The results indicate that the increase in scabbing was due to the addition of organic matter to the soil, although the amount of organic matter applied in the case of agar was very small.

Field trials in 1937:

In field experiments in 1937 the inoculum was added to the soil on sterile oat sprouts (6 to 12 inches long) in the case of Actinomycetes and fungi and four of the bacteria, and in beef-peptone ~~agar~~ broth in the case of the other five bacteria. Besides untreated controls there were additional controls to which sterile oat sprouts were applied on the one hand, and beef-peptone ~~agar~~ broth on the other. About 800 cc. of sterile oat sprouts culture was used for 10 hills, and about 800 cc. of broth culture for the same length of row. There were three randomized 10-hill plots for each treatment.

The results of these trials are given in Table XXXVIII.

The controls were about four times as scabby as those of the previous year. The controls to which organic matter had been added in the form of oat sprouts or beef-peptone broth gave more scab than the untreated controls, but not significantly so. In fact, none of the differences approached statistical significance.

Field trials in 1938:

As shown in Table XXXIX, various media and cultures of various ages were employed in this experiment. The severity of scabbing of individual plots was not recorded since it was very obvious that there were no differences that approached significance. The incidence of scabbing was 100% in every case. The severity of scabbing was extreme in the plots where potatoes had been grown annually for several years. However, 12 Actinomycetes were inoculated into soil of a plot where potatoes had not been grown in many years, if ever. This was intended as a pathogenicity trial, but the incidence of scabbing was 100% in both treated and control blocks, so it may be recorded here that under the conditions of the experiment, these Actinomycetes did not influence the scabbing of potatoes one way or the other. Their effect on scabbing of other hosts will be discussed in a later chapter.

TABLE XXXV

Source of Organisms used in Biological Studies

Organism	source
Actinomyces viridis Millard and Burr Actinomyces praecox Millard and Burr A. Setonii Millard and Burr A. bovis Harz Proactinomyces minimus Jensen P. paraffinae Jensen Micromonospora chalceae Jensen M. fusca Jensen	National Type Culture Collection, London, England
Actinomyces citreus Gasperini A. viridochromogenus Krainsky A. reticuli Waksman and Curtis A. albidus Duché A. farcinicus (de Toni et Trevisan) Westerdijk	Centraalbureau voor Schimmelcultures, Baarn, Holland
Actinomyces S2, S6, S7, S8, S9 S10, S14, S17, S18, S20, S24, S28, S32, S48, S49, S51, S52, S60, S62, S63.	Isolated from soil at East Lansing Mich., 1935-1936.
Actinomyce T1 Bacillus megatherium Ps. fluorescens Ford	Isolated from scab on potatoes, Kalahdin potato, E. Lansing, Mich. 1935. Bacteriology Department, Mich. State College, East Lansing, Michigan
Bacteria 1-14	Isolated from soil, East Lansing, Mich.
Bacteria 1401, 1402, 1403, 416, 484.	F.E. Clark, (U.S.D.A.), Manhattan, Kansas
Actinomyces C#23 " C"63	L. Schaal, (U.S.D.A.) St. Paul, Minn.
Weindling's Trichoderma lignorum	Weindling,
Fusarium lycopersicum Mucor mucedo Rizopus batatas Rhizoctonia solani	Botany Department, Mich. State College, East Lansing, Mich.
Dendryphium sp. Alternaria sp. Yeast 1 & 2.	Isolated from soil at East Lansing, Mich.

TABLE XXXVI

Attempts at Biological Control of Potato Scab in a Pot Experiment in the Greenhouse, 1936

Organism	Soil not artifically infested with scab			Soil artificially infested with scab		
	Severity of scabbing					
Control	T*	L*	0*	M*	M*	-*
Bacterium (1)	T	0	0	H	H	0
" (2)	T	T	-	T	T	0
" (3)	L	T	-	M	0	-
" (4)	T	T	T	H	H	M
" (5)	T	T	-	L	-	-
" (6)	L	-	-	M	-	-
Fusarium lycopersicam	L	-	-	H	T	T
Dendryphium	L	0	0	H	H	T

* Severity of scabbing: T= trace, L = light, M = medium, H = heavy, 0 = no trace of scab. 3 pots were employed in each replication. The missing readings are due to the plants having died from Fusarium wilt.

TABLE XXXVII

Attempts at Field Control of Potato Scab through Antibiosis in 1936

Soil infested with		1	2	T	$\bar{X}$
Control		8	8	16	8.0
Actinomyces	S2	11	31	42	21.0
"	S6	19	22	41	20.5
"	S7	16	18	34	17.0
"	S8	9	13	22	11.0
"	S9	22	13	35	17.5
"	S10	7	18	25	12.5
"	S11	33	14	47	23.5
"	S14	19	13	32	16.0
"	S17	28	38	66	33.0
"	S18	23	11	34	17.0
"	S20	10	18	28	14.0
"	S24	17	15	32	16.0
"	S28	23	24	47	23.5
"	S32	19	32	51	25.5
"	S48	15	16	31	15.5
"	S49	20	29	49	24.5
"	S51	20	16	36	18.0
"	S52	21	29	50	25.0
"	S60	13	12	25	12.5
"	S61	14	12	26	13.0
"	S62	24	32	56	28.0
"	S63	36	8	44	22.0
"	T1	39	12	51	25.5
Dendryphium	sp.	29	8	37	18.5
Pythium		27	30	57	28.5
Bacterium	1	2	11	13	6.5
"	2	17	2	19	9.5
"	3	6	40	46	23.0
"	4	24	37	61	30.5
B		571	582	1153	19.2

ST^2=49,671 SB^2=664,765 Sx^2=27,503

$C = (Sx)^2 \div n = 1,329,409 \div 60 = 22,156.82$

$Sx^2 - C = (ST^2 \div 2) - C \div (SB^2 \div 30) - C \div$ sum of squares due to error.

Analysis of variance

Variation due to	Degrees of freedom	Sum of squares	Mean square	Error
Total	59	5346.18		
Replication	1	2.01		
Treatment	29	2678.68	92.368	
Error	29	2665.49	91.91	9.5869

F = 92.37 ÷ 91.91 = 1.005 t = 2.045

Standard error of difference between means =

$9.59\sqrt{2} \div \sqrt{2} = 9.59$

Difference between means required for statistical significance = 9.59 x 2.005 = 19.56

TABLE XXXVIII

Attempts at Field Control of Potato Scab through Antibiosis in 1937

Soil infested with	% of surface area of tubers scabbed			
	replicates			
	1	2	3	mean
(cultures grown on sterile oat sprouts)				
Control (no treatment)	47	26	24	32.3
Control (sterile oat sprouts)	34	54	26	38.0
Weindling's Trichoderma *	53	18	-	35.5
Fusarium lycopersicum	24	37	13	24.7
Alternaria sp.	44	17	-	30.5
Dendryphium sp.	52	25	33	36.7
Mucor mucedo	63	48	37	49.3
Rhizopus batatas	53	43	25	40.3
Actinomyces praecox*	55	44	51	50.0
" viridis	70	36	28	44.7
" Setonii	63	18	20	33.7
" 3	28	33	40	37.0
" 9	39	22	8	23.0
" 12	60	35	27	40.7
" 35	38	9	15	20.7
" 37	13	30	-	23.0
" 38	64	54	32	50.0
" 39	57	15	17	30.7
" 68	28	48	15	30.3
" 69	48	23	23	31.3
Pseudomas fluorescens **	58	62	30	50.0
Bacterium (7)	57	18	-	37.5
" (8)	49	29	29	39.0
" (9)	38	27	-	32.5
(cultures grown on beef-peptone broth)				
Control (no treatment)	41	34	37	34.0
Control (beef-peptone broth)	52	32	40	44.7
Bacterium 10	39	38	19	32.0
" 11	36	23	25	26.0
" 12	44	29	21	31.3
" 13	37	39	42	39.3
" 14	42	20	-	31.0
"				

* This culture had a bacterial contaminant.
** The organism made poor growth.
By inspection: No statistically significant differences.

TABLE XXXIX

Attempts at Field Control of Potato Scab through Antibiosis in 1938

Soil infested with	medium	age culture	planting date	no. of replicates (10 hills)
(Trials on scab-infested soil: incidence of scab of potatoes 100%, severity extreme).				
Clark's bacteria: 1401, 1402, 1403; 416, 484.	(1)	3 days	6-9	2
Ibid	(1)	9 days	6-14	2
Clark's bacteria:	(2)	3-4 days	6-27 to 6-28	4
1401, 1402, 1403;	(2)	"	6-27 to 6-28	4
Bacillus megatherium;	(2)	"	6-27 to 6-28	4
Ps. fluorescens;	(2)	"	6-27 to 6-28	4
Sclerotinia sclerotinium;	(2)	4 weeks	6-27 to 6-28	4
Weindling's Trichoderma;	(2)	"	6-27 to 6-28	4
Rhizoctonia solani;	(2)	"	6-27 to 6-28	4
yeast (a);	(2)	"	6-27 to 6-28	4
yeast (b);	(2)	3-4 days	6-27 to 6-28	4
Act. praecox.	(2)	4 weeks	6-27 to 6-28	4
(Trials on land not previously planted to potatoes in many years, if ever; incidence of scab of potatoes 100%; scabbing not deep nor over large areas of tuber surfaces)				
Actinomycetes nos.: 2, 5, 9, 35, 39, 42, 43, 57, 64.	(3)	8 weeks	5-12	none (one 20-foot row)
Actinomycetes nos.: 3, 6, 8, 35, 42, 43.	(4)	4 weeks		ibid

TABLE XXXIX
(continued)

Media:

(1) Glucose, 1%; maltose 1%, peptone 0.5% in tap water, pH adjusted to 7.0.

(2) 100 grams mashed potatoes, 10 grams glucose per liter of tap water, pH unadjusted.

(3) Fresh horse manure, autoclaved 4 hours a day for 3 days.

(4) Glucose, 1%, peptone 0.5% in tap water.

All cultures applied at the rate of 500 cc. per 10 hills.

Field trials with green and stable manures:

In 1937 cut blue grass and alfalfa were applied in the potato planting furrows at the rate of 20 tons per acre in triplicate, randomized, 10 hill plots. In addition, blue grass was applied with calomel (6 lbs.) and with bacterial cultures. The bacteria were mixed ~~with~~ cultures produced by incubating a small quantity of rotting oat sprouts in beef-peptone broth for three days. Bacteria (a) were from sprouts rotting under aerobic conditions, and bacteria (b) from sprouts rotting under anaerobic conditions. The bacterial cultures were applied at the rate of one liter per ten hills. All treatments caused a significant increase in scabbing over that produced by the controls. (Table XLI). There were few tubers produced on any of the treated blocks that had less than 50% of their surface areas deeply scabbed.

At the same time, one and five tons per acre of fresh horse manure were applied in 10 hill blocks with ten replications. The manured plots gave slightly less scab than the controls, but the differences did not approach significance. (Table XLII).

TABLE XLI

Effect of Green Manures on Scabbing of Potatoes

Planted June 9, harvested Sept. 10
East Lansing, 1937

Treatment	1	2	3	T (sum)	mean
Control	65	17	32	114	38.00
Blue grass*	49	53	64	166	55.33
Blue grass* & calomel**	70	65	65	200	66.67
Blue grass* & bacteria(a)	67	66	65	198	66.00
Blue grass & bacteria(b)	66	70	59	195	65.00
Blue grass, bacteria(a) & calomel	70	67	64	201	65.33
Alfalfa*	57	65	57	179	59.67
B (sum)	444	403	406	1253	
mean	63.4	57.6	52.3		59.67

* At rate of about 20 tons per acre.

** At six lbs. per acre.

$C = (Sx)^2/21 = 74762.33$, $Sx^2 = 78209$, $SB^2 = 524381$, $ST^2 = 230223$.

Effect of Green Manures on Scabbing of Potatoes

Analysis of Variance

Variations due to	Degrees freedom	Sum of squares	Mean square	Error
Total	20			
Replication	2	149.24	74.62	
Treatment	6	1978.67	329.7783	
Error	12	1318.76	109.8967	10.4831

$F = 329.7783/9.8967 = 3.0008$, which is significant.

Standard error of the mean $= 10.483/\sqrt{4} = 5.2415$

S.E. of the difference between means $= \sqrt{2(5.2415)^2}$

$= 7.41$. Tabular value of $t = 2.179$

Required difference for statistical significance between means $= 2.179 \times 16.146 = 16.146$

Therefore every treatment gave significantly more scab than did the control.

TABLE XLII

Effect of Fresh Stable Manure on Scabbing of Potatoes

Planted June 9, harvested Sept. 10
East Lansing, 1937

	% surface area tubers scabbed		
	Control	Manure	
		1 ton	5 tons
1	62	38	48
2	41	56	36
3	38	31	36
4	27	50	43
5	28	7	27
6	59	57	34
7	29	64	27
8	35	26	65
9	49	56	44
10	46	30	18
Mean	45.3	41.5	37.8

By inspection: No significant differences.

Studies on the associative action of Clark's bacteria with Actinomycetes

Of the five bacterial cultures received from Clark, three (1401, 1402, and 1403, - Bacillus subtilis vulgatus group) were said by him to be strongly antibiotic to Phymatotrichum and other fungi on solid media. He had made no tests with Actinomycetes.

In a preliminary study the writer inoculated sets of 20 tubes of beef-peptone broth with each of the five bacteria, and a week later plated pairs of these with actinomycete-isolates, saving two tubes of each bacterium as controls. Although the bacteria in no case altered the reaction of the medium greatly, the majority of the Actinomycetes made little or no growth. In consequence, the writer considered these organisms promising material for studies in antibiosis. However, it was later observed that it is commonplace for bacteria to inhibit actinomycetes in bullion cultures whenever the bacteria are given several days start. The mechanism of this is probably one of exhaustion of nutrients, since the bacteria make very rapid growth as compared with the actinomycetes.

Clark's bacteria were employed not only in field

trials in 1938, but each was inoculated also into both sterilized and unsterilized soil in the greenhouse at the rate of 100 cc. of a 5-day beef-peptone-broth culture for each of five 6-inch pots, making a total of 60 pots in the experiment. 100 cc. of sterile beef-peptone-broth was added to each control pot. One week after soil samples were poured in the case of control and for two of the five bacteria. Each sample was a composite of five samples, - one from each pot of the treatment. The medium employed was Waksman's egg albumin agar, and the dilutions 1-10000 and 1-100000.

The results are given in table XLIII. 1-100,000 was not a sufficiently high dilution to obtain a good count from the control in the unsterilized soil set, for at that dilution there was only one plate out of 5 that had not been completely overrun by fungi in 6 days. On this one plate there were 16 fungal, 155 actinomycetal, and 770 bacterial colonies. It is likely that the count for all three groups should have been higher, the numbers here being influenced by crowding. The most striking thing about the plate was the marked reduction in numbers of fungi in bacteria-infested

(unsterilized) soil. This reduction was so great that one set could be counted at the 1-10,000 dilution. It will also be noted that there was a marked reduction in the total bacterial count, and a still greater reduction in the actinomycetal count as compared with the control.

The platings indicated that sterilized (flowing steam for 15 hrs.) soil to which sterile beef-peptone-broth had been added had a soil population of about 900,000 to 1,000,000 bacteria per gram at the end of one week, whereas the actinomycetal and fungal populations were too small to be measured with a 1-10,000 dilution. On the other hand, the bacterial populations in steamed soil that had been infested with bacterial cultures were 38 and 8 millions respectively for cultures 1402 and 1403. This would indicate that a soil population of these particular organisms was actually established. The results might be taken to indicate too that Bacillus 1402 favored actinomycetal contamination as compared with the control (Table XLIII).

The pots were then planted with formaldehyde-treated Katahdin tubers. When the crop was harvested eight weeks later, all tubers in the 60 pots were scabby, even though the soil in 30 of the pots had

been steam-sterilized and not subsequently artificially infested with scab-organisms.

Later the associative action of two of Clark's bacteria (1401 and 1403) with various Actinomycetes was again studied in duplicate tubes of beef-peptone broth. The final results of these trials are given in Table XLIV. The tubes were heavily seeded with aerial mycelium of the Actinomycetes and lightly seeded with bacteria. Controls were also run for each organism employed. 1403 was antibiotic to seven of the Actinomycetes, compatible with one and was itself inhibited by Act. #5 and 16. Bacterial culture 1401 was antibiotic to four Actinomycetes (all except one of which were slow-growers), compatible with four, and was itself inhibited by Act. #4 and 16. Act. #16 was antibiotic to both bacteria made an initial visible growth though this disappeared later in some tubes. (Table XLIV).

TABLE XLIII

Plate Counts of Soil infested with Bacteria#

Soil infested with	dilution (1: 100,000) bact.	act.	fungi	dilution (1: 10,000) bact.	act.	fungi
(soil steam-sterilized prior to infestation)						
(control)	8	0	1	107	0	1
	6	0	0	91	1	5
	12	0	0	111	0	0
	11	0	1	110	0	0
	8	0	0	91	0	0
Mean	9.0	0	0.4	102.0	0	1.2
Bact. 1402	384	2	0	-	6	0
	405	1	0	-	2	0
	386	1	0	-	0	0
	354	0	0	-	0	0
	373	0	0	-	1	0
Mean	380.4	0.8	0	-	1.8	0
Bact. 1403	103	0	0	-	0	0
	33	0	0	-	0	0
	97	0	0	-	0	0
	86	0	0	-	0	0
	99	0	0	-	0	0
Mean	83.6	0	0	-	0	0
(soil not steamed prior to infestation)						
-- (control)	770	115	16*	-	-	-
Bact. 1402	169	42	1	-	-	-
	324	46	1			
	350	48	2			
	334	69	2			
	320	54	0			
	299.4	51.8	1.2	-	-	-
Bact. 1403						

(continued on next page)

TABLE XLIII (continued)

Soil infested with	dilution 1:100,000 bact.	act.	fungi		dilution 1:10,000 bact.	act.	fungi
Bact. 1403	22	5	0	**	-	160	4
	33	2	0		-	116	4
	29	5	2		-	96	6
	32	10	0		-	83	2
	35	9	0		-	110	3
	30.2	6.2	0.4		-	113	3.8

* Other plates in this series had too many fungi for a count to be made.

** Dilution 1 to 1,000,000 through error.

\# Plated 7 days after infestation on egg albumin agar (Waksman's), pH 7.0. Plates incubated at 32 C.; counts made on 6th day.

TABLE XLIV

Associative Action of Actinomycetes with Clark's Bacteria in Beef-peptone Broth, pH 6.8

Condition 2 weeks after seeding

Act. #	source	Bacillus 1401	Bacillus 1403
4	Mich. (soil)	B	A-
5	Mich. (beet)	C	B
7	Mich. (turnip)	C	C
12	Mich. (potato)	C	A-
16	Mich. (Solanum nigrum)	B	B
35	Mich. (potato)	A-	A-
37	Mich. (potato)	A-	A-
43	Mich. (radish)	A-	A
C#66	Maine (potato)	C	A-
50	Mich. (Amaranthus retroflexus)	A	A

Symbols:

A = Actinomycete making no perceptible growth, bacteria uninhibited.

A- = Actinomycete inhibited, but making perceptible growth, bacteria uninhibited.

B = Bacteria making no visible growth, actinomycete uninhibited.

C = Bacteria and actinomycete compatible, both growing fairly well.

Discussion of Biological Control

Attempts to control scab through antibiosis met with no success. The addition to the soil of organic matter (even as a substrate of microorganisms) tended to aggravate scabbing, This is what one would expect ~~except in some instances~~ on the basis of no antagonism between scab organisms and the natural or introduced soil flora.

In view of frequent reports of reductions in scabbing as a result of green-manuring, in 1937 the organisms employed in this study were, for the most part, introduced into the soil in a green manure composed of 6 to 12-inch oat sprouts. Although Millard and Taylor (~~99~~ 100) reported that green manure was the best medium they had tried for culturing Actinomycetes, several actinomycete-isolates from Michigan soil and potatoes refused to grow on the medium. Millard and Taylor found that green manure was effective in controlling scab under their conditions only in the presence of Act. praecox,-a saprophytic species isolated from a potato scab. This species and many other Actinomycetes from various sources were used in green manure and in various other media to infest soil, but none of them gave any indication of control under local conditions.

Since frequently the addition of huge quantities of scab-inoculum to heavily scab-infested soil results in no increase in scabbing, it seems logical that the destruction of large numbers of scab organisms in heavily infested soil would not result in any marked reduction in scabbing. Since scab may be caused by numerous strains or species of Actinomyces, Act. praecox might antagonize a great many of these without causing a reduction in scabbing if other uninhibited strains or species were sufficiently numerous.

The results of these experiments are in accord with those of Goss (48) who obtained no control of scab with Act. praecox under Nebraska conditions. However, Millard and Taylor's report of control of scab with this organism was substantiated in a certain degree by the work of McCormick (82) who in studying the associative action of Actinomycetes with bacteria, fungi, and other Actinomycetes, found that Act. praecox was antibiotic on solid media to Act. viridis and Act. intermedius, phytopathogenic European species, and to the majority of other Actinomycete-isolates against which he tested it. However, it seems significant that he found that it was not antibiotic to N23, a parasitic actinomycete obtained from Dr. Goss in Nebraska.

The mechanism of antagonism between one organism and another may be due to (A) a change in the medium rendering it unfit for the one, (B) production of a toxin inhibitive to the one, or(C) exhaustion of nutrients. The latter case can hardly be taken into consideration here since organic matter was added to the soil at the time of soil infestation.

The production of toxins by organisms has been shown to be dependent to a large extent on the conditions of culture and the medium employed (74,76) so that organisms that are antagonistic towards certain other organisms in one soil might not necessarily be so in another soil. Furthermore, some toxins are readily destroyed by aeration (4,152) and consequently the toxins produced by soil organisms might be much more quickly destroyed in one soil than in another. Also, many toxins are readily absorbed on charcoal (4, 152) so that perhaps they are absorbed in some soils.

The absence of biological control may have been due in these experiments to failure to establish the "antagonistic" organisms in the soil or possible failure to show antagonism to all parasitic actinomycetes in the soil. Many Actinomycetes are themselves antagonistic to various other Actinomycetes, fungi, and bacteria (3,51,75,81,140,147), so that antagonism

may have operated in the wrong direction. Organisms not well suited to the soil conditions could hardly be expected to gain a foothold in soil already well-populated with microbes. The situation of trying to establish a predominant flora of a given saprophytic species in unsterilized fertile soil is perhaps analagous to attempting to obtain a stand of wheat by planting the seed on unbroken prairie sod.

In 1937 an attempt was made to determine the extent of soil infestation with the fungi (Fusarium, Alternaria, Dendryphium, Mucor, and Rhizopus) used in the "antibiosis experiment" by making soil dilution plates four to six weeks after soil infestation. Although remnants of the original oat sprouts used as a culture medium were found in the soil samples, in no instance was the organism recovered in plating from soil into which the organism had been inoculated. This is a criticism of the plating method rather than any good indication that the organisms were not present because in soil plating at low dilutions, the plates are completely overrun in a few days by a few species of Penicillium and Aspergillus (and occasionally other genera), crowding out the fungi that do not grow so rapidly. A wide variety of fungi may be observed on soil platings at high dilutions (1-100,000

or higher); the number of fungal colonies per plate is so small, however, and the number of plates required for deduction of conclusions is so large that most workers are discouraged in including generic identification in plate counts.

That parasitic microbes can be established in field soil, is, of course, demonstrated every time one establishes a disease garden for any pathogen. As will be discussed in a later chapter, this field soil well infested with Actinomycetes parasitic on potatoes but not on beets or radishes was later successfully infested with species of Actinomyces that attacked the latter hosts. However, that a large population of any of the sparophytic species employed in these tests were established in field soil was not demonstrated.

In the pot experi ent with Clark's bacteria it was expected that the potatoes in the sterilized soil would scab since that is the rule in the greenhouse employed for the test. Soil platings indicated that Clark's bacteria were strongly antibiotic to other soil organisms, especially fungi, and also Actinomycetes and even other bacteria. That these bacteria were antibiotic to some Actinomycetes

isolated from plant hosts but not to others was demonstrated in culture media. That they did not prohibit natural infestation of initially sterilized soil with Actinomycetes was indicated by soil plating, and that at least some Actinomycetes that became established in the soil were pathogenic was proven by the fact that scab occurred on every tuber in every pot at the end of eight weeks.

Recalling that Kiessling's (70) mixed cultures of bacteria controlled scab except for a type of scab caused by a species other than "Act. scabies", it seems probable that if practical control of scab is ever obtained by biological means in soil infested with several species of parasitic Actinomycetes, it will be with mixed cultures, since it appears unlikely that a single organism will be found that is antagonistic to all strains of the scab organism.

In the absence of antagonistic action of other soil organisms on parasitic Actinomycetes it would normally be expected that addition of organic matter to infested soil would result in an increase of scabbing. Green manure caused a statistically significant increase in scabbing in 1937. The small amount of nutrient agar with various organisms caused an undoubted increase in scabbing in 1936. In 1938 the

controls on the heavily infested soil ran about 55-60% of the surface area of the tubers scabbed, and with controls so scabby it is unusual for any treatment to make it worse. In view of the fact that other forms of organic matter in 1937 tended to increase scabbing, it seems strange that fresh horse manure at one and five tons per acre showed no such tendency.

STUDIES ON THE HOST RANGE OF PHYTOPATHOGENIC ACTINOMYCETES

Review of the literature

Actinomyces scab of the potato (Solanum tuberosum) is probably the most wide-spread disease of the crop, and is of economic importance in every important potato-producing region in the world. The malady was formerly considered to be caused by a single species of Actinomyces which is now generally called A. scabies (Thaxter) Güssow, although it is occasionally referred to as A. chromogenus Gasperini. However, nearly everyone who has done extensive work with Actinomycetes isolated from potato scab lesions has noted differences between isolates. American authors have generally regarded all of these isolates as strains or physiologic races of A. scabies, while some European investigators have considered them as separate species. These other named species which have been alleged to be parasitic on the potato are A. aerugineus Wollenweber, A. incanescens Wr., A. tricolor Wr., A. xanthostroma Wr., A. intermedius (Krüger) Wr., A. nigrificans (Krüg.) Wr., A. albus (Rossi Doria) Gasp., A. albus (R. D.)

Gasp. var. cretaceus (Krug.) Wr., A. Albus (R.D.) Gasp. var. ochroleucus (Neukirch) Wr., A. roseus Krainsky, A. Setonnii Millard et Burr, A. viridis M. et B., A. fimbriatus M. et B., A. flavus M. et B., A. marginatus M. et B., A. Loidensis M. et B., A. Wedmorensis M. et B., A. coniformis M. et B., and A. clavifer M. et B. (98,163,164).

Actinomyces scab on the roots of beets (Beta vulgaris) is of widespread occurrence in the United States and abroad, and, although it is rarely of much economic importance in America, the so-called "girth scab" of sugar beets has frequently been reported to cause severe damage in central Europe (31.44). As in the case of potato scab, American authors refer to the causal organism as A. scabies (Thax.) Güss. while European investigators have regarded several other species as parasitic on beets: A. intermedius (Krug.) Wr., A. nigrificans (Krug.) Wr., A. tumuli M. et Beeley, Oospora (Act.) tenax Kruger, O. (Act.) rosella Krug., and the two varieties of A. albus noted above. (72,99). In

America beets are also regarded as affected by the Actinomyces pox of sweet potatoes. (1,135).

Lesions caused by Actinomycetes are comonly observed on the roots of turnips (Brassica rapa) and rutabagas (B. campestris) (54,103) and less frequently on the roots of radishes (Raphanus sativus) (50,54,103); while scab of carrot (Daucus carota) roots is rarely reported although apparently of wide-spread occurrence. (61,85,103). In addition, parsnips (Pastinaca sativa)have been reported as susceptible (171), and there is one mention of an Actinomycete-lesion on a leek bulb (Allium porrum) (103).

Taubenhaus (135) found that Act. poolensis Taub. was associated with soil rot or pox, a destructive disease of sweet potato (Ipomoea batatas) roots,-a disease which is said to include white potatoes, beets, and turnips in its host range. Adams (1) considered Act. poolensis a saprophyte but demonstrated that typical symptoms of the disease could be produced by another actinomycete which he referred to as "Actinomyces

p.". Martin and Person (94) were able to reproduce the disease with various isolates of Actinomyces from pox lesions. In striking similarity to frequent reports regarding scab of white potatoes, Poole found that the pox disease was worse in dry than in wet years (109), that soils that pack hard after rains are favorable to its development (107), and that the malady could be controlled by sulphur soil treatments (108).

Act. Totschidlowskii Serbinov attacks the fruits of chile pepper (Capsicum annum) in Europe, sometimes causing considerable damage (121-122,125). Act. brasiliensis Spencer rots Brasil nuts (Bertholletia nobilis and B. excelsa) (130). Root nodules of Elaeagnus angustifolia and various species of Alnus in Europe are said to be caused by Act. alni (18,56,71,105,114), which, it is claimed, is capable of fixing atmospheric nitrogen. In addition, Beijerink (10) isolated Act. chromogenus in practically pure culture from the roots of various plants (including ferns, shrubs, and trees), although he did not consider

the organism parasitic. Furthermore Banga (7) isolated an acid-fast actinomycete from living tissues (leaves, petioles, roots) of strawberry, potato, tomato, bean and various other plants,- the organisms occuring in apparently healthy as well as in diseased tissues.

The relationship of Actinomyces scab of various hosts to that of potatoes has long been recognized, due to the fact that certain of these hosts frequently show symptoms of the disease when grown in soil infested with the potato scab organisms. Consequently the scabs of beets (5,77,167), turnips (168), rutagagas (169), radishes (170), carrots (172), and parsnips (171) have been considered to be caused by Act. scabies, although in the case of beets and carrots other species of Actinomyces also have been implicated (61,99,. However, relatively few reports have been made of attempts to prove the identity of the causal organisms by cross-inoculations.

The relationship of plant-pathogenic Actinomycetes to those causing animal diseases has not been extensively investigated. However, it is worthy of note that Gordon and Hagan (46)

reported that an acid fast actinomycete-isolate from a potato scab lesion was highly pathogenic to rabbits.

Field experiments and observations

Since susceptible plants other than potatoes probably play an important part in the maintenance of scab infestation in soils, it seemed desirable to obtain information regarding the susceptibility of the various reported hosts of scab to the soil-borne Actinomycetes in Michigan. In order to do this, examinations were made of the roots of all crops grown on scab-infested soil in the plant pathology field plots at East Lansing. In 1937 100-foot rows of each of the following crops and weeds were planted on heavily infested soil: Crimson Giant Globe, White Icicle and Early Scarlet Turnip radishes (Raphanus sativus), Early Purple Top turnips (Brassica rapa), American Purple Top rutabagas (B. napobrassica var. solidifolia), Golden Acre and Danish Ball Head cabbage (B. oleracea var. capitata), Burpee's Black Beauty eggplant (Solanum melongena), Jimson weed (Datura Stramonium), Savoy-leaved

spinach (Spinacia oleracea), Chantenay carrots (Daucus carota), Hollow Crown parsnips (Pastinaca sativa), Ohio Yellow Globe and Yellow Sweet Spanish Valencia onions (Allium cepa), and redroot(Amaranthus retroflexus). Since lime and mercury compounds as soil treatments generally cause a marked increase in both incidence and severity of scabbing of potatoes in Michigan, a portion of each row in the host range experimental plot was treated with lime at one ton per acre and calomel at 20 lbs. per acre, spearately and in combination.

The results of the trials for radishes, turnips, rutabagas, beets, and eggplants are given in Table XX in which it is shown that there was a considerable amount of scab on all of these hosts. Scabbing of eggplant roots is apparently noted here for the first time. The lesions appeared similar to those on the roots of turnips and rutabagas and invariably yielded almost pure cultures of Actinomycetes on plating. As in the case of potato scab, the incidence and severity of scabbingof eggplant roots was markedly increased by soil treatments with lime and with calomel.

That what appears to be scab lesions may occasionally be observed on the roots of Amaranthus retroflexus was first called to the attention of the author by Dr. Muncie who brought two scabbed roots into the laboratory in 1936. Both yielded an Actinomyces of the albus group on plating. In a 100-foot row of this plant on scab-infested soil in 1937 there were only a few plants that appeared to have scab lesions on the roots, A few other similarly diseased plants were found as weeds in the potato plots.

A poor stand of parsnips was obtained, there being only about 20 plants in the 100-foot row. The roots of these were free from lesions resembling scab, as were also the roots of the carrots, spinach, and cabbage, and the bulbs of the onions. Examination of roots of other plants on scab-infested soil revealed no scab on red peppers (Capsicum annuum), tomatoes (Lycopersicum esculentum), wild ground cherry (Physalis spp.), nor on any weeds except Amaranthus retroflexus and

Solanum nigrum. The occurrence of what appeared to be typical scab lesions on the roots of the latter was commonplace both in the field and greenhouse, and the lesions always yielded Actinomycetes on plating. Dr. muncie called to the attention of the writer that the nuts of a few hills of peanuts planted on scab-infested soil by Dr. Grigsby were covered with small raised lesions with a proliferation of tissue that resembled scab. Many of the lesions had a white powdery appearace due to fruiting Actinomycetes.

In 1938 potatoes (Katahdin), beets (Burpee's Extra Early), turnips (Early Purple Top), rutabagas (American Purple Top), eggplant (Burpee's Black Beauty), two varieties of radishes (French Breakfast and Crimson Giant), and Amaranthus retroflexus were planted in 20-foot rows in scab-infested soil, with and without yellow oxide of mercury as a soil treatment, and with two randomized repetitions of each treatment. Altho A. retroflexus, eggplants, and the crucifers were scabbed, as noted at mid-season, after heavy rainfall in August the roots were so damaged by fungus rots and maggots that it was not possible to

determine with accuracy the incidence of the disease. The effect of the treatment on scabbing of beets is shown in Table XXI and is discussed in the text preceding the table.

The roots of red peppers planted on scab-infested soil in 1938 showed a high incidence of lesions that somewhat resembled potato scab and which yielded Actinomycetes as well as divers other organisms on plating. Although many roots of these plants were examined in July, no trace of scab was noted at that time. The author doubts that the Actinomycetes were in this case primary invaders.

Pathogenicity tests

The results of the writer's pathogenicity tests of strains of Actinomycetes were rather unsatisfactory due to difficulty of preventing contamination of sterilized soil with parasitic Actinomycetes. Furthermore, in most cases Koch's postulates were not carried out to the extent of reisolation and identification of the organisms. Nevertheless, some of the results are worthy of note.

On May 13, 1937 sprouts from potato tubers (Katahdin) were washed and layered in steam sterilized soil in six-inch pots. Three or four sprouts were layered in each pot in order to insure a good stand, but only one plant was permitted to mature. One half of the pots (82) were placed out of doors on a lawn and the others were left in the greenhouse. On July 8, duplicate pots, both indoors and out doors, were infested with each of 8 isolates of Actinomycetes on agar. No agar was added to the control pots. Both sets of pots were harvested August 3. All tubers produced in the pots in the greenhouse, including those in the 30 control pots that were not artifically infested with scab, were severely scabbed. Out of doors there was scab in only three out of the thirty control pots whereas there was scab in both pots for each of 7 out of 8 of the Actinomycetes tested.

The results of this test were discounted by the writer because of the appearance of contamination in 10% of the controls and because a too

high percent of the Actinomycetes tested appeared to be pathogenic. Although hands and implements were rinsed in 0.1% bichloride of mercury before and after soil inoculation in the case of each actinomycete tested for pathogenicity, it seems likely that clouds of spores were released into the air whenever a culture (agar plate) was opened and that one pathogenic species in the group might have contaminated all of the pots. About 20 cc. of agar was added to the soil in each pot with the inoculum, but no agar was added to the controls. A pathogenic contaminant probably could become more readily established in soil to which agar was added than it could in the uninoculated soil, and consequently it seems likely that scabbing in some of the pots with inoculated soil may have been due to contamination. Act. #31 was the first culture to be opened. That this actinomycete appeared to be nonpathogenic (whereas all the others appeared to be pathogenic) may have been due to the fact that this von pathagenic culture was inoculated into the soil and the agar covered over

before a pathogenic culture was opened.

On July 20, 1938, a number of plants of several weeds were transplanted into scab-infested soil in the greenhouse. The soil was not sterilized, nor were any plants left as controls, the test being one to determine whether the weed roots would scab rather than a pathogenicity test of scab-isolates. The soil in one half of the pots was heavily inoculated with cultures of Act. #42 (isolated from an Amaranthus retroflexus root) and the other half with Act. #2 (isolated from a potato scab-lesion). The pots were watered heavily and the soil then allowed to dry until the plants began to wilt and then watered heavily again, this process continuing 5 weeks. Many of the plants died, but of those that lived every one had what appeared to be scab lesions on the roots. A few lesions were plated but they yielded several types of Actinomyces instead of only the one inoculated into the soil. The hosts included were Amaranthus retroflexus, A. graecizans, Solanum nigrum, and Brassica arvensis.

In 1938 an attempt was again made to test the pathogenicity of Actinomycetes in the greenhouse. In order to avoid contamination the floor of the greenhouse was covered with asphalt roofing. The soil was steamed in 6-inch pots for 15 hrs. and the pots were placed four each in galvanized pans specially constructed for the purpose and were watered from the bottom. The surface of the pots were covered over with rock wool with the hope that this would prevent contamination with Actinomycetes from the air. The pots were planted with formaldehyde-treated Katahdin tubers, and the soil (steam-sterilized 15 hrs. before infestation) in the pots was kept rather wet throughout the experiment. The soil was inoculated with spore-suspensions of the Actinomycetes.

The results are given in Table XLV. It will be noted that tubers in 5 out of 16 of the control pots were somewhat scabby. This, of course, renders the results of the entire experiment questionable. However, Act. viridis and nos. 5, 7

and 38 produced much more scab than did any of the controls. No. 43 gave no indication of pathogenicity at all, yet it is the one that appeared to be pathogenic to radishes under field conditions (Table XLV).

TABLE XLV

Pathogenicity Tests of Actinomycetes on Potato Tubers

Greenhouse, 1938-1939

Act. #	source	severity of scabbing
A. viridis	potato (England)	severe, up to 50 lesions per tuber.
2	potato	trace
5	beet	severe
6	eggplant	trace
7	turnip	severe
8	soil	trace
9	soil	trace
10	air	moderate
12	potato	trace
20	soil	moderate
35	potato	moderate
36	potato	trace
38	potato	severe
43	radish	none
59	rutabaga	light
63	air	light
A. Setonii	potato (England)	moderate
Control (1)	.....	trace
Control (2)	.....	moderate
Control (3)	.....	none
Control (4)	.....	none

Each treatment consisted of 4 6-inch. pots.
There were 16 control pots of which all the tubers in 11 were free from scab.
Planted Nov. 22 to Dec. 12; harvested Feb. 28, 1939.

Referring back to Table XXXIX it will be noted that 12 Actinomycetes were inoculated (some on manure, some in broth and some in both) into soil of a plot that had not been planted to potatoes in many years, if ever. Along with potatoes were planted 5-foot rows of other hosts: beets, turnips, rutabagas, and radishes, but only two or three of these four were tested as potential hosts for each organism. It was intended that this would also be a test of the pathogenicity of these Actinomycetes on potatoes, but not a single clean tuber was found in any of the blocks, treated and control alike, at harvest. Every third block was left as a control (sterile manure of beef-peptone broth added to soil in the same quantities as in treated blocks). None of the beets, turnips, rutabagas, or radishes scabbed except: 5 out of 9 of the beets in soil infested with Act. #5 (isolated from beet scab), 2 out of 6 rutabagas in soil infested with Act. #42 (isolated from scab on root of Amaranthus retroflexus), and 3 out of 11 radishes in soil in-

fested with Act. #43 (isolated from radish scab); Table XLVI would indicate that this soil, although infested with organisms capable of scabbing potatoes, was not infested with ~~organ~~ organisms capable of infecting these other hosts of Actinomyces scab and that only certain species are capable of infecting these other hosts. As has been noted before, in a plot a few rods distant the soil was naturally infested with Actinomycetes capable of scabbing all of these hosts, indicating the presence in that soil of parasitic Actinomycetes differing in host relationships from those that were so uniformly abundant in the soil of the plot that had not recently (if ever) produced a potato crop.

TABLE XLVI

Pathogenicity of Actinomycete-isolates on Hosts other than Potatoes under Filed Conditions, 1938

Act. #	Source	No. of roots scabbed beet	turnip	baga	radish
2	potato	0	-	-	0
3	soil	0	-	0	-
5	beet	5/9	-	-	0
6	eggplant	0	-	-	0
8	soil	0	0	-	-
9	soil	0	-	-	0
35	potato	0	0	-	0
39	potato	0	-	-	0
42	Amaranthus retroflexus	0	-	2/6	0
43	radish	0	-	0	3/11
57	soil	0	0	-	-
64	potato	0	-	-	0

Symbols: 0=:no infestion.
- : not tested
5/9 : 5 roots out of 9 showing scab infection

Planted May 12; partially replanted June 21 due to partial drowning out of first planting.

Discussion of pathogenicity trials

The problem of testing the pathogenicity of actinomycete-isolates was complicated by invariable contamination of the controls under greenhouse conditions. This was not avoided by covering the soil in the pots with rock wool, nor by planting detached potato sprouts rather than tubers. Afanasiev (2) who experienced similar difficulty obtained fairly satisfactory results by using a greenhouse with a concrete floor which he could disinfect. The writer had much less contamination when the greenhouse floor was covered with asphalt coated roofing paper than when it was left uncovered, although the results even then were inconclusive. Little contamination of the controls occurred in a trial conducted out of doors, and also there was little evidence of contamination by "Michigan scab organisms" of soil infested with "Long Island scab organisms" in pots placed in an orchard in 1938. It seems probable that further work could be done successfully out of doors.

The results of these experiments indicate that not all Actinomycetes that attack potatoes are capable of parasitizing the roots of other hosts of Actinomyces scab, but that some of the species of Actinomyces attacking potatoes are also capable of infecting other hosts seems evident since these other hosts commonly become scabby on soil infested with potato scab,- though they do not always do so.

OBSERVATIONS FROM ISOLATIONS OF ACTINOMYCETES AND DILUTION-PLATE COUNTS

During the course of this study a great many media were employed for plating-out soil for samples for actinomycetal counts and for isolating Actinomycetes from plant materials. In general, synthetic media containing no proteins were superior to other media. In one instance two composite soil samples, one from heavily scab-infested woil and the other from lightly infested soil, were plated on various media for comparison. The results of the counts of the 1-100,000 dilution are given in Table XLVII and the formulae for the media in Table XLIX. The effect of reaction of the medium and temperature of incubation for a single sample from scab-infested soil is given in Table XLVIII. Other dilution-plate counts with beef-peptone agar (Tables XXIV, XXV, XXX), sodium asparaginate agar (Tables XXIII, XXXIII), egg albumin agar (Tables XXII, XVIII), and glucose agar (Tables XXVII*XXIX) are also recorded in this paper.

Beef-peptone and potato glucose agars were the poorest media tried for plating of both soil samples and plant material because of the high incidence of "spreader" bacterial colonies. As illustrated in Table XLVIII beef-peptone agar of neutral reaction is somewhat worse in this respect than in the same medium slightly acidified. Furthermore, a low temperature of incubation was less favorable for the spreading bacteria, although damage from them is not always avoided by incubation at temperatures as low as 16°C.

Sodium asparaginate agar gives less trouble from spreaders than does beef-peptone, but where the prime consideration is the actinomycetal count, synthetic media with either glucose, glycerine, or sucrose as the source of carbon are much preferable. No spreaders have been observed on glucose or glycerine agars, and only occasionally a few on sucrose agar, although the latter medium is sometimes distorted by gas-producing organisms. These media have given approximately the same actinomycetal counts as have sodium asparaginate and beef-peptone when incubated at the same temperature.

It has been observed frequently that high temperatures of incubation of media (32°C. and 37°C.) generally give higher dilution-plate counts of actinomycetes than do lower temperatures (Tables XXIV, XXV, XLVII, and XLVII) with 32°C. probably about the optimum. On the other hand, bacteria frequently give higher counts at low temperatures of incubation (same tables). That highest counts of Actinomycetes should be obtained at about 32°C. is not surprising since that is near the optimum temperature for growth of the majority of soil Actinomycetes on nutrient media. On the other hand, 16°C. is certainly below the optimum for the majority of soil bacteria which appear on dilution plates, and the high counts obtained at that temperature may be due to a reduction in antagonistic action between the organisms at lower temperatures. This seems reasonable in view of the fact that the inhibitive action of toxins generally increases with rise in temperature. That the actinomycetal

count is not affected in this way may be an indication that the Actinomycetes in general are less subject to antagonism by other organisms than are the bacteria. This too seems probable in view of the fact that the actinomycete count is often apparently not reduced by "spreader" bacteria which eliminate fungi and greatly reduce the number of other bacteria appearing on the plates.

Considering that Actinomycetes in general are reported to have rather high thermal death points (80) whereas fungus spores are more sensitive to heat (149), it was thought that possibly fungi could be removed from soil dilutions without greatly reducing the actinomycetal counts by heating the soil dilution. This would make it possible to count plating at fairly low dilutions. Sterile water blanks in test tubes (9cc. each) were incubated in water baths held at constant temperatures of 60°, 70°, and 80°C. respectively. After the water in the test tubes had reached that of the baths, 1 cc. of 1-100 dilutions of a soil sample were added. At intervals of 5, 10, and 20 minutes tubes were removed and plunged in ice water. When all had been cooled they were plated.

The results of this test are given in Table L. A short period of heating reduced the bacterial count; a longer period caused an increase in bacterial count

at all three temperatures. This increase may have been due to breaking up of bacterial clumps since the colonies from heated tubes were definitely smaller than those of the control. The number of fungi was very markedly reduced by heating five minutes at 60 C., but since the reduction in actinomycetal count was almost equally as great, the method had no partical application in the way intended. It will be noted that five minutes at 70°C. reduced the actinomycetal population to such an extent that no colonies appeared even in the 1-1000 dilution, whereas the control gave 4,100,000 Actinomycetes per gram. It is perhaps worthy of note that Actinomycetes in soil samples plated by Lutman et al (80) were much less sensitive to heat.

Actinomycetes isolated from plant hosts at East Lansing commonly produced little or no pigmentation of potato media. A few of them produced black, greenish-black, blue, purple, or red pigmentation in various liquid and solid media. Actinomycetes isolated from potatoes from Lake City mostly produced blue, red, or purple pigmentation on various media.

In plating soil samples from New Jersey and from Long Island, New York, it was noted that Actinomycetes which produced brown to black pigmentation of protein media were much more abundant in these soils than in any scab-infested Michigan soils that had been plated.

Likewise, Actinomycetes which produced coiled aerial mycelium (both clockwise and counter clockwise spirals) were more common in eastern than in local soils.

TABLE XLVII

Comparison of Media for plating Soil Samples

Plate counts from two soil samples (July 25), one from a plot heavily infested with scab, the other lightly infested.
Incubated 7 days. Dilution: 1-100,000.

Medium	pH	Temp. C.	HEAVILY INFESTED bact.	act.	fungi	LIGHTLY INFESTED bact.	act.	fungi
Beef-peptone	7.0	25°	31	8	8	55	12	1
			38	12	0	*	(4)	1
			*	-	-	*	-	-
			*	-	-	*	-	-
Potato-glucose	7.0	25°	51	4	8	7	5	2
			**	(8)	9	12	3	3
			*	(4)	(1)	12	6	2
			*	(10)	(3)	17	7	5
Sodium asparaginate	7.0	25°	52	10	2	65	9	0
			54	10	1	43	10	3
			55	8	2	54	10	3
			42	18	6	46	9	1
Sodium asparaginate	7.0	32°	46	21	7	79	19	1
			59	19	0	55	17	3
			61	18	8	45	11	4
			*	-	-	43	15	1
Sodium asparaginate	7.0	37°	31	19	1	43	13	1
			32	20	0	60	15	0
			81	20	11	*	-	(1)
			44	18	6	54	15	1
Sodium asparaginate	7.0	40°	21	21	0	20	9	0
			(21)**	(4)**	2	32	13	0
			23	6	0	27	5	0
			22	6	0	37	8	0
Sodium asparaginate	5.4	25°	18	11	6	9	7	5
			15	10	3	10	12	5
			14	12	4	2	12	5
			10	5	8	6	11	7
Sodium asparaginate	5.0	25°	3	7	9	2	9	1
			6	4	7	1	3	5
			2	12	2	3	5	2
			4	7	6	3	6	2

* "Spreader" bacteria made the count doubtful or worthless
** Fungi overran the plate.

TABLE XLVII continued

Medium	pH	Temp. C.	HEAVILY SCABBY bact.	act.	fungi	LIGHTLY SCABBY bact.	act.	fungi
Tyrosinate	7.0	25°	32	12	2	6	10	1
			37	9	2	0	12	3
			30	13	3	8	12	2
			44	20	0	8	13	0
Sucrose	6.6	25°	54	11	5	24	17	2
			32	21	3	15	5	6
			27	27	0	24	10	1
			51	15	3	13	7	3
Glycerine	6.7	25°	53	11	11	80	9	7
			58	10	6	65	12	10
			53	8	5	137	13	9
			**	(5)	10	160	5	5
Glucose	6.8	25°	17	16	8	2	13	6
			42	11	2	13	9	2
			29	17	3	37	11	7
			31	15	7	14	12	4
Succinic acid	7.0	25°	14	6	5	2	7	3
			15	6	5	3	3	3
			10	4	10	0	4	2
			14	6	4	3	4	3
Tartaric acid	7.0	25°	3	0	3	0	0	1
			4	0	5	0	0	2
			2	2	5	0	0	0
			3	0	2	3	0	2
Citric acid	7.0	25°	1	0	1	0	0	0
			1	0	0	0	0	0
			0	0	0	0	0	0
			5	0	0	0	0	0
Oxalic acid	7.0	25°	1	0	8	0	0	3
			0	0	5	0	0	4
			0	0	15	0	0	4
			0	0	6	0	0	5
Sodium asparaginate + 10 p.p.m. $HgCl_2$	7.0	25°	20	14	2	42	8	2
			20	4	4	48	11	2
			18	11	1	47	13	2

* "Spreader" bacteria made the count doubtful or worthless
** Fungi overran the plate.

TABLE XLVIII

Effect of Reaction of Medium and Temperature of Incubation on Plate Counts

Plate counts from a single soil sample; count made on 6th day and checked on 10th. Dilution: 1-100,000
Beef-peptone agar.

Temp.	pH 6			pH 7			pH 8		
	bact.	act.	fungi	bact.	act.	fungi	bact.	act.	fungi
37°	44	23	1	*	*	*	14	3	0
	(14)*	(6)*	0	*	*	*	5	9	0
	43	12	0	*	*	*	9	8	0
	45	23	0	*	*	*	14	1	0
	*	*	*	*	*	*	10	7	0
$\bar{x}$	36.5	16.0	0.25	-	-	-	10.4	5.6	0
32°	71	18	1	4	14	12	55	10	1
	44	24	0	29	16	0	14	9	1
	*	*	*	76	20	4	(11)*	(2)*	(0)*
	*	*	*	*	*	*	(27)*	(1)*	(0)*
	*	*	*	*	*	*	(25)*	(11)*	(1)*
$\bar{x}$	57.5	21.0	0.5	36.3	13.3	2.0	26.4	6.6	0.4
25°	76	9	1	74	6	1	43	1	3
	86	7	1	57	11	2	48	11	0
	56	7	1	45	5	4	56	1	0
	*	*	*	*	*	*	42	2	1
	*	*	*	*	*	*	44	5	0
$\bar{x}$	72.7	7.7	1.0	58.7	7.3	2.3	46.6	3.6	0.8
16°	107	5	2	101	1	2	52	2	0
	58	5	3	89	6	1	38	0	1
	62	5	1	64	7	0	33	0	2
	87	13	2	67	0	3	55	0	2
	88	2	5	70	2	0	40	1	1
$\bar{x}$	80.4	6.0	2.6	78.2	3.2	1.2	43.6	9.6	1.2

* count damaged or ruined by "spreader" bacteria.

TABLE XLIX

Media Referred to in this Paper*

Medium	grams per liter of dist. water
Beef-peptone	
beef extract	10
Bacto peptone	10
NaCl	3
agar	12
Potato-glucose	
glucose	20
extract (filtered through cotton) of 240 gms. pared potatoes boiled ½ hr.	
agar	15
Sodium asparaginate	
sodium aspar.	1
glycerine	10
$NH_4H_2PO_4$	1.5
$CaCl_2$	0.1
$MgSO_4 \cdot 7HOH$	0.2
KCl	0.1
$FeCl_3$	trace
agar	12.
Cook's #II	
peptone	10.
glucose	20
$MgSO_4 \cdot 7HOH$	0.25
KH_2PO_4	0.25
agar	30
Glucose Sucrose Glycerine	
carbohydrate	20
KH_2PO_4	1
$NaNO_3$	2
$MgSO_4 \cdot 7GOH$	0.5
$FeCl_3$	0.01
KCl	0.5
agar	15.
Egg albumin (Waksman's)	
egg albumin	0.15
glucose	10.
KH_2PO_4	0.5
$MgSO_4 \cdot 7HOH$	0.2
$Fe_2(SO_4)_3$	trace
agar	15.
(egg albumin disolved in N/10 NaOH until neutral to phenolphthalein, then added to the warm medium)	

*Various hydrogen-ion concentrations of some of these media were employed, and consequently the reaction of the medium employed is recorded individually for each plating.

TABLE L

Effect of Heating the Soil Dilution on Plate Counts

Temperature	Time in minutes	1000's per gram bacteria	fungi	Actinomycetes
room	-	833	600.0	4100
60 C.	5	650	57.7	600
	10	687	10.3	370
	20	870	6.3	220
70 C.	5	675	4.0	0*
	10	853	2.0	0*
	20	1413	1.3	0*
80 C.	5	307	0.7	0*
	10	797	1.0	0*
	20	920	0.7	0*

* No Actinomycetes at any of the dilutions poured: 1-100,000, 1-10,000, and 1-1,000.

Beef-peptone agar, pH 7.0; plates incubated at room temperature; counts made on 6th day and checked on 10th. Each number is the average count of 3 plates. Bacteria and actinomycetes counted a dilution of 1-100,000; fungi at 1-1000 (3rd day).

DISCUSSION

Parasitic Actinomycetes in Michigan attack potato tubers, and the roots of beets, turnips, rutabagas, radishes, and eggplants, and evidence is presented that they attack also the roots of Amaranthus retroflexus, A. graecizans, Solanum nigrum, and Brassica arvensis, and the nuts of peanut plants. It is probable that further study would disclose more hosts. The parasitism of Actinomycetes on the roots of various crop plants and weeds likely plays an important part in the maintenance of infestation in scab-infested soils, and may also have some bearing on the occurrence of Actinomycetes pathogenic to potatoes in soils where potatoes were never previously grown.

Frequent mention is made in the literature of the multiplicity of species or strains of Actinomyces attacking potatoes. These species or strains have been shown to differ widely in their physiological as well as in their morphological characteristics. It is only with this in mind that the literature on potato scab becomes comprehensible. As was pointed out in reviewing the literature, various investigators have reported very divergent results concerning the effect on scabbing of potatoes of soil moisture, soil reaction, soil

aeration, and soil treatments with manures and antiseptics. In fact, scarcely anything that has been said about potato scab has been found to be true by all investigators.

Not only are several species of Actinomyces involved in scabbing of potatoes in a given locality as has been shown to be the case in Germany (30,61,163,164), England (98), Italy (24) and in New York (81), and Michigan, and which is probably true elsewhere also, but apparently most or all of the parasitic Actinomycetes in one locality may differ in some physiological characteristics from most or all of those in another locality. For example, the results from mercurial soil treatments in this investigation clearly indicate that such "geographical differences" in the actinomycetal soil flora exist in regard to the tolerance of Actinomycetes for mercurials as antiseptics. Broad differences in the frequency of Actinomycetes with certain cultural characteristics were also noted from platings of scab-infested Michigan soils as compared with those from Long Island, New York, and from New Jersey. Similar, tho less marked, differences were evident between the Actinomycetal floras of scab-infested soils from East Lansing and Lake City, Michigan, although those soils reacted similarly to mercurial soil treatments.

The various Actinomycetes attacking potatoes have been considered as separate species by several European authors, whereas American investigators have mostly considered them as "strains" or physiologic races of a single species, Act. scabies. In no other group of plants would such a heterogeneous population be considered as a single species. Objection has been raised to the practice adopted by Millard and Burr of establishing species differences in Actinomyces on the basis of every constant difference, since by that means hundreds, perhaps thousands, of species could be named (62,144); but whether these Actinomycetes are called species, strains, or physiologic races, their differences must be taken into consideration in attacking the potato scab problem. In this connection the following quotation from Millard and Burr (98) is especially apt:

"In any consideration of the incidence of scab, the physiological characteristics of many species must now be included. Certain investigators assert that the primary factor in the occurrence of scab is soil reaction, and have tried to correlate the latter

with the range of hydrogen-ion concentration with in which a few 'strains' of A. scabies develop. Such a correlation can obviously hold only for the particular species of scabbing Actinomyces present in the soil under consideration, and not for those which thrive in other types of soil."

The effect of soil reaction on scabbing is most curious. Some investigators find no effect of soil reaction on scabbing over a wide pH range (68), others show a consistent trend of increasing scabbiness with rise in soil reaction, while one author reports the opposite trend (47), and many find an optimum reaction with scabbing decreasing as the pH deviates from the optimum in either direction (14,128,129,157). This optimum reaction is extremely variable from one locality to another as is also the limiting acid reaction for scabbing. In interpreting these results several factors must be taken into consideration. A portion of these discrepancies must certainly be due to differences in the optimum and limiting soil reactions for various species of parasitic Actinomyces which have different geographical distributions, for differences so great as that of optimum reaction for

scabbing of pH 4.5 in one soil (17) while in another the limiting reaction on the acid side for scabbing was pH 5.8 (55), can scarcely be attributed to differences in the physical or chemical properties of the soil or to different associations of soil microorganisms. However, soil differences may be, in part, responsible for these discrepancies, since Wingerberg (162) found that Actinomycete-isolates not only had different optimum reactions, but that the optimum reaction of a given isolate depended upon the medium and the temperature of incubation. Soil may possibly also have an indirect effect on scabbing. An acid soil condition with a high moisture content favor the growth of certain fungi such as Trichoderma lignorum, which has been shown to be antibiotic to "A. scabies" on culture media (28); whereas other soil reactions may favor other organisms which might antagonize certain of the parasitic Actinomycetes, or would at least compete with them for nutrients and limit their development in that manner.

Observations on the effect of soil moisture on scabbing are as divergent as those for soil reaction. Scabbing is most severe in some localities when the

soil is wet (77,20), and especially after heavy, packing rains that tend to encrust the soil (20,48). In other places a moderate amount of soil moisture appears to be optimum for development of the disease (9); while in other localities drought conditions appear to favor scabbing (88,96,116,118); and under irrigation fluctuations in soil moisture may aggravate scabbing (84). In pot experiments severity of scabbing may be unrelated to soil moisture when under field conditions in the same soil scabbing is correlated with a dry soil condition (96). In some soils little or less scabbing occurs during wet years (118,96,90); in others there is less scabbing during dry seasons (48); while in others scabbing is likely to be severe in both wet and dry years (124). At East Lansing, Michigan, the disease is rather severe in infested soil every year regardless of rainfall. It appears to be worst in encrusted soil, but where no encrusting takes place, a moderate amount of soil moisture appears to be optimum. In 1938, when the fall was wet, both the dampest and the dries parts of the field plots produced less scab than did the remainder.

These discrepancies might be interpreted as an indication of different geographic distributions of parasitic Actinomycetes with different optimum soil moisture levels for growth or infection. Possibly some of the strongly aerobic Actinomycetes are inhibited in wet woils thru a lack of oxygen as suggested by Millard (96) and by Sanford (116-118). Perhaps some of the parasitic Actinomycetes are suppressed by certain bacteria which thrive in wet soils, as suggested by Dippenaar (32). Increased scabbing in dry soil may be due in part to the higher soil temperature that obtains in dry seasons as pointed out by Millard (97). Likely much of the increased scabbing associated with drying out of soils results from the ability of the Actinomycetes to produce spores and survive and cause infection of tubers which come in contact with them in soils too dry for activity of other organisma which might interfere with them in more moist soils. Furthermore, certain Actinomycetes may be capable of parasitizing potatoes only when the latter are injured by drought as indicated in the statement of Millard and Burr that inoculation experiments with A. flavus were "entirely negative

under normal conditions of summer heat and soil moisture, whereas when the soil was allowed to dry out so much that the plants died of drought, the inoculation was successful" (98). A wet soil condition does not inhibit scabbing in some soils probably because of the presence in such soils of parasitic Actinomycetes which are compatible with the other microorganisms, or the absence in such soils of the organisms which antagonize them in some wet soils.

Successes (59,95-97,100,160) and failures (32,112,119) in control of scab have been reported for green-manuring experiments. That antibiosis is the factor involved when control obtains seems highly probable, for, if it were not for other organisms, one would expect an increase rather than a decrease in number of Actinomycetes (including facultatively parasitic species) and a consequent increase in scabbing as occurred in the writer's green-manuring experiment with both alfalfa and blue grass.

The importance of antibiosis in natural control of scab has been a matter of much speculation. Its possible role in causing variable reports on the effect of environmental factors and of green-manuring

on scabbing has been discussed in the preceeding paragraphs. Sterilization of soil before infestation with scab-organisms generally results in more severe scabbing than does similar infestation of unsterilized soil. A similar increase in scabbing may be obtained in Michigan and western New York soils by applications of mercury compounds. In both cases the probable reason for aggravation of the disease is that sterilization or disinfection destroy soil microorganisms which interfere with the development of the scab organisms. However the writer's attempts at biological control of scab using 71 organisms inoculated into the soil on various media, were wholely unsuccessful.

The failure to readily obtain biological control of scab artifically under Michigan conditions is probably primarily due to the presence of numerous parasitic species of Actinomyces which are not all antagonized by any one organism used as an antibiotic. For another thing, Actinomycetes are in general relatively tolerant to toxins while they are themselves commonly antibiotic to other organisms.

The writer's attempts at biological control were by the "cut and try" method. Not knowing which Actinomycetes were primarily responsible for scabbing of potatoes in Michigan, it was considered rather useless to precede field trials with extensive laboratory experiments to study the associate action of the organisms with Actinomycetes in culture media or in sterile soil. Furthermore, toxins produced in culture media might not be produced in the soil, or if produced might be absorbed on soil particles; and the associative action of an organism with Actinomycetes might be greatly modified by other organisms in unsterile soil. Consequently, a large number of organisms, including several well known in the literature on antibiosis, were inoculated into scab-infested soil with the hope of finding one that would lessen the incidence of the disease.

In view of the evidence for biological control of scab in nature, it does not seem improbable that the disease could be controlled by modifying the soil flora. Since many strains or species of Actinomycetes are involved in scabbing, it may be difficult

to find an organism antibiotic to all of them. There may be greater promise in use of mixed cultures. One of the two reports of successful experimental control of scab by antibiosis,-that of Kiessling (70), was obtained by use of mixed cultures of bacteria, but even in this case there was, in the treated plots, some scab which Kiessling considered due to Actinomycetes other than A. scabies.

Mercury compounds and various other antiseptics and disinfectants have given no promise of control in Michigan. The theory of Daines and Martin (27) that mercurials are inneffective in controlling scab in some soils through "mercury-binding" capacity of the soil which is associated with the oxidation-reduction potential of the soil was not substantiated for Michigan soils. Likewise, the statement of Störmer (133) that mercury compounds are efficacious only in an acid medium (in her case in superphoysate in soil of pH 5.0) is not in harmony with the results of field and pot experiments in Michigan. It was shown that calomel would control scab in Michigan soil that was infested with scab organisms from Long

Island, whereas increased scabbing similar to that occurring under field conditions in Michigan occurred in Long Island soil infested with scab organisms from Michigan. Therefore the diametrically opposite results obtained from mercurial soil treatments in scab-infested soil in the two areas (Long Island and Michigan) appears to arise from differences in the species of Actinomyces infesting those soils. This is substantiated by the fact that Actinomycetes from plant hosts in Michigan were, in general, far more tolerant of mercuric chloride as an antiseptic than were Actinomycetes isolated from plant hosts in areas where mercurials are reported to control scab.

Stormer's failure to control scab in any but acid soil may have been due to the occurrence of numerous species causing scab rather than to an immobilization of the fungicide at higher pH values. It is not common for scab to be of consequence in soil of pH 5.0 such as that in which she obtained control with mercuric chloride. It is possible that there were parasitic species of Actinomyces there which were tolerant of the mercurial and so were not

controlled in neutral soils or when the mercurial was applied with an alkaline fertilizer. If these species could not tolerate an acid soil condition whereas other species that were sensitive to mercurials could cause scabbing in soil of pH 5.0, the results of mercurial soil treatments would bear a relation to soil reaction such as she reported.

The failure of mercury compounds to cause an increase in scabbing when applied with sulphur may be due to the sulphur having an inhibitory action on the particular Actinomycetes that are normally benefited by the mercurial treatment without controlling other parasitic Actinomycetes in the soil. That sulphur does sometime have a depressing effect on scabbing without greatly modifying the soil reaction frequently has been shown (35,49,136).

Control of scab by acidification of the soil has not met with much favor in Michigan. The trials that have been made with sulphur soil treatments have generally been of only one year duration on a given plot, and in most instances reduction in scabbing,

if any, was not sufficient to justify the cost of the treatment (134,150,158). Since a single application of sulphur has been shown to reduce scabbing in some instances (150,158), the question arises as to whether several successive sulphur treatments might not increase the acidity of the soil to a point where scabbing would no longer be of much consequence.

The problem is complicated by the common practice of growing alfalfa in rotation with potatoes. Although potatoes are relatively tolerant of an acid soil condition (~~36~~,55,126,129,157) the optimum reaction for alfalfa is near neutral. Liming the soil for the benefit of the alfalfa not only aggravates scabbing of potatoes, but buffers the soil with carbonates so that a large quantity of sulphur is requires to lower the soil reaction appreciably for the next potato crop. Thus it appears that where common scab is a limiting factor in the production of marketable potatoes, alfalfa and potatoes are not especially compatible in the same crop rotation. Whether, in general, Michigan soils could be economically acidified to a reaction at which potato scab

would not be troublesome, and whether that soil reaction could be economically maintained using acid tolerant crops in rotation with potatoes, is a problem that appears worthy of further study.

Crop rotations in which potatoes occur not oftener than every 4-6 years frequently have been reported to reduce scabbing (47 ,77,113), although they certainly do not always do so. Why the organisms that cause scab should die out or lose their virulence in some soils and not in others has not be determined. It might be explained by differences in species of Actinomyces causing scab, or differences in soil conditions such as soil reaction, soil moisture, soil aeration, or the presence in some soils of organisms antagonistic to parasitic Actinomycetes. At any rate, it is clear that with our present knowledge crop rotation cannot be depended upon to control scab under all conditions. In fact, reports of the occurrence of pathogenic Actinomycetes in virgin soils are not rare (11,36,64,79,110).

In recent years much emphasis has been placed on breeding potatoes for resistance to scab in the United States, both by the United States Department

of Agriculture and by various state institutions. Aside from difficulties in the breeding of potatoes (such as bud abscission, pollen sterility and polyploidy), the problem is complicated by the fact that many species of Actinomyces, and it has already been shown by other investigators that a variety which is resistant to scab in one locality may not be so in another (132), and evidence has been presented that this is due to "physiologic races of A. scabies", or species as some authors would term them (73). Much can be done in the way of improving varieties as regards resistance to scab, but an early complete solution of the problem by plant breeding is not to be expected.

In this discussion no attempt has been made at completeness in reviewing the literature but only sufficient citations have been made to show the great diversity of opinion regarding the influence of various factors on scabbing. More thorough reviews on the biology and morphology of Actinomycetes (33,34,38,75,166) antibiosis (45,82,111,146,148,156), and the effect of soil conditions on numbers of soil Actinomycetes (145) will be found by referring to the papers cited here.

SUMMARY

1. In field trials at East Lansing, Michigan, on scab-infested soil of pH 6.9-7.3, no control of potato scab has been obtained from soil treatments with compounds of aluminum, arsenic, boron, cerium, chromium, copper, fluorine, lead, manganese, mercury, nickel, and zinc, nor with tetrachloroethane, various phenol derivaties, sulphur, and sulphuric, hydrochloric, and oxalic acids. In greenhouse trials "Formacide", gentian violet, malachite green, wood creosote, and coal tar creosote were also ineffective, while nickel cyanide apparently gave control at 500 lbs. per acre,- a rate too high to be considered for practical control.

2. In field trials at Lake City, Michigan, in 1937 on soil of pH 5.2-5.8, sulphur, red copper oxide, and powdered zinc all were apparently of some value in reducing scabbing at all rates of application although none of the reductions in scabbing reached statistical significance by analysis of variance. The following year, after this field had been limed, none of these treatments showed any tendency to reduce scabbing.

3. Lead acetate was of no value as a soil treatment at Lake City. Ammonium thiocyanate caused a marked reduction in yield without effecting the severity of scabbing.

4. Mercury compounds as soil treatments have generally caused a marked increase in scabbing of potatoes at both East Lansing and Lake City at all rates of application from 6 to 100 lbs. per acre. Yellow oxide of mercury has been somewhat more effective in aggravating scabbing than have calomel, corrosive sublimate, and ethyl mercury iodide.

5. Combinations of oxidizing and reducing agents with mercurials and other combinations of chemicals as soil treatments were of no value in controlling scab. At East Lansing in 1937 singnificant increases in scabbing were obtained with combinations of calomel with zinc, with sodium nitrite, and with potassium permanganate, Sulphur alone and combinations of sulphur with calomel and with sodium nitrite, and a combination of copper sulphate with sulphuric acid had no significant effect on scabbing.

6. At Lake City in 1937, on unlimed soil, where sulphur tended to reduce scabbing, the mixture of mercury

compounds with sulphur in seed and soil treatments was in no case better than sulphur alone. Combinations of zinc with yellow oxide of mercury and with calomel, and combinations of both calomel and corrosive sublimate with red copper oxide, and corrosive sublimate with oxalic acid had no significant effect on scabbing.

7. In 1937 scab-infested soil was obtained from Long Island, New York, and from New Jersey from fields were calomel had been effective in controlling potato scab. Both soils had been fumigated with carbon disulphide before shipment. In a pot experiment calomel as a soil treatment caused an increase in scabbing in proportion to the amount applied in Long Island soil that had been artificially infested with scab organisms from Michigan potatoes. Calomel had little, if any, effect on scabbing in similar soil that had not been artificially infested. The results with New Jersey soil were about the same as those for Long Island soil. Evidence is presented that the soils that had not been artifically infested were nevertheless contaminated with local parasitic Actinomycetes, this explaining the failure of calomel

to control scab under those conditions. Calomel did not cause an increase in scabbing of potatoes in local soil in a parallel experiment probably only because the controls were excessively scabby.

8. In 1938 scabby potatoes as well as scab-infested soil were obtained from Long Island, New York, for further pot experiments. In these trials, calomel mixed thoroughly through the soil controlled scab in both Long Island and Michigan soils that had been steam-sterilized and infested with strains of Actinomyces from Long Island. Conversely, calomel aggravated scabbing in both Long Island and Michigan soils, infested with Michigan strains of Actinomyces, at the same time controlling Rhizoctonia scurf.

9. In a placement test at Lake City in 1938, yellow oxide of mercury caused a highly significant increase in scabbing when (a) mixed thoroughly through the soil along the planting row, (b) applied on the surface of the soil in a band 6 inches wide after the tubers were planted, (c) banded two inches from the seed pieces and on a level with them, (d) applied in the planting furrow, and (e) applied in a band two

inches directly below the seed pieces. However, when the mercurial was placed four inches below the seed pieces, the increase in scabbing was small and not significant.

10. In general, in soil treatment experiments, results obtained from pot experiments were comparable to those obtained in field trials. A few exceptions are noted. Calomel even at 50 p.p.m. gave complete control of Rhizoctonia scurf in East Lansing soil in a pot experiment; whereas calomel and yellow oxide of mercury applied in the planting furrow showed no tendency to control scurf under field conditions at Lake City in 1937.

In pots calomel caused a significant increase in yield of potatoes in Michigan soil that had been fumigated to make it more comparable to the Long Island soil. No increase in yield occurred in soil that had not been fumigated with carbon disulphide. Mercurial soil treatments have shown no tendency to increase yield under field conditions in Michigan; at rates up to 50 lbs. per acre they have had no effect on yield. It is noteworthy in this connection that significant increases in yield from mercurial soil treatments

have been obtained in western New York.

11. Combinations of calomel with zinc tended to reduce scabbing in both Long Island and Michigan soils in pots. Apparently this was due to the zinc rather than to the combination. Zinc alone also caused a reduction in scabbing. Zinc, but not combinations of zinc with mercurials, has shown a tendency to reduce scabbing under field conditions in some instances.

12. In pots there was much more Rhizoctonia scurf in local soil that had been fumigated with carbon disulphide than in similar soil which had not been fumigated.

13. In field trials in 1937 calomel did not greatly affect either the incidence or the severity of scabbing on the roots of radishes, turnips, and rutabagas, but greatly aggravated scabbing on the roots of beets and eggplants. On this approximately neutral soil liming caused no appreciable increase in scabbing of these hosts except in the case of beets. A combination of calomel with lime aggravated scabbing of all of these hosts except one of the three varieties of radishes (White Icicle), and caused a greater increase in scabbing than did either calomel or lime applied

separately except in the case of White Icicle radishes and eggplants.

14. In 1938 yellow oxide of mercury caused a significant increase in scabbing of beets.

15. Several attempts were made to test the hypothesis that increased scabbing of potatoes as a result of mercurial soil treatment is due to predisposition of the host plant to the disease. Although the results of these trials were rather unsatisfactory, no evidence was obtained that would support this hypothesis.

16. Dilution plate counts of soil microorganisms from field, pot, and glassware experiments showed that calomel even at rates up to 1 part per 100 parts of soil did not eliminate Actinomycetes from local soil, and that at lower rates of application there was in some instances a marked increase in the total number of Actinomycetes as compared with the control. That an increase in number of parasitic Actinomycetes usually occurs as a result of mercurial soil treatments might be inferred from the effect of such treatment on scabbing of susceptible hosts.

17. Mercurial soil treatments, even at low rates of application, greatly affect the bacterial soil flora causing a decrease of some types of soil bacteria and an increase of others. In several instances

the total bacterial count of treated soil was considerably higher than that for untreated soil.

18. Media containing proteins or peptones or asparagin have been unsatisfactory for soil plating studies. Synthetic media with glucose or glycerine as a source of carbon have been most satisfactory for soil plating in which the desired count is that of Actinomycetes, and also for isolation of Actinomycetes from scab lesions on plant tubers and roots.

19. In a pot experiment sodium thiocarbonate reduced scabbing of potatoes in proportion to the amount applied, but only at rates at which the yield was materially reduced. In this instance the number of Actinomycetes in the soil, as determined by dilution-plate counts, closely paralleled the severity of scabbing as measured by number of lesions per tuber. However, as a general rule, there appears to be no marked correlation between the total Actinomycetal count of a soil and the severity of soil infestation with parasitic strains of Actinomyces.

20. A study was made of the tolerance of 31 Actinomycetes to mercuric chloride in nutrient solutions. These included two from England (Act. Setonii M. & B. and Act. viridis M. & B.), two from Maine, three from

Long Island, New York, and 24 others isolated from various plant hosts and from soil in Michigan. The results, for the most part, bear out the theory that mercurials control scab in England and on Long Island because the parasitic species (or strains) of Actinomyces in the soils there are relatively susceptible to mercury as an antiseptic, whereas mercurials do not control scab in Michigan soils because the parasitic Actinomycetes are highly tolerant of mercury.

21. The Actinomycetes from England and from Long Island were all less tolerant of mercuric chloride as an antiseptic than 12 out of the 13 Actinomycetes isolated from plant hosts in Michigan. Some of the actinomycete-isolates from Michigan tolerated more than 100 times the concentration of mercuric chloride than did some of the isolates from regions where mercurial soil treatments control scab.

22. The evidence for natural biological control of potato scab is discussed.

23. Field soil was infested with various organisms which were added to the soil in broth, on agar, on sterilized manure, and on sterilized oat sprouts.

In trials over a three year period no indication of biological control was obtained.

24. In 1936 all organisms added to the soil on agar or on oat sprouts resulted in a striking increase in scabbing whereas those added in broth had no effect on scabbing. A slight tendency was noted also in later trials for organic matter alone to cause an inorease in scabbing, but, because the controls were severely scabbed in later trials, no such great differences were to be observed.

25. In all, 21 bacteria, 41 Actinomycetes, seven fungi, and two yeasts were employed in attempts at biological control of scab under field conditions. Many of these organisms were not identified, and a few may be duplicates except in the case of the fungi, each of which was a representative of a different genus. Among the organisms employed were Act. praecox, Trichoderma lignorum, Pseudomonas fluorescens, and Bacillus megatherium,- all of which are well-known in the literature on the associative action of microorganisms.

26. In plating out media containing proteins it has been constantly observed that Actinomycetes in general are less subject to the inhibitive effects of "spreader" bacteria than are other organisms.

27. In liquid media containing sugars some bacteria inhibited Actinomycetes by changing the reaction of the medium.

28. In broth to which no sugars were added the majority of bacteria tested were not antibiotic to various Actinomycetes when the bacteria and Actinomycetes were seeded simultaneously. The Actinomycetes that were inhibited were in most cases those that made slow growth. Frequently the bacteria were inhibited. In many cases the bacteria and Actinomycetes were compatible. When bacteria were seeded one or more days in advance of the Actinomycetes the latter were generally inhibited, probably due to competition for nutrients.

29. A bacterium which is antibiotic to one Actinomycete-isolate may itself be inhibited by another.

30. Five bacteria, three of which had been shown by Clark to be strongly antibiotic to Phymatotrichum and other fungi on agar media, failed to inhibit scabbing of potatoes even when inoculated heavily into sterilized soil that was not artifically infested with Actinomycetes, the latter presumably contaminating the soil from dust in the air of a dusty greenhouse in which the soil was infested with scab organisms.

31. Green manures of both blue grass and alfalfa applied at the rate of 20 tons per acre caused a significant increase in scabbing.

32. Evidence is presented that Solanum melongena,

S. nigrum, Amaranthus retroflexus, and possibly also A. graecigans, Brassica arvensis, and Arachis hypogaea are hosts of phytopathogenic Actinomycetes.

33. The results of pathogenicity tests with pure cultures of Actinomyces were not very satisfactory due to contamination of sterilized soil with parasitic Actinomycetes under greenhouse conditions. This dificulty was less conspicuous in pots placed out of doors.

34. Evidence is presented that several Actinomycetes were pathogenic to potatoes. These include Act. viridis and several Actinomycete-isolates from plant hosts and soil.

35. Actinomycetes isolated from plant hosts at East Lansing commonly produced little or no pigmentation of potato media. A few of them produced intense black, greenish black, blue, purple, or red pigmentation in various liquid and solid media.

36. Actinomycetes isolated from potatoes from Lake City mostly produced blue, red, or purple pigmentation in various media.

37. Differences were noted in the abundance of certain types of Actinomycetes in eastern soils as compared with local soils.

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