THE INFLUENCE OF SOIL REACTION UPON THE GROWTH OF ACTINOMYCETES CAUSING POTATO SCAB¹

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The close correlation between soil reaction and the growth of specific microorganisms in the soil has recently been made the subject of investigations from various viewpoints. By expressing the soil reaction in terms of hydrogen-ion concentrations, we are employing a factor which has greater significance in biochemical soil processes than merely the amount of acid substance present. In studying the relation between the hydrogen-ion concentration of the soil and the activities of microorganisms, we must differentiate between the limiting reaction, at which beneficial microorganisms become active in the soil, from that reaction at which microorganisms injurious in one way or another to the soil microflora or the growth of higher plants are checked in their development. The work of Gainey (7) and others takes up the influence of reactions upon the growth of *Azotobacter*, Fred and associates (6) and others on *B. radicola*, Meyerhof (24) on *Nitrosomonas* and *Nitrobacter*. The limitation of the growth of actinomycetes causing potato scab can serve as an excellent illustration of the second group of phenomena. However, when we attempt to adjust the reaction of the soil to a certain hydrogen-ion concentration with a view of limiting the growth of an injurious organism, we ought not omit from consideration the influence that this reaction may exert upon the growth of beneficial organisms.

The organism causing potato scab was first described by Thaxter (28, 29) under the name *Oospora scabies*. In a study of "scab" of sugar beets, Krüger (17) suggested that *Oospora* can probably be classified with the streptothrices (actinomycetes). It was later identified by Cunningham (5) with *Actinomyces chromogenus* of Gasperini. However, the term *A. chromogenus* based upon the fact that the organism produces a dark brown pigment on organic media is hardly appropriate, since, as pointed out by Krainsky (16), Waksman (31, 32), and Conn (4), there is a large number of actinomycetes in the soil, which are able to produce this pigment. This factor was readily recognized by Güssow (12), who very appropriately suggested the term *Actinomyces scabies*, which should be used in the interests of an accurate nomenclature. Even with a correct nomenclature, we must be on the lookout for other actinomycetes not of the *A. scabies* type. From 15 to 40 per cent of the soil's microbial flora consists of actinomycetes and one may readily isolate from a diseased potato tuber another *Actinomyces* which will not be "scabies" at all and which may differ much in its

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metabolism, including the limiting reaction for growth. Only recently, Wollenweber (38) pointed out the potato scab is caused not by one species of *Actinomyces*, but by a number of them. At least 6-7 *Actinomyces* species have been isolated from diseased potatoes and from the soil, and their pathogenicity demonstrated by inoculation tests. As a result of these studies of Wollenweber and of the writer, which will be published at a later date, we may have to limit the term *A. scabies* (Thaxter) Güssow to one specific type, from which other actinomycetes that are able to cause potato scab may be differentiated. However, for the present, we may speak of the organism causing common potato scab both in America and in Europe (as pointed out by Wollenweber), as the *Actinomyces scabies* group.

One of the most important and most promising methods of controlling the development of potato scab in the field is the use of acid forming substances. Fertilizers which leave the reaction of the soil alkaline have been looked upon as injurious, while those which leave the soil acid, like ammonium sulfate and acid phosphate, are generally preferred. The use of sulfur for combating scab has been recognized by Halsted (13, 14). But the results obtained by the various investigators are lacking in uniformity. Even Halsted (15) found that an application of 300 pounds of sulfur per acre was effective, within one year under certain conditions, while an application of 600 pounds of sulfur in 1896 was ineffective, but became effective in 1898.

These discrepancies were due to the fact that the basic underlying principles were not recognized, namely that the sulfur is oxidized in the soil by certain microorganisms to sulfuric acid, and the increase in soil acidity leads to a decrease in scab. Two factors are to be considered here; the presence of the proper sulfur-oxidizing organisms in the soil and the reaction and character of the soil itself. The amount of sulfur to be used will depend upon the latter factor.

This lack of correlation between the application of sulfur and control of scab is also true of the other experiments where the addition of sulfur failed to diminish the amount of scab as those of Garman (8) and Brooks (1). However, where enough sulfur was used year after year and the soil conditions were proper, a definite reduction of the potato scab was effected by Wheeler and Adams (36), Sherbakoff (26, 27) and Lint (18, 19). Even in as late a publication as that of Wollenweber (38) in 1920, where the importance of making the soil acid for the control of potato scab has been well recognized, the application of sulfur was not found to give uniform results.

Only with the development of methods for determining the correct reaction of the soil as expressed in terms of hydrogen-ion concentration, and with an increased knowledge of the microorganisms concerned in the oxidation of sulfur in the soil, could we understand the underlying principles and place our knowledge of scab control on a systematic foundation. A third factor should be considered here which is not of least importance, and that is the metabolism of the causative agent of potato scab, *A. scabies*. Our information on the soil reaction has been advanced by the researches of Sørensen and Michaelis in Europe, Clark and Lubs and others and particularly that of Gillespie (10, 11) in this country, as applied to scab control. Our knowledge of the mechanism of sulfur oxidation in the soil by crude cultures of microorganisms has been advanced by the work of Lipman and his associates (20, 23) and McLean (24), and with pure cultures by Waksman and Joffe (33, 34, 35). As to the metabolism of *Actinomyces scabies* itself, it has been taken up by Lutman and Cunningham (21) and by Waksman (30).

Considering the practical application of the subject, the following questions are important:

1. Is there a limiting acid reaction for the species of *Actinomyces scabies* which will absolutely prevent their development?
2. By what certain means can we obtain this reaction?

3. What is the influence of type of soil, amount of organic matter in the soil, moisture content, aeration, etc.?

These three points will be taken up in order and experimental results presented.

LIMITATION OF GROWTH OF *A. SCABIES* BY ACIDITY OF MEDIUM OR SOIL

Gillespie and Hurst (10) were the first to call attention to a characteristic difference of hydrogen-ion concentration shown by the extracts of soils of the types Caribou loam and Washburn loam. These types, similar in texture and derived from the same glacial till, differ, however, due to local topographic differences. In reaction, the Caribou loam is more intensely acid than the Washburn, with a relative freedom from scab of potatoes grown in the more acid soil. Gillespie and Hurst (11) have further shown that soils having a hydrogen-ion concentration equivalent to pH 5.2 rarely produced scabby potatoes, while less acid soils generally produced scabby potatoes, unless it was new land. In another paper, Gillespie (9) found that, by growing the organisms causing potato scab on two synthetic media and upon a medium prepared from potato extract at reactions of pH = 5.2 and less, the growth was much slower and generally less vigorous than in less acid media. It is interesting to note that Gillespie (9) observed a marked decrease in acidity accompanying the growth of the organisms. This change in reaction of media was pointed out by Waksman (31) for the *Actinomyces* group as a whole. Wollenweber (38) stated that actinomycetes causing potato scab are very sensitive to acid (0.05 per cent) and much less sensitive to alkali (0.25 per cent).

EXPERIMENTAL

The influence of reaction upon the growth of actinomycetes, in general, and species causing potato scab, in particular, was studied both in solution and in soil. The cultures used were obtained from Dr. Morse of the Maine Agricultural Experiment Station and Mr. M. Shapovalov of the U. S. Department of Agriculture except for three which were isolated by Waksman and Curtis (32).

The following cultures were used in one or another of the experiments:

A. scabies 222 and 254 were obtained from Dr. Morse, who isolated them from potatoes in Maine in 1906 and in 1914 respectively.

A. scabies 259 was obtained from Dr. Morse, who isolated it from potatoes coming from Ohio in 1915.

A. scabies 281, 283 and 295 were obtained from Mr. Shapovalov, who isolated them from potatoes infected with common scab in Maine.

A. griseus was isolated by the writer and Curtis (32) from the soil in 1916. This culture has a strong proteolytic power (30).

A. violaceus-ruber and *A. viridochromogenus* were also isolated in 1916 by the writer and Curtis from the soil and described in detail elsewhere (31). These two cultures are quite similar, in description, to Wollenweber's *A. tricolor* and *A. aerugineus*.

The organisms were grown on synthetic agar, Czapek's or dextrose agar (31), and were then inoculated into the sterile liquid medium. For the inoculation of sterile soil, the cultures were grown on the liquid synthetic media, in 250-cc. Erlenmeyer flasks and, for inoculation, 1 cc. of the culture, well shaken previously, was introduced, under sterile conditions, into each flask.

Experiment 1

The medium used in the first experiment had the following composition:

	grams
Glycerin.....	20.0
Asparagin.....	5.0
Mg SO ₄	0.5
KCl.....	0.5
Fe SO ₄	0.01

Phosphates in varying amounts. Distilled water to make 1000 cc. (after adding the buffers).

Phosphoric acid, KH₂PO₄, K₂HPO₄ and NaOH were used to adjust the reaction of the medium to the desired hydrogen-ion concentration, as shown in table 1.

TABLE 1

The amounts of buffer solutions used in experiment 1 and the hydrogen-ion concentration of the media

MEDIUM NUMBER	BUFFER SOLUTIONS				REACTION
	H ₂ PO ₄ 1.0 N	KH ₂ PO ₄ 1.0 M	K ₂ HPO ₄ 1.0 M	NaOH 1.0 N	
	cc.	cc.	cc.	cc.	pH
1	4	16			3.2
2	2	18			3.6
3		20			4.2
4		19.5	0.5		4.6
5		19.0	1.0		5.2
6		18.0	2.0		5.6
7		16.0	4.0		6.4
8		4.0	16.0		7.0
9			20.0		7.5
10			16.0	4	8.2

In all media, a total of 20 cc. of the buffer solutions were added per liter. The media were distributed, in 100-cc. portions into 250-cc. Erlenmeyer flasks and sterilized at 15 pounds pressure for 15 minutes. The flasks were then inoculated from slants with a culture 7-14 days old by means of a platinum loop. They were incubated at 25-27°C. for 21 days and filtered through weighed filter paper. The growth on the filter paper was dried at 80°C. to constant weight. Under "growth", the relative amount of growth is given, while under "weight" actual weight of the mycelium is given, in milligrams. The pH values were obtained by the use of the indicator method, as outlined by Clark and Lubs (2). Results are presented in table 2.

The limiting acid reaction for the two cultures of *A. scabies* seems to be at a pH between 4.6 and 5.0, while the limiting acid reaction for *A. griseus* and *A. violaceus ruber* isolated from the soil lies between pH 4.2 and 4.6. This indicates a greater acid resistance for the saprophytic forms. As to the

alkaline limit, *A. scabies* 259 did not develop above pH 7.4. When good growth took place, the reaction of the medium was invariably changed to alkaline. This is due to the fact that the actinomycetes as a group, with perhaps few exceptions, do not seem to form any acid from the carbon sources, as in the case of many bacteria and fungi, but produce alkaline substances from the nitrogen sources [Waksman (30)]. In this case, it is the production of ammonia from the asparagin which makes the medium alkaline. By determining the ammonia formed, we can account almost quantitatively for the change in reaction.

TABLE 2

Growth of actinomycetes in synthetic media at various hydrogen-ion concentrations and changes of the reaction produced.

MEDIUM	REACTION OF CONTROL	A. SCABIES 259			A. SCABIES 222			A. GRISEUS			A. VIOLACEUS RUBER		
		Relative growth*	Wt.	Final reaction	Relative growth	Wt.	Final reaction	Relative growth	Wt.	Final reaction	Relative growth	Wt.	Final reaction
	pH		mgm.	pH		mgm.	pH		mgm.	pH		mgm.	pH
1	3.4	0	0	3.4	0	0	3.4	0	0	3.4	0	0	3.4
2	3.8	0	0	3.8	0	0	3.8	0	0	3.8	0	0	3.8
3	4.2	0	0	4.2	0	0	4.2	0	0	4.2	0	0	4.2
4	4.6	0	0	4.6	0	0	4.6	T	1†	4.6	T	2†	4.6
5	5.0	T	1	5.0	2	268	5.0	T	1†	5.0	1	15	5.0
6	5.6	T	1	5.6	3	346	5.8	T	1†	5.6	2	47	5.8
7	6.4	1	2	6.4	4	377	6.8	2	214	6.4	3	184	6.6
8	6.9	2	5	7.1	5	601	8.4	3	253	8.0	4	314	7.4
9	7.4	1	2	7.4	5	499	8.6	2	177	8.2	3	233	7.6
10	8.0	0	0	8.0	5	547	9.2	2	110	9.0	1	99	8.0

* T = Trace, 1-5 indicates the relative amounts of growth, 5 being the maximum.

† Approximate weight.

Experiment 2

The medium used in this experiment had the same composition as that used in the previous one, only the amount of asparagin was reduced to 2 gm. per liter of medium and the amounts of buffers in the media were slightly changed, as shown in table 3. The cultures were grown for 21 days at 25-27°C. The results are given in table 4.

We find a decided difference between the various cultures of *A. scabies* in their resistance to acidity. While *A. scabies* 254 did not begin to develop till the pH of the medium was 5.3, *A. scabies* 281 made some growth even at 4.6.

TABLE 3
The amounts of buffer solutions used in experiment 2

MEDIUM NUMBER	BUFFER SOLUTION		REACTION OF MEDIUM
	KH_2PO_4 1.0 M	K_2HPO_4 1.0 M	
	cc.	cc.	pH
1	20.0	0.0	4.2
2	19.5	0.5	4.6
3	19.0	1.0	5.0
4	18.5	1.5	5.4
5	18.0	2.0	5.6
6	16.0	4.0	6.2
7	10.0	10.0	6.8

TABLE 4
Growth of A. scabies in synthetic media at various hydrogen-ion concentrations

MEDIUM NUMBER	REACTION OF CONTROL	A. SCABIES 254		A. SCABIES 259		A. SCABIES 281	
		Relative growth	Final reaction	Relative growth	Final reaction	Relative growth	Final reaction
	pH		pH		pH		pH
1	4.0	0	4.0	0	4.0	0	4.0
2	4.6	0	4.6	0	4.6	1	5.2
3	4.9	0	5.0	1	6.6	2	5.8
4	5.3	2	5.4	2	6.1	3	6.2
5	5.6	3	6.8	2	6.2	3	6.6
6	6.1	3	6.6	3	7.4		
7	6.8			3	7.4		

Experiment 3

The above experiment was repeated with larger amounts of buffer (50 cc. of the mixture) and 2 gm. of asparagin per liter. The flasks contained only 60 cc. of culture medium. Five cultures of *A. scabies* were used, with three or four flasks for each culture and each reaction. The amount of buffer used in experiment 3 is given in table 5 and the results are presented in table 6.

TABLE 5
The amount of buffer solutions used in experiment 3

MEDIUM NUMBER	KH_2PO_4 1.0 M	K_2HPO_4 1.0 M	REACTION OF MEDIUM
	cc.	cc.	
1	50.00	0.00	4.2
2	48.75	1.25	4.8
3	47.00	3.00	5.2
4	45.00	5.00	5.6
5	40.00	10.00	6.4

From the results presented in table 6, we may definitely establish the fact that, when the nutrients present in the medium are favorable for the development of actinomycetes, the limiting acid reaction, for the majority of strains, lies at a pH between 4.8 and 5.2. While some strains (281) may develop to

TABLE 6
Growth of A. scabies in synthetic media at various hydrogen-ion concentrations

INCUBATION PERIOD	REACTION OF MEDIUM	A. SCABIES 222			A. SCABIES 254			A. SCABIES 281			A. SCABIES 283		A. SCABIES 295	
		gr.*	wt.	pH	gr.	wt.	pH	gr.	wt.	pH	gr.	pH	gr.	pH
days	pH		mgm.			mgm.			mgm.					
5	4.2	0			0			0			0		0	
	4.8	0			0			0			0		0	
	5.2	1			0			0			1		0	
	5.6	2			T			0			2		0	
	6.4	3			2			1			3		1	
10	4.2	0			0			0			0		0	
	4.8	0			0			0			0		0	
	5.2	2			1			1			2		0	
	5.6	3			2			2			3		T	
	6.4	4			3			3			5		2	
15	4.2	0			0			0			0		0	
	4.8	0			0			T			0		0	
	5.2	3			1			2			2		0	
	5.6	4			2			3			4		1	
	6.4	5			3			4			5		2	
20	4.2	0		4.2	0		4.2	0		4.2	0	4.2	0	4.2
	4.8	0		4.8	0		4.8	T	4	5.2	0	4.8	0	4.8
	5.2	3	80	5.4	1	10	5.4	2	18	5.8	2	5.5	0	5.2
	5.6	5	190	6.5	3	60	6.3	4	42	6.7	4	6.2	1	5.9
	6.4	5	225	6.9	3	48	6.8	4	120	7.1	5	7.0	2	6.6
30	4.2	0		4.2	0		4.2	0		4.2	0	4.2	0	4.2
	4.8	0		4.8	0		4.8	2	30	6.3	T	5.0	0	4.8
	5.2	3	70	5.4	2	40	5.8	3	60	7.3	3	5.4	0	5.2
	5.6	5	200	7.1	4	120	6.6	3	65	6.8	5	6.4	1	6.0
	6.4	5	250	7.3	4	125	6.8	4	100	7.4	5	7.0	2	6.6

* gr. = relative growth; wt. = actual weight of mycelium; pH = pH value of culture at the end of the particular period.

a slight extent at 4.8 and, given a long enough period of incubation may even make a fair growth at that reaction, others (295) will begin to develop only at a much higher point, namely at about pH 5.6. It is quite possible that in the first case, the organism is able to change rapidly the reaction of the medium to less acid and make conditions more readily favorable for its de-

velopment than do the other strains. Culture 295 made a rather weak growth even under optimum conditions; this is due probably to the fact that this strain has lost its power of producing aerial mycelium, perhaps on account of long cultivation on artificial media. When a piece of substratum growth is inoculated into a flask with medium, conditions have to be very favorable for its development, before growth can take place. Aerial spores, however, soon germinate and develop into a mycelium, if conditions are only favorable. This is the reason why so many variable results are obtained with various cultures. It is due more to the length of time the organism has been kept on artificial culture media than to a difference in strain. Wollenweber (38) has obtained such a culture from Amsterdam, this culture coming originally from Dr. Whetzel's laboratory at Cornell University. Unable to obtain any scab on potatoes by the use of this culture, Wollenweber merely suggested that it is possible that the American strain does not cause potato scab in Germany.

The change in reaction of medium to less acid confirms the previous observations.

Experiment 4

In addition to the study of the growth of actinomycetes in solution, a series of experiments were carried out, in which actinomycetes, chiefly *A. scabies*, were grown in sterile soil previously adjusted to various reactions, with or without the addition of organic matter. A greenhouse soil and a Sassafras loam were used for this work. A quantity of the two soils were air dried, sieved and brought into the laboratory. The total moisture holding capacity of these soils was 60 and 30 per cent respectively. During the experiment the soils were kept at 60 to 70 per cent saturation unless otherwise stated. The soils, with or without the organic matter, were placed, in 100-gm. portions, in 250-cc. Erlenmeyer flasks, moisture was added, and flasks were plugged with cotton and sterilized in the autoclave at 15 pounds pressure for 1 hour. The flasks were then inoculated with 1-cc. portions of well shaken liquid cultures of the proper organisms, 7 to 14 days old, and incubated at 25 to 28° for the desired length of time. Sterile water was added, at definite intervals, to keep the moisture at an optimum. At the end of the incubation period, some of the soil was used for determinations of ammonia. The method of Gillespie (10) for obtaining the soil solution combined with the colorimetric method for the determination of reaction were used. Ammonia was determined by distillation with MgO into standard acid solution. In some cases the Folin aeration method was used; the results checked up fairly well with those obtained by the distillation method, but were somewhat lower. Since the ammonia formation is given only as an indication of the growth of the organisms and is not an absolute value only the results obtained from the distillation with MgO are reported.

In the first experiment, the greenhouse soil was used; the reaction was adjusted to various pH values by means of sulfuric acid and CaO. Ten-gram portions of soil were placed in beakers, and various amounts of acid and CaO added. To each soil 3 cc. of water was added (the acid was added in the water to make 3 cc.), the beakers covered and allowed to stand 4 to 7 days, when the pH values were determined. Titration curves for the two soils are given in figure 1.

Ten points were selected on the curve, so as to give a series of values, ranging from pH 3.0 to pH 9.8. The proper amounts of acid and CaO were then added to 1-kgm. portions of the soil, the optimum amount of water was then

FIG. 1. TITRATION CURVES FOR THE TWO SOILS

The hydrogen-ion exponent (pH value) are given as ordinates and the amounts of 2 *N* acid, in cubic centimeters, or powdered CaO, in milligrams, added to 10-gm. portions of soil, as abscissae.

introduced and the soils allowed to stand in jars till air-dry. The soils were then placed in 100-gm. portions, in flasks, 2 gm. of alfalfa meal or 1 gm. of dried blood were then added, the soils were brought to optimum moisture, sterilized as usual, inoculated with *A. scabies* 222 and the three soil Actinomyces and incubated for 28 days. The flasks were well shaken at the end of 7 days, so as to make the inoculation more uniform. The results are given in table 7.

The sterilization influenced to some extent the reaction of the soil, particularly in the case of the soils made alkaline. In these the reaction became less alkaline, probably because of the absorption of the calcium. The limiting acid reaction is found to lie at about pH 5.0 to 5.2. While *A. scabies* produced,

at this reaction, only a trace of growth with the alfalfa meal, it made a fair growth with the dried blood. This is no doubt due to the rapid decomposition of the latter, with the formation of ammonia which accounts for the change in reaction. As much as fifty per cent of the nitrogen of the dried blood has been liberated in 28 days in the form of ammonia by various actinomycetes when the initial reaction was at an optimum. Although the figures given under growth are only relative, the amounts of ammonia formed are good indications of actual growth that has taken place. Under these conditions, we find that the optimum reaction for both *A. scabies* and the saprophytic actinomycetes is at a pH 5.8 to 7.7, while the limiting reactions are pH 5.0, on the acid side, and pH 8.8, on the alkaline. Above and below these two points no growth took place.

The alkaline limit is so high that it would be impossible to obtain it for any practical purposes, without ruining the fertility of the soil and is comparable only to black alkali soils. The acid limit is that found by Gillespie and Hurst (10, 11) for the potato soils of Maine which give good crops of potatoes practically free from scab. The application of this limit is not only possible, but practical and of immense economic importance.

Experiment 5

To eliminate the rapid change in reaction, due to the presence of easily decomposable organic matter, the experiment 4 was repeated with sassafras soil to which no organic matter had been added. Five strains of *A. scabies* were used. Since the amounts of ammonia formed from the soil organic matter were rather small, the actual numbers of actinomycetes were determined in the flask by the plate method. Of course, we do not get here absolute figures, since no differentiation is made between actinomycetes spores and mycelium. The results are, however, striking even with the relative figures. The method of plating out is similar to that used in the routine soil bacteriological counts, modified egg albumen agar being used for preparing the plates. The details of the method are found elsewhere (31). The results are reported in table 8.

The differences found in the liquid cultures are also observed on sterile soil. *A. scabies* 281 makes a fair growth at pH 5.1, while 295 begins to develop only at pH 5.3. The rapidity of formation of aerial mycelium accounts for the difference in numbers. Cultures 222 and 283 readily form an abundant aerial mycelium and gave, therefore, a count of 80 millions per gram, with an optimum reaction. Culture 295, which formed no aerial mycelium in pure culture, grew much more slowly and gave a maximum of only 1,500,000 per gram. The limiting reaction for the majority of the strains is found at pH 5.0. Culture 281, however, grew at pH 4.8 and others developed only when the reaction was distinctly less acid, pH 5.3 to 5.5. The difference in the strains may also account for some of the discrepancies in scab control by making the soil acid.

TABLE 8
The influence of reaction upon the growth of *A. scabies* in the soil

ORIGINAL REACTION OF MEDIUM	A. SCABIES 222			A. SCABIES 254			A. SCABIES 281			A. SCABIES 283			A. SCABIES 295		
	Gr.*	Numbers†	Final reaction pH	Gr.	Numbers	Final reaction pH	Gr.	Numbers	Final reaction pH	Gr.	Numbers	Final reaction pH	Gr.	Numbers	Final reaction pH
4.8	0	15,000	4.8	0	8,000	4.8	0	7,000	4.9	0	110,000	4.9	0	3,000	4.9
5.1	1	800,000	5.3	0	9,000	5.1	1	48,000	5.4	?	330,000	5.2	0	20,000	5.1
5.3	2	1,700,000	5.6	1	500,000	5.4	2	500,000	5.6	1	22,000,000	5.5	T	36,000	5.3
5.6	3	51,000,000	5.8	3	1,000,000	6.0	3	1,700,000	5.6	3	40,000,000	5.7	1	250,000	5.5
6.2	4	80,000,000	6.2	3	1,500,000	6.3	3	2,500,000	6.2	3	47,000,000	6.4	2	300,000	6.0
7.2	4	85,000,000	7.4	4	5,000,000	7.6	3	10,300,000	7.7	4	80,000,000	7.5	2	1,500,000	7.6

* gr. = relative amount of growth.

† Organisms per gram of soil.

THE REDUCTION OF SOIL REACTION BY THE UTILIZATION OF THE OXIDATION OF SULFUR BY MICROORGANISMS

The use of acidic fertilizers, or such that tend to make the soil reaction more acid has been recommended. These may do for soils that are acid to start with and where the amount of scab may be small. In this case the use of green manures may suffice to control scab, because organic acids formed in the process of the decomposition of the organic matter may be sufficient to exert a temporary check upon the development of scab. However, this acidity is not of a lasting nature, since the organic acids are rapidly broken down in the soil.

To be able to control scab in slightly alkaline, neutral or only slightly acid soils, acid has to be added to the soil so as to make the reaction acid enough to prevent growth of *A. scabies*. The direct use of acid is both unpractical in method of application and injurious in its action upon the beneficial microorganisms. A substance has to be added which gradually becomes acid. Such a substance is sulfur, which will be oxidized to sulfuric acid, provided the proper sulfur oxidizing organisms are present.

A brief review of the literature on the use of sulfur for the control of potato scab has been given above. We might cite here two typical experiments which have a direct bearing upon the problem at hand.

Wheeler, Hartwell and Moore (37) found that by applying sulfur at the rate of 600 pounds per acre in 1896, no benefit was obtained in limiting scab, in several instances, although it seemed to have been of some value in other cases. When 300 pounds of sulfur per acre were applied again to the same soil in 1897, the number of scabbed tubers was reduced 9 per cent and the number of badly scabbed ones, 31 per cent. They suggested then, quite correctly, that sulfur is oxidized in the soil to sulfuric acid and the germicidal action of the sulfur was probably caused by this acid; therefore, a given amount of sulfur would be expected to exert a more marked influence as a preventative of scab on a neutral or slightly alkaline soil than on one which was very alkaline or which contained such large quantities of carbonates as not to be materially affected by the limited amount of acid produced. However, they pointed out that the application of sulfur to soils, for preventing potato scab regardless of the character of the reaction of the soil is liable to occasionally cause much injury.

By applying sulfur to soils and studying the resulting reaction Martin (22) found that an increase in soil acidity resulting from oxidation of sulfur led to a marked decrease in the amount of scabby potatoes. Where the initial hydrogen-ion concentration of the soil before the sulfur application was pH 5.8 or less, the lighter applications 300 to 500 pounds of sulfur gave approximately as good control of scab as the heavier applications (700 to 1200 pounds); where the initial reaction of the soil was greater than pH 6.0, the heavier applications of sulfur gave the best control of scab.

Experiment 6

In a study of the influence of oxidation of sulfur of the soil upon the activities of the various groups of soil microorganisms, it was found that small additions of sulfur, as long as the reaction does not become too acid (not below pH 5.0), stimulate the growth of microorganisms as indicated by numbers and the decomposition of organic matter. Of course such microorganisms,

like the nitrifying and nitrogen fixing bacteria, which are active at an optimum reaction above pH 6.0 and more, may be slightly injured in their activities (this has not been demonstrated yet). But as soon as the reaction becomes acid enough to bring down the pH value to less than 5.0 or 5.2, the relative number of actinomycetes to the total bacterial numbers rapidly decreases, as indicated by the results in table 9, which represents a typical instance.

TABLE 9

The influence of sulfur oxidation upon the relative numbers of bacteria and actinomycetes in the soil

SULFUR APPLIED PER ACRE	REACTION	MICROORGANISMS PER GRAM	ACTINOMYCETES	
			Numbers per gram	Per cent of total numbers
<i>lbs.</i>	<i>pH</i>			
None	6.2	13,600,000	6,100,000	44.8
300	5.6	12,600,000	6,500,000	51.6
600	5.1	4,800,000	1,200,000	25.0
900	4.8	4,000,000	900,000	22.5

Experiment 7

Several experiments were carried on for the purpose of demonstrating the fact that the oxidation of sulfur by a pure culture of *Thiobacillus thiooxidans* will result in preventing or limiting the growth of pure cultures of *A. scabies* in the same soil. But experiments of this nature are rather difficult to carry out. When sulfur and *Th. thiooxidans* are added first to the soil, and the *A. scabies* is introduced only after the oxidation of the sulfur has gone so far as to make the reaction of the soil acid enough, no growth of *A. scabies* will take place. This treatment is equivalent to making the soil acid by means of sulfuric acid. When *A. scabies* is introduced in the beginning of the experiment, it will make the reaction of the soil alkaline enough, particularly in the presence of a protein-rich substance like dried blood, to neutralize the acidity formed from the oxidation of sulfur. This is particularly true of sterile soil, where the actinomycetes begin to develop rather rapidly while the action of *Th. thiooxidans* is slow at first. These experiments are therefore not representative of what actually takes place in normal soil.

An experiment will therefore be reported, in which the greenhouse soil was used, both with and without the addition of 1 per cent dried blood. Where sulfur was used (100–150 mgm.), it was added to 100-gm. portions of the soil, before sterilization, which took place as usual. The relative amount of growth, ammonia content, and the numbers of actinomycetes per gram of soil are reported as an index of the activities of *A. scabies*. The flasks were inoculated with 1-cc. portions of a 14-day old liquid culture of *Th. thiooxidans* and *A. scabies* strains.

Whenever sulfur was used in connection with *Thiobacillus thiooxidans*, there was found a limitation of the growth of the various strains of *A. scabies*,

TABLE 10

The influence of sulfur oxidation by Thiobacillus thiooxidans upon the development of A. scabies

TH. THIOOXIDANS	A. SCABIES STRAIN NO.	RELATIVE GROWTH	FINAL REACTION	NH ₃ IN 100 GM. SOIL	ACTINOMYCETES PRESENT IN 1 GM. OF SOIL
<i>Soil itself</i>					
No sulfur					
			pH	mgm.	
+		0	5.6	7.3	0
+	254	1	5.9	7.5	660,000
+	257	2	6.0	13.6	13,000,000
+	259	2	6.0	9.7	14,500,000
—	259	2	6.0	14.7	17,000,000
0.1 per cent sulfur					
+		0	2.8	7.1	0
+	254	T	5.8	7.1	18,000
+	257	T	5.8	9.5	0
+	259	T	5.8	7.5	500,000
—	259	1	5.8	10.3	3,000,000
<i>Soil with 1 per cent dried blood</i>					
No sulfur					
+		0	5.6	8.7	0
+	254	3	6.7	42.0	22,000,000
—	254	3	7.2	65.8	40,000,000
+	257	3	7.5	79.5	400,000,000
—	257	3	7.5	89.6	100,000,000
+	259	3	7.3	82.0	100,000,000
—	259	3	7.5	82.6	124,000,000
0.15 per cent sulfur					
+		0	5.6	8.6	0
+	254	T	5.8	12.8	275,000
—	254	1	6.0	23.2	9,000,000
+	257	0	5.6	8.0	0
—	257	3	6.4	32.3	17,000,000
+	259	2	5.8	39.0	5,000,000
—	259	3	6.3	56.0	130,000,000

* + indicates flasks inoculated with sulfur oxidizing organism; —, uninoculated.

as indicated by numbers and ammonia formation. However, the pH values are not those that we would expect from the previous experiments. We would expect that the reaction, in which the limitation of the growth of *A.*

scabies took place, should be at a pH of 5.0 or less. We find flasks, however, in which no growth of *A. scabies* took place, to have a pH value of 5.6 and even 5.8. The only explanation for that phenomenon is the very complexity of the problem and the fact that there was present such a large amount of organic matter. Possibly the sulfuric acid formed from the oxidation of sulfur was present on the surface of the soil particles, sufficient to exert a preventative action upon the germination and growth of *A. scabies*. When the soil is shaken up with water, for the pH determination, the acid may be rapidly neutralized by the buffers present in the soil, in the form of proteins and inorganic colloids. We will be able to answer this complicated problem more definitely when our information of the relation between the acid formation and surface phenomena of the soil as well as the diffusion of the acid will be more extended.

The important conclusion to be drawn from this experiment is the fact that the presence of sufficient sulfur with the sulfur oxidizing bacterium can prevent the development of Actinomyces species that cause potato scab.

INFLUENCE OF SOIL TYPE, MOISTURE, ETC., UPON THE DEVELOPMENT OF ACTINOMYCETES SCABIES

According to Wollenweber (38), scab is not abundant in heavy loam and clay soils and humus-rich moor soils. Light sandy soils, especially in dry years are inclined very favorably to scabbiness. Millard (25) called attention to the fact that peat soils are usually free from scab, while in sandy or gravelly soils, especially where these have been liberally supplied with lime, the disease is prevalent. He explains this by the fact that the introduction of fresh organic matter remedies this defect since it serves as a food (acting as a decoy for the fungus), thus the potato escapes the attack.

It would seem to be rather contradictory to observations of those who studied the activities of actinomycetes in the soil. Conn (3), Waksman (31), Krainsky (16) found them to be quite abundant in soils rich in organic matter. Of course, the observation of Millard (25) that the organic matter serves as a sort of decoy for the *A. scabies* is untenable, both from the principles underlying activities of microorganisms and from available data.

The very accurate studies of Gillespie and Hurst (10) on the Caribou and Washburn soil types in Maine would seem to point to just the opposite results from the observations of the other investigators. The Washburn loam which, due to its low situation, favors an accumulation of muck and peat, is much more subjected to infection by *A. scabies*. Gillespie and Hurst pointed out quite accurately that the reasons for this difference are to be looked for in the difference in soil reaction as measured by the hydrogen-ion concentration. No attempt in this direction has been made by either the German (38) or the English (25) investigators.

We must also keep in mind that, in the presence of an abundance of organic matter, there is an abundant development of fungi and acid producing bacteria. These may produce enough acid from the decomposition of the organic matter to limit the growth of *A. scabies*. As a matter of fact, in slightly acid soils, a small amount of scab may be controlled by the use of green manure, which is rapidly decomposed by various soil microorganisms with the formation (temporary probably) of sufficient organic acids to control scab.

As to the influence of moisture and soil type, a brief summary of experiments carried on for that purpose will suffice here.

The optimum condition for the growth of various strains of *A. scabies* is found at a moisture content of about 60 to 65 per cent of the maximum moisture holding capacity of the soil. This optimum is not much different from that commonly employed as the optimum for the activities of soil bacteria and fungi.

When various amounts of sand were added to a medium loamy soil, the best growth of *A. scabies* was obtained with the mixture containing 25 per cent of sand. Upon a further addition of sand, growth rapidly decreased, till, in pure sand, the amount of growth (as indicated by the formation of ammonia from dried blood) was less than one-fourth that in the soil itself.

SUMMARY

1. The limiting acid reaction for the growth of *Actinomyces scabies* in culture solutions, properly buffered, and in soil, varies with the strain. For the majority of strains, the limiting acid reaction is about pH 5.0-5.2; some strains may grow even at pH 4.8, while others will begin to develop only at pH 5.3-5.6. These results bear out, in the main, the results of Gillespie.

2. The saprophytic soil actinomycetes seem to be more acid resistant than *A. scabies* strains.

3. By the use of the proper amount of sulfur inoculated with *Thiobacillus thiooxidans*, an acid reaction is obtained which will control the growth of *A. scabies* in the soil.

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