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A STUDY OF FACTORS INFLUENCING INOCULATION EXPERIMENTS WITH AZOTOBACTER

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A STUDY OF FACTORS INFLUENCING INOCULATION EXPERIMENTS WITH AZOTOBACTER

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INTRODUCTION

For the past twelve years the Department of Bacteriology of the Kansas Agricultural Experiment Station has been conducting experiments designed to ascertain the factors controlling the natural distribution of *Azotobacter* in the soil. Many interesting facts have been brought to light in these investigations, some of which have been presented in a series of papers *(6), (7), (8) , (9), (10), (11),* and *(12)*. The experimental work connected with these investigations has involved many hundreds of tests, the result of which could not be presented in the series of brief papers just cited. It is proposed to bring together in this bulletin the results of such of these experiments as bear directly upon the introduction of *Azotobacter* into soils not containing them and the necessarily closely related tests having to do with the elimination of *Azotobacter* from soils in which they are abundant.

It is hoped that in making available these data further light may be thrown upon the factors controlling the distribution of this group of organisms. It is believed that the data here presented will substantiate that previously submitted, and, coupled with it, offer a satisfactory explanation for many of the unsuccessful *Azotobacter* inoculation experiments now on record.

The literature relative to inoculating soils with *Azotobacter* has recently been reviewed by Brown and Hart (1); hence there is no necessity for an exhaustive summary here. There are a few points, however, in connection with previous work to which it is wished to call attention.

The ultimate aim of any soil inoculating procedure is, of course, to increase the crop-producing ability of the soil. Presumably, in the case of *Azotobacter* inoculation, this end would be reached only by increasing the nitrogen-fixing ability of the soil. In most of the previously reported experiments successful inoculation has been measured in terms of increased nitrogen-fixing or crop-producing

1. Contribution No. 116 from the Department of Bacteriology.

ability of the soil, little significance being attached to the possibility that the soil in question might be incapable of sustaining the introduced organisms.

One may hope to increase the nitrogen-fixing ability only by the introduction of *Azotobacter* into a soil where they previously did not exist, or by increasing the number or efficiency of those present. Many soils have been reported that apparently are devoid of *Azotobacter*. These would seem to offer the best chances of success. However, with the extremely wide natural distribution of the group, its absence from a particular soil would seem most likely to be due to failure to find there conditions suitable for development. The introduction of *Azotobacter* into such a soil could not be expected to be attended with success unless conditions had been so altered as to make them more favorable. As to the second condition, it would seem logical to expect that any particular group of organisms in a given soil would soon reach a more or less constant level as to numbers and vigor, this level depending upon the equilibrium between the beneficial and harmful factors operating, and that only upon upsetting this equilibrium could one expect any appreciable change in the bacterial constant. Successful practical *Azotobacter* inoculation, then, could hardly be hoped for before the fundamental reasons for their absence or low level in any particular soil were understood and remedied.

Lipman and Brown *(18),* the first to attempt soil-inoculation experiments with *Azotobacter,* realized the lack of sufficient information relative to the physiological requirements of these organisms. After failure, in most instances, to recover *Azotobacter* following their introduction into a soil, they said, "They (their experiments) do show that these organisms *(Azotobacter)* will not survive or remain prominent in soils which do not offer suitable conditions for their growth, and further experiments must be directed toward a better understanding of these suitable conditions." It is not surprising, then, that many experiments in which no attempt was made to render the soil more suitable for bacterial growth have failed to show marked beneficial effects from inoculation.

That beneficial results may possibly be obtained when a suitable habitat is provided is indicated by the results secured by Makrinoff *(20)* and Hutchison *(17).* Unfortunately, these investigators made no qualitative tests to ascertain the presence or absence of the introduced organisms following inoculation, using as the only criterion of successful inoculation the resulting plant growth.

Still more recently Brown and Hart *(1)* have endeavored to demonstrate the beneficial effects of introducing *Azotobacter* into a soil already containing large numbers of these organisms, without in any way altering the soil. Their results tend to bear out the contention that a material change in a flora need not be expected without altering the physical or chemical makeup of the soil, for only moderate or questionable increases, either in numbers or activity of the *Azotobacter,* could be detected following the introduction of large numbers of these organisms.

Briefly, the data available might be summarized by stating that inoculation experiments with *Azotobacter,* whether designed to test their longevity, their effect upon the nitrogen content of the soil, nitrogen-fixing ability, or crop-producing power of the soil have given rather indefinite results. Frequently the results have been conflicting due, no doubt, to the failure of investigators to realize the necessity of so altering the soil as to make it a suitable pabulum or due more probably to the absence of the necessary information for effecting such an alteration.

OBJECT OF INVESTIGATIONS

The *Azotobacter* investigations that have been under way at the Kansas Station were stimulated primarily by the desire to find out why certain soils invariably yielded typical *Azotobacter* cultures while other soils, just as regularly, failed to show any indications of the presence of these organisms when cultured in exactly the same manner. It was thought possible that an answer to the above query might lead to an ultimate explanation of why certain soils, notably in Colorado and other semiarid regions, apparently possessed such active nitrogen-fixing floras and likewise suggest the means whereby these organisms might be used as an aid in maintaining the nitrogen content of nitrogen-deficient soils.

A preliminary examination of 100 soils collected under the most varied conditions immediately available indicated that the absolute reaction of the soil solution was probably the most important single factor influencing the local distribution of this group of organisms. Similar studies were then extended to include soils from widely varying geographic, geologic, and climatic conditions. These studies have been duplicated and the results verified in other laboratories, notably by Christensen *(2), (3), (23), (22), (24), (28).* The apparently well-established and fundamental relation between the reaction of the soil solution and distribution of *Azotobacter* has formed

the basis upon which subsequent inoculation experiments have been made.

Experiments have been conducted primarily along four distinct lines, namely: (1) The effect of reducing the hydrogen-ion concentration of the soil solution upon the longevity of introduced *Azotobacter,* (2) the effect of mixing in varying proportions two soils, one containing, the other not containing *Azotobacter,* upon the subsequent flora of the mixture, (3) the effect of increasing the hydrogen-ion concentration of soils upon their *Azotobacter* flora, and (4) field-inoculation experiments with acid soils both with and without preliminary treatment to alter the reaction.

EXPERIMENTAL METHODS

Certain of the methods employed in these investigations are applicable to practically all the experiments and will be described here.

Collecting Soils.—When large quantities of soil were desired, the exposed surface was scraped off and the soil taken to a depth of approximately six inches. Quantities of soil were taken from several points within a radius of a few yards in order to secure a more representative sample. The entire volume of soil was then passed through a coarse sieve to pulverize, remove any foreign material, and thoroughly mix it. No special effort was made to protect the larger quantities of soil from contamination.

In collecting samples from small plots for cultural purposes, sterile spatulas were used to remove the exposed surface soil; the soil in a small area was stirred to a depth of six inches and the desired quantity removed to sterile bottles. Collection was made from two to six points, depending upon the size of the plot, and the composite sample thoroughly mixed. The total quantity taken usually varied from one-half to one pound. The removal of larger quantities from small plots at frequent intervals would soon cause a marked depression, resulting in the undesirable collection of water.

Culturing for Azotobacter.—If soil from field plots was to be cultured the composite sample was thoroughly mixed and two or four 300 cc. Erlenmeyer flasks containing 50 cc. of mannite culture solution were inoculated from each sample. Ten cubic centimeters of a suspension prepared by shaking one part of soil with two parts of sterile water were used as the inoculum. The suspension was allowed to stand a few minutes to let the heavier soil particles settle out. Four flasks were inoculated when the quantity of nitrogen

fixed was to be determined, in which case two cultures were immediately sterilized in the autoclave as controls.

In culturing laboratory samples the procedure was similar except that approximately five grams of soil were used directly as the inoculum, the quantity of soil available not being sufficient to prepare a suspension.

The moisture content of all samples of soil incubated in the laboratory was made up to approximately the optimum for aerobic bacterial activity, *i. e.,* one-half to two-thirds saturated. The moisture lost by evaporation was restored from time to time.

The culture medium employed had the following composition:

Magnesium sulphate	0.2 gm.
Dibasic potassium phosphate	0.2 gm.
Sodium chlorid	0.5 gm.
Ferric chlorid	Trace.
Calcium chlorid	Trace.
Mannite	20.0 gm.
Distilled mater	1,000.0 cc.

This medium was rendered slightly alkaline to phenolphthalein with sodium hydroxid. A small quantity of sterile calcium carbonate was added to each culture flask before inoculating. In all cases the cultures were incubated at room temperature for three weeks, after which total nitrogen determinations were made according to the modification of the Kjeldahl method suggested by Latshaw (19). The quantities of nitrogen reported represent the average of duplicate cultures after deducting the average of duplicate controls.

During the incubation period frequent examinations were made both macroscopically and microscopically, to ascertain the character of the growth. After approximately two weeks of incubation a heavy fungous growth usually appeared, especially where no *Azotobacter* growth or a nontypical *Azotobacter* film developed. The growth afterwards became so complex that it was difficult to detect *Azotobacter* either macroscopically or microscopically. Where difficulty was experienced in distinguishing between *Azotobacter* and other organisms their presence is reported as questionable, as indicated in the tables by a question mark.

The microscopic examinations were made by placing on a slide a loopful of that part of the surface growth which appeared most characteristic of *Azotobacter,* covering with cover glass, and examining with the 1/6 objective. If typical *Azotobacter* were present in appreciable numbers, the picture was so striking as to be almost

unmistakable. If *Azotobacter* are not present in a soil in sufficient numbers and vigor to develop a visible film or to produce sufficient growth to be observed microscopically by the methods employed, it is questionable whether they are of any significance in the nitrogen economy of a soil.

In the examination for *Azotobacter* it will be noted that three methods of detecting their presence were employed; the formation of a film, the microscopic examination, and the quantity of nitrogen fixed. In the tables a ++ has been placed where, in the opinion of the writer, the major evidence indicated the existence of an *Azotobacter* flora in the soil and a — — where the evidence did not indicate the presence of *Azotobacter.* A +— indicates that one of the duplicate cultures contained *Azotobacter,* while the other did not.

Determination of Reaction. — The colorimetric hydrogen-ion determinations were made by the Clark and Lubs *(4)* method as modified for soils by Gillespie *(14)*. A weighed quantity of the soil was mixed with five times its weight of water, shaken well and centrifugalized until the supernatant liquid was practically clear. The water used in the preparation of the soil extract and suspension was freshly distilled from a mixture of potassium hydroxide and potassium permanganate. The pH of water thus obtained was from 5.7 to 6.0 and was affected by the minutest trace of acid or alkali. All glassware coming in contact with the soil extract was washed in this water. In part of the work buffer solutions were prepared according to Clark and Lubs *(4)* and were checked, and adjusted if necessary, at frequent intervals on a Leeds and Northrup type K potentiometer. It was found difficult to maintain such standard solutions in a sterile condition and this method was later abandoned in favor of a combination of the methods suggested by Gillespie *(15)* and Medalia *(21)*. Difficulty was sometimes experienced in checking readings where two indicators overlapped, but inasmuch as no special effort was being made to determine absolutely the critical pH such discrepancies can in no way alter the general conclusions. Such variations were always less than 0.5 pH.

Electrometric hydrogen-ion concentration determinations, or differences in potential between the soil solution and the hydrogen electrode, were made by using a Leeds and Northrup type K potentiometer in connection with saturated KC1-calomel and hydrogen electrodes similar to the one described by Hildebrand *(16)*. The ratio of soil to water used was the same as employed in colorimetric determinations; i. e., 1 to 5. Six hydrogen electrodes

were connected by switches to the potentiometer so that six samples could be run at the same time. Hydrogen was bubbled through the cells continuously at a rather rapid rate, the cells being constantly shaken. A maximum difference in potential was usually recorded in 10 to 30 minutes, after which the difference decreased very slowly. The length of time required to reach the maximum reading apparently depended, other things being equal, upon the rate of flow of hydrogen. The influence of the rate of flow of hydrogen

TABLE I.—TIME REQUIRED FOR ELECTRODES TO RECORD MAXIMUM DIFFERENCE IN POTENTIAL; HYDROGEN PASSED OVER ELECTRODES SLOWLY (READINGS RECORDED AS MILLIVOLTS)

Soil No.	Time (in minutes)													
	15	20	30	35	40	45	50	55	60	70	80	90	95	100
376	613	.....	625	.....	.....	.....	655	.....	673	683	687	690	.....	a693
376	618	.....	654	.....	.....	.....	678	.....	685	690	694	695	.....	a696
380	503	.....	515	.....	.....	554	563	565	a566	.....	.....	.....	.....	.....
380	517	.....	540	.....	.....	563	a566	566	566	.....	.....	.....	.....	.....
381	.....	520	.....	523	.....	a524	.....	524	.....	.....	.....	.....	.....	.....
381	.....	500	.....	524	.....	a526	.....	526	.....	.....	.....	.....	.....	.....
410	.....	633	.....	.....	642	.....	.....	670	.....	695	.....	.....	a703	703
410	.....	654	.....	.....	691	.....	.....	697	.....	a702	.....	.....	702	700

a Maximum reading.

TABLE II.—TIME REQUIRED FOR ELECTRODES TO RECORD MAXIMUM DIFFERENCE IN POTENTIAL; HYDROGEN PASSED OVER ELECTRODES RAPIDLY (READINGS RECORDED AS MILLIVOLTS)

Soil No.	Time (in minutes)													
	5		10		15		20		25		30		35	
74		678		688	(a)	691		690		690		.....		.....
74		668		681		680	(a)	682		682		.....		.....
75		674		683	(a)	688		688		688		.....		.....
75		670		685		688		690	(a)	692		.....		.....
76		583	(a)	587		586		585		584		.....		.....
76		590	(a)	592		591		590		588		.....		.....
24		648		.....	(a)	675		.....		674		.....		.....
24		630		.....	(a)	667		.....		664		.....		.....
26		662		.....	(a)	670		.....		666		.....		.....
26		665		.....	(a)	670		.....		670		.....		.....
28	(a)	690		.....		690		.....		686		.....		.....
28		682		.....		680		.....	(a)	688		.....		.....
209	(a)	576		574		573		.....		.....		.....		.....
209		535		570	(a)	577		.....		.....		.....		.....
210		540	(a)	546		546		.....		.....		.....		.....
210		536		546	(a)	550		.....		.....		.....		.....
191		.....		.....	(a)	562		.....		562		.....		560
191		.....		.....		564		.....	(a)	566		.....		564
192		.....		.....	(a)	562		.....		560		.....		558
192		.....		.....		560		.....	(a)	564		.....		564
265		.....		.....	(a)	515		.....		515		514		.....
265		.....		.....	(a)	515		.....		515		514		.....
166		575		.....		.....	(a)	588		.....		586		.....
166		565		.....		.....	(a)	586		.....		584		.....
38		556		.....		.....	(a)	563		.....		562		562
38		525		.....		.....		557		.....	(a)	561		560
8		548		.....		.....		582		.....	(a)	585		585
8		548		.....		.....		577		.....		584	(a)	585

(*a*) Maximum reading.

upon the length of time necessary to obtain maximum difference of potential is illustrated in the data presented in Tables I and II. Neutral or alkaline soils usually required a longer time to reach the maximum difference in potential, and the agreement between duplicates was not, as a rule, so close as it was with acid soils.

TABLE III.—EFFECT ON ELECTRODE READINGS OF VARYING THE METHOD OF SATURATING THE ELECTRODE AND SUSPENSION WITH HYDROGEN

Soil No.	Hydrogen run over electrode continuously					
	Electrode No.					
	1	2	3	4	5	Average
401	615	608	595	661	619	608
362	557	550	552	553	554	553
378	542	544	541	541	538	541
377	545	542	549	541	551	546

Soil No.	1,000 cc. hydrogen run over electrode					
	Electrode No.					
	1	2	3	4	5	Average
401	(a) 597	(a) 611	(a) 611	(a) 615	611	609
362	550	550	550	550	.....	550
378	543	542	530	540	538	539
377	550	(a) 530	(a) 537	(a) 538	554	542

Soil No.	1,000 cc. hydrogen run over electrode, then run continuously					
	Electrode No.					
	1	2	3	4	5	Average
401	629	620	622	624	614	622
362	553	553	557	555	.....	554
378	543	543	533	543	530	538
377	555	554	550	552	552	553

(a) These samples apparently did not have sufficient hydrogen passed through to saturate the electrode and suspension, as is evidenced by the increased reading when further passage of hydrogen took place.

The platinum electrodes used were coated with platinum black and tested on a standard acetate solution before using. Several determinations could usually be run with one coating. Duplicate samples of soil were always run, and as a rule the results agreed within 10 millivolts. If the disagreement were much greater than this, the sample was again run. Sharp and Hoagland *(27)* state that "Duplicate determinations of soil suspension usually agreed within 0.01 to 0.02 volt." Plummer *(25)* says "Duplicate readings on the same sample of soil could easily be read to 0.02 volt," while "It was almost impossible to get such closely agreeing results as 0.02 volt

with different samples of the same soil." The data presented in Tables I, II, III, and IV give the millivolt readings of duplicate samples run on different electrodes. In Table IV are shown the millivolt readings of samples of the same soil run on different dates and also the slight effect upon the reading of varying the ratio of soil to water.

TABLE IV.—DIFFERENCE IN POTENTIAL OF SAME SOIL DETERMINED ON DIFFERENT DATES, EFFECT OF VARYING RATIO OF SOIL TO WATER, AND VARIATIONS IN DIFFERENCE IN POTENTIAL AS DETERMINED WITH FIVE DIFFERENT ELECTRODES

Soil No.	Ratio of soil to water	Electrode No.					
		1	2	3	4	5	Average
390 (*a*)	1 to 5	534	544	541	539	532	537
390 (*b*)	1 to 5	538	540	539	532	536	537
389	1 to 5	678	679	677	678	676	678
395	1 to 5	530	528	528	528	529	529
395	1 to 10	536	533	536	536	539	536
387	1 to 5	493	494	493	493	493	493
387	1 to 10	495	494	494	495	494	494
384	1 to 5	650	653	650	652	652	651
397	1 to 5	563	566	567	566	566	566

(*a*) April 5. (*b*) May 31.

In converting volt readings into pH use had been made of the tables prepared by Schmidt and Hoagland *(26)* adding 91 millivolts to the readings to convert them into N/10 KC1-calomel electrode readings. Some investigators regard the difference in potential between saturated and N/10 KC1-calomel electrodes to be of a value other than 91 millivolts. To convert the pH values here recorded into those of any other difference in potential between saturated and N/10 KC1-calomel electrodes, it is only necessary to add or subtract, as the case may be, 0.017 from the figure here recorded for each millivolt above or below 91. All determinations were made at room temperature, and Schmidt and Hoagland temperature correction factors were used to convert room temperature readings into 25° C. readings,

Hydrogen was purchased in cylinders and washed through a saturated solution of mercuric chlorid, alkaline potassium permanganate solution, alkaline pyrogallic acid solution, and distilled water before entering the hydrogen electrode cell. The connection between the calomel and the hydrogen electrodes was made through a glass stopcock. The end of this immersed in the soil suspension was drawn out to a capillary opening and the cock was kept closed during the determination. Between successive determinations, however, the connection was refilled with fresh saturated potassium chlorid.

The pH of a large number of soils was determined both by the colorimetric and by the electrometric methods for a comparison of the two methods. Table V gives the resulting data for 70 different soils. A similar comparison for another series of 418 soils may be summarized as follows:

Number of soils examined	418
Number of soils electrometric pH 6.0 or above	207
Number of soils colorimetric pH 6.0 or above	206
Number of soils electrometric pH below 6.0	211
Number of soils colorimetric pH below 6.0	212
Number of soils electrometric pH 6.0 or above containing *Azotobacter,*	165
Number of soils colorimetric pH 6.0 or above containing *Azotobacter,*	166
Number of soils electrometric pH below 6.0 not containing *Azotobacter.*	177
Number of soils colorimetric pH below *6.0* not containing *Azotobacter*	179
Number unlimed soils electrometric pH *6.0* or above containing *Azotobacter*	18
Number unlimed soils colorimetric pH 6.0 or above containing *Azotobacter*	18
Number of soils electrometric pH below 6.0 containing *Azotobacter*	34
Number of soils colorimetric pH below 6.0 containing *Azotobacter*	33
Average electrometric pH	6.09
Average colorimetric pH	6.11
Association coefficient based on electrometric pH determinations	0.959
Association coefficient based on colorimetric pH determinations	0.961

The data may be taken to show that for a study of this nature, where the exact hydrogen-ion concentration is not essential, the colorimetric method will serve the purpose almost as satisfactorily as the electrometric and is much less time-consuming. Therefore, most of the pH determinations reported in this paper were made by the colorimetric method. However, sufficient experiments with the electrometric method were conducted to show quite conclusively that data secured by either method will lead to the same general conclusions. This does not mean that the colorimetric method is as accurate as the electrometric for the determination of the absolute reaction of a given sample of soil or that one always secures such close agreement between the two methods, for such is certainly not the case. However, where a large number of soils and soil treatments are to be compared, it is believed that the gross results of the two methods will approach each other very closely. Furthermore, a few tenths error in pH one way or the other could in no wise alter the fundamental conclusions arrived at in these investigations.

In all the tables where pH readings are recorded, if the numerical

TABLE V.—TYPE OF GROWTH, NITROGEN FIXED, AND REACTION OF SOILS OF SERIES I

Soil No.	Type of film	Microscopic picture	*Azotobacter*	pH colorimetric	pH electrometric
20	None	No *Azotobacter*	—	5.4	5.10
21	do	do	—	5.6	5.05
22	do	do	—	5.6	5.27
23	do	do	—	5.6	5.10
24	do	do	—	5.6	4.90
25	Typical *Azotobacter*	Typical *Azotobacter*	+	7.0	6.45
26	do	do	+	6.6	6.13
27	do	do	+	6.1	5.88
28	do	do	+	6.2	5.86
29	do	do	+	7.6	7.27
30	None	No *Azotobacter*	—	5.6	5.39
31	Typical *Azotobacter*	Typical *Azotobacter*	+	7.5	7.71
32	None	No *Azotobacter*	—	5.9	5.85
33	do	do	—	6.7	6.56
34	do	do	—	6.8	6.74
35	Typical *Azotobacter*	Typical *Azotobacter*	+	7.6	7.62
36	do	do	+	6.0	6.12
37	None	No *Azotobacter*	—	6.2	6.07
38	Nontypical	*Azotobacter* present	—	5.6	5.59
39	do	do	+	6.1	6.05
40	do	do	+	7.0	6.72
41	do	Typical *Azotobacter*	+	6.0	5.98
42	do	do	+	7.4	7.05
43	Typical *Azotobacter*	do	+	7.7	8.75
44	Nontypical	do	+	7.5	7.71
45	Typical *Azotobacter*	do	+	7.4	7.45
46	None	No *Azotobacter*	—	5.9	6.03
47	Typical *Azotobacter*	Typical *Azotobacter*	+	7.4	7.52
48	Nontypical	do	+	6.4	6.52
49	None	No *Azotobacter*	—	5.5	5.03
50	do	do	—	5.8	5.32
51	do	do	—	5.3	5.41
52	Nontypical	*Azotobacter* present	+	7.3	7.27
53	Typical *Azotobacter*	Typical *Azotobacter*	+	7.7	7.79
54	Nontypical	do	+	6.0	6.18
55	Typical *Azotobacter*	do	+	7.5	7.50
56	Nontypical	do	+	7.4	7.18
57	Typical *Azotobacter*	do	+	7.5	7.30
58	None	No *Azotobacter*	—	5.5	5.46
59	Typical *Azotobacter*	Typical *Azotobacter*	+	7.4	7.49
60	None	No *Azotobacter*	—	5.8	5.73
61	do	do	—	5.5	5.51
62	Typical *Azotobacter*	Typical *Azotobacter*	+	7.5	7.55
63	do	do	+	7.4	7.43
64	do	do	+	6.1	6.35
65	None	No *Azotobacter*	—	5.7	5.94
66	do	do	—	5.7	5.81
67	do	do	—	5.5	5.32
68	Nontypical	Typical *Azotobacter*	+	6.1	6.05
69	None	No *Azotobacter*	—	5.6	5.19
70	Typical *Azotobacter*	Typical *Azotobacter*	+	6.8	6.93
71	Nontypical	do	+	5.6	5.61
72	do	do	+	7.0	6.72
73	None	No *Azotobacter*	—	5.9	5.88
74	Nontypical	Typical *Azotobacter*	+	7.4	7.25
75	Typical *Azotobacter*	do	+	7.5	7.30
76	Nontypical	do	—	5.5	5.63
77	None	No *Azotobacter*	—	5.6	5.34
78	Nontypical	Typical *Azotobacter*	+	7.7	7.54
79	Typical *Azotobacter*	do	+	7.7	8.48
80	Nontypical	*Azotobacter* present	+	7.6	7.84
81	Typical *Azotobacter*	Typical *Azotobacter*	+	7.4	7.77
82	do	do	+	7.7	7.60
83	do	do	+	7.6	7.72
84	do	do	+	7.5	7.47
85	do	do	+	7.5	7.62
86	do	do	+	7.5	7.60
87	do	do	+	7.8	7.59
88	do	do	+	6.9	6.88
89	do	do	+	7.5	7.76
90	do	do	+	7.3	7.06

value is recorded to tenths only the determination was made colorimetrically, whereas if the values are recorded to the second decimal place the determinations were carried out by the electrometric method.

EFFECT UPON THE LONGEVITY OF INTRODUCED AZOTOBACTER OF ADDING BASIC MATERIALS TO ACID SOILS

Several experiments were conducted in which to acid soils, known not to contain *Azotobacter,* there were added varying quantities of basic materials, principally calcium carbonate. The soils were then inoculated with *Azotobacter* and cultured for *Azotobacter* after varying periods of incubation. During incubation the moisture content was restored at frequent intervals. Either at the beginning or conclusion of the experiment the pH of the soil was determined,

Six different soils were used for this purpose. Soil "B" is an upland silt loam that would normally be neutral or slightly alkaline, but years ago it was thickly set with pine trees and the decomposition of the acid pine needles has brought about a strong acid condition while the high organic matter content, gives it an exceptionally highly buffered condition. Approximately 1 per cent calcium carbonate is required to bring the soil back to the neutral point.

Soil "G," also an upland silt loam, is fairly typical of large areas of upland soil from central and eastern Kansas; it is slightly acid but not highly buffered, therefore not requiring very much lime to alter its reaction.

Soil "1000" came from what was originally a swamp that was drained, cleared and cultivated for a short period of years, during the early part of which it produced high yields, but the yields rapidly decreased and it was soon abandoned. It is now largely covered with broomsedge *(Andropogon),* dwarf bamboo *(Arundinaria)* and deciduous species of smilax *(Smilax).* Were it not for the annual fires that destroy practically all vegetation, it would probably become reforested with loblolly pine *(Pinus tæda).* The soil is made up almost entirely of organic matter akin to muck or peat, and coarse sand, which apparently underlies the entire area, cropping out at every slight elevation.

Soil "1001" is a light sandy loam, acid but low in organic matter, hence poorly buffered and very low in productivity.

Soil "1002" could probably be best described as a sandy silt loam since it contains appreciable coarse sand, yet sufficient silt and clay to render it in its deflocculated condition practically impervious to water, remaining water-logged most of the year within a few

yards of open drainage ditches. This type of soil is sometimes spoken of as "crayfish soil" because of a species of crayfish that burrow in it, throwing up mounds of dirt as the water level falls. It is highly acid and well buffered.

Soil "1003" can hardly be called a soil since it is almost pure quartz sand from pine barrens of the coastal plain, acid but very low in organic matter. The characteristic flora of this soil are long-leaf pine *(Pinus pulastris)* and black jack oak *(Quercus nigra)*.

The last four soils all came from Cumberland county, North Carolina, near the western edge of the coastal plain and were selected because of their acid condition. Only "1001" and "1003" may be regarded as typical of large areas, though the other two are fairly abundant.

EXPERIMENTAL RESULTS

Two experiments were conducted with soil "G" to which different quantities of bases were added and afterward inoculated with *Azotobacter*. For the latter purpose *Azotobacter* were prepared by grinding up films from typical crude cultures. Two hundred grams of soil were used in each case. The details of these experiments and the results are presented in Tables VI and VII. It may be noted that soil "G" has a pH only slightly below that regarded as the

TABLE VI.—EFFECT UPON THE LONGEVITY OF INTRODUCED AZOTOBACTER OF ADDING BASIC MATERIALS TO SOIL "G"

Sample No.	Base added	Grams base added	pH at end	Presence of *Azotobacter*					
				2-9-20 (*b*)		3-19-20		5-8-20	
1	$CaCO_3$	.02	5.9	+	+	+	+	−	−
2	do	.10	6.0	+	+	+	+	−	−
3	do	.20	6.2	+	+	+	+	+	+
4	do	1.00	7.4	+	+	+	−	+	+
5	do	2.00	7.4	+	+	+	+	+	+
6	do	5.00	7.4	+	+	+	?	+	+
7	Na_2CO_3	.02	5.8	+	+	+	−	+	−
8	do	.10	6.0	+	+	+	+	+	+
9	do	.20	6.2	+	+	+	+	+	−
10	do	(*a*) 1.00	7.6	+	+	+	+	+	+
11	do	(*a*) 2.00	8.6+	+	?	+	−	+	+
12	do	(*a*) 5.00	8.6+	+	?	−	?	−	−
13	$MgCO_3$	.02	5.8	+	+	?	+	−	−
14	do	.10	6.0	+	+	+	+	+	+
15	do	.20	6.4	+	+	+	+	+	+
16	do	1.00	7.6+	+	+	+	+	+	−
17	do	(*a*) 2.00	8.0	+	+	+	+	+	+
18	do	(*a*) 5.00	8.6+	−	?	+	−	−	−
19	NaOH N/1	.02	5.8	+	+	−	−	−	−
20	do	.10	5.8	+	+	+	−	−	−
21	do	.20	5.8	+	+	+	−	−	−
22	do	1.00	5.8	+	+	+	+	−	−
23	do	2.00	5.8	+	+	+	−	−	−
24	do	5.00	5.8	+	+	+	−	−	−
25	None		5.8	+	+	−	−	−	−
26	None		5.8	−	−	−	−	−	−

(*a*) Gave highly colored extract.
(*b* Experiment started 1-21-20.

critical pH for *Azotobacter,* and it may, therefore, be expected that when these organisms are introduced into it some of them would survive for a time.

The larger applications of Na_2CO_3 and $MgCO_3$ were sufficient to impart a dark or black color to an extract of the soil and may have been present in toxic quantities. The heaviest application of NaOH was not sufficient to appreciably alter the reaction.

TABLE VII.—EFFECT UPON LONGEVITY OF AZOTOBACTER OF ADDING $CaCO_3$ TO SOIL "G"

Sample No.	Grams $CaCO_3$	Pounds per A	pH at end	Presence of *Azotobacter*							
				9-2-20 (*a*)		10-22-20		1-5-21		2-18-21	
1	.02	300	5.7	+	+	+	+	—	—	+	?
2	.04	600	5.7	+	+	+	—	—	?	+	?
3	.06	900	5.8	+	+	+	—	—	—	+	+
4	.08	1,200	5.8	+	+	+	—	+	?	+	+
5	.10	1,500	5.8	+	+	+	—	+	?	+	—
6	.12	1,800	5.8	+	+	+	—	+	+	+	+
7	.14	2,100	5.8	+	+	+	—	+	+	+	—
8	.16	2,400	5.8	+	+	+	—	+	+	+	+
9	.18	2,700	5.8	+	+	+	—	+	+	+	+
10	.20	3,000	5.8	+	+	+	—	+	+	+	?
11	.30	4,500	5.9	+	+	+	—	+	+	+	+
12	.40	6,000	5.9	+	+	+	—	+	+	+	+
13	.00	000	5.6	+	+	+	—	—	—	+	?
14	.00	000	5.6	+	+	+	?	—	—	+	?

(*a*) Experiment started 8-21-20.

The results of these two experiments, while somewhat irregular in part, indicate that the addition of around 0.05 per cent or larger quantities of $CaCO_3$, $MgCO_3$ or Na_2CO_3 will enable this soil to support a typical *Azotobacter* flora for at least six months, provided the base is not present in sufficient quantity to produce toxic conditions. The pH determinations indicate that such additions resulted in lowering the H^+ concentration to approximately 10^{-6} or less. With smaller quantities of the base or with the original soil the *Azotobacter* either disappeared or were incapable of producing a typical film when cultured a short time after inoculation.

The two experiments with soil "B" were identical with the preceding. The results are given in Tables VIII and IX. Again the large applications of Na_2CO_3 and $MgCO_3$ caused a very dark-colored extract and were probably toxic to microorganisms in the soil. The quantities of the various basic substances necessary to materially alter the reaction were very much greater, as were also the quantities necessary to maintain suitable living conditions for the introduced *Azotobacter,* than were required in the preceding experiments. It appears that approximately 1 per cent of the carbonates were required to raise the pH to 6.0 and that similar quan-

tities were sufficient to maintain an *Azotobacter* flora for the longest test period, except in the case of Na_2CO_3, where the quantity necessary to render the reaction favorable was probably toxic.

In the experiment with $CaCO_3$ a smaller quantity than was necessary to adjust the pH to 6.0 was found sufficient to maintain favor-

Table VIII.—EFFECT UPON THE LONGEVITY OF INTRODUCED AZOTOBACTER OF ADDING BASIC MATERIALS TO SOIL "B"

Sample No.	Base added	Grams added	pH at end	Presence of *Azotobacter*					
				2-9-20 (*b*)		3-18-20		5-8-20	
1	$CaCO_3$	.02	4.3	—	—	—	—	—	—
2	do	.10	4.4	—	—	—	—	—	—
3	do	.20	4.7	—	—	—	—	—	—
4	do	1.00	5.3	+	+	—	?	—	—
5	do	2.00	6.5	+	+	+	+	+	—
6	do	5.00	7.0	+	+	+	+	+	+
7	Na_2CO_3	.02	4.3	—	—	—	—	—	—
8	do	.10	4.8	—	—	—	—	—	—
9	do	.20	5.2	—	—	—	—	—	—
10	do	(*a*) 1.00	5.8	—	—	—	—	—	—
11	do	(*a*) 2.00	6.5	—	—	—	—	—	—
12	do	(*a*) 5.00	(*b*) 8.6	—	—	—	—	—	—
13	$MgCO_3$	.02	4.3	—	—	—	—	—	—
14	do	.10	4.3	—	—	—	—	—	—
15	do	.20	4.3	—	—	—	—	—	—
16	do	1.00	5.4	+	+	—	—	—	—
17	do	(*a*) 2.00	6.6	+	+	+	?	+	+
18	do	(*a*) 5.00	7.8	+	+	—	—	—	—
19	NaOH N/1	.02	4.3	—	—	—	—	—	—
20	do	.10	4.2	—	—	—	—	—	—
21	do	.20	4.2	—	—	—	—	—	—
22	do	1.00	4.2	—	—	—	—	—	—
23	do	2.00	4.8	—	—	—	—	—	—
24	do	5.00	4.8	—	—	—	—	—	—
25	None		4.3	—	—	—	—	—	—
26	None		4.3	—	—	—	—	—	—

(*a*) Soil solution slightly to highly colored by base added.
(*b*) Experiment started 1-21-20.

TABLE IX.—EFFECT UPON THE LONGEVITY OF AZOTOBACTER OF $CaCO_3$ ADDED TO SOIL "B"

Sample No.	Grams $CaCO_3$	pH at end	Presence of *Azotobacter*							
			9-1-20 (*a*)		10-21-20		1-4-21		2-18-21	
1	.0	4.1	+	?	+	?	—	—	—	—
2	.2	4.3	+	+	+	?	—	—	—	—
3	.4	4.4	+	+	+	?	—	—	—	—
4	.6	4.5	+	+	+	?	—	—	—	—
5	.8	4.7	+	+	+	+	—	—	—	—
6	1.0	4.8	+	+	+	+	—	—	—	—
7	1.2	5.2	+	+	—	+	+	?	+	+
8	1.4	5.3	+	+	+	+	+	+	+	+
9	1.6	5.6	+	+	—	+	+	+	+	+
10	1.8	5.7	+	+	+	+	+	—	+	+
11	2.0	5.7	+	+	+	—	+	—	+	+
12	2.2	5.9	+	+	+	+	+	—	+	+
13	2.4	6.1	+	+	+	+	—	—	+	+
14	2.6	6.5	+	+	+	+	+	+	+	+
15	2.8	6.6	+	+	+	+	+	+	+	+
16	3.0	6.7	+	+	+	+	+	+	+	+

(*a*) Experiment started 8-20-20.

able conditions for *Azotobacter* during the experimental period of six months. However, where such large quantities of insoluble $CaCO_3$ are added it is difficult to get an absolutely even distribution, which probably leads to marked variations in the reaction of soil particles. Hence it is conceivable that limited portions of the soil may have a favorable reaction even though the soil as a whole may be too acid for the existence of *Azotobacter*. There are strong indications that as the incubation period lengthens, larger quantities of $CaCO_3$ are necessary to maintain an *Azotobacter* flora, at least until the quantity added is sufficient to maintain a pH of approximately 6.0 or higher.

A single experiment was conducted with the highly buffered and strongly acid soil "1000." One hundred grams only of soil were used in this case. So high was the H^+ concentration that 2 per cent or more $CaCO_3$ was necessary to maintain favorable conditions for *Azotobacter,* even for a short period of time, while 3 or 4 per cent was essential for prolonged longevity. The quantity of $CaCO_3$ necessary to raise the pH from 3.4 to above 6.0 was also 3 or 4 per cent. The details are given in Table X.

TABLE X.—EFFECT UPON THE LONGEVITY OF AZOTOBACTER OF ADDING $CaCO_3$ TO SOIL "1000"

Sample No.	Grams $CaCO_3$	pH at end	Presence of *Azotobacter*							
			6-3-24 (*a*)		8-13-24		10-23-24		1-17-24	
1	0	3.4	—	—	—	—	—	—	—	—
2	.1	3.4	—	—	—	—	—	—	—	—
3	.2	3.4	—	—	—	—	—	—	—	—
4	.3	3.6	—	—	—	—	—	—	—	—
5	.4	3.6	—	—	—	—	—	—	—	—
6	.5	3.6	—	—	—	—	—	—	—	—
7	.6	3.6	—	—	—	—	—	—	—	—
8	.7	3.8	—	—	—	—	—	—	—	—
9	.8	3.9	—	—	—	—	—	—	—	—
10	.9	4.0	—	—	—	—	—	—	—	—
11	1.0	4.0	—	—	?	+	—	—	—	—
12	2.0	5.5	+	+	—	?	—	?	+	?
13	3.0	6.0	+	+	+	+	—	?	+	+
14	4.0	6.1	+	+	+	+	+	+	+	+
15	5.0	6.4	+	+	+	+	—	?	+	+

(*a*) Experiment started 3-12-24.

The decidedly acid but poorly buffered soil "1001" was used in one experiment. One hundred grams of soil were used in each sample. The results are presented in Table XI. *Azotobacter* were capable of surviving short periods of time with no addition of base, while the quantity of lime necessary for prolonged longevity apparently lay between 0.1 per cent and 0.2 per cent, the former giving a pH of 6.2 and the latter 6.6.

TABLE XI.—EFFECT UPON THE LONGEVITY OF AZOTOBACTER OF ADDING $CaCO_3$ TO SOIL "1001"

Sample No.	Grams $CaCO_3$	pH at end	Presence of *Azotobacter*			
			6-4-24 (a)	8-13-24	10-23-24	1-17-25
1	0	5.6	+ −	− −	− −	− −
2	.1	6.2	+ +	− −	− −	− −
3	.2	6.6	+ +	+ +	+ +	+ +
4	.3	6.7	+ +	+ +	+ +	+ +
5	.4	7.0	+ +	+ +	+ +	+ +
6	.5	7.1	+ +	+ +	+ +	+ +
7	.6	7.2	+ +	+ +	+ +	+ +
8	.7	7.1	+ +	+ +	+ +	+ +
9	.8	7.2	+ +	+ +	+ +	+ +
10	.9	7.2	+ +	+ +	+ +	+ +
11	1.0	7.1	+ +	+ +	+ +	+ +
12	2.0	7.3	+ +	+ +	+ +	+ +
13	3.0	7.2	+ +	+ +	+ +	+ +
14	4.0	7.3	+ +	+ +	+ +	+ +
15	5.0	7.2	+ +	+ +	+ +	+ +

(a) Experiment started 3-12-24.

A single experiment with the strongly acid and fairly well buffered soil "1002" was also conducted. The results are recorded in Table XII. As the quantity of $CaCO_3$ increased the length of time *Azotobacter* remained viable also increased, 0.4 per cent with a pH of 5.6 being necessary to maintain a typical flora for ten months. A slightly higher quantity was necessary to raise the pH to 6.0, and would also probably be necessary to prolong indefinitely the *Azotobacter* in vigorous condition.

TABLE XII.—EFFECT UPON THE LONGEVITY OF AZOTOBACTER OF ADDING $CaCO_3$ TO SOIL "1002"

Sample No.	Grams $CaCO_3$	pH at end	Presence of *Azotobacter*			
			6-4-24 (a)	8-13-24	10-23-24	1-17-25
1	0	4.0	+ −	− −	− −	− −
2	.1	4.0	+ +	− −	− −	− −
3	.2	4.4	+ +	− −	− −	− −
4	.3	5.4	+ +	+ +	− −	− −
5	.4	5.6	+ +	+ +	+ +	+ +
6	.5	5.7	+ +	+ +	+ +	+ +
7	.6	6.0	+ +	+ +	+ +	+ +
8	.7	6.1	+ +	+ +	+ +	+ +
9	.8	6.2	+ +	+ +	+ +	+ +
10	.9	6.4	+ +	+ +	+ +	+ +
11	1.0	7.0	+ +	+ +	+ +	+ +
12	2.0	7.1	+ +	+ +	+ +	+ +
13	3.0	7.1	+ +	+ +	+ +	+ +
14	4.0	7.1	+ +	+ +	+ +	+ +
15	5.0	7.1	+ +	+ +	+ +	+ +

(a) Experiment started 3-12-24.

As shown in Table XIII, which gives the results of adding $CaCO_3$ to soil "1003," about 0.1 per cent $CaCO_3$ made conditions suitable for *Azotobacter* for a period of 10 months, whereas all had died in four

untreated controls in a much shorter time. The pH was changed from 5.0 to 6.0.

TABLE XIII.—EFFECT UPON THE LONGEVITY OF AZOTOBACTER OF $CaCO_3$ INTRODUCED INTO SOIL "1003"

Sample No.	Grams $CaCO_3$	pH at end	Presence of *Azotobacter*							
			6-4-24 (a)		8-13-24		10-23-24		1-17-24	
1	0	5.0	+	+	−	−	−	−	−	−
2	.1	6.0	+	+	+	+	+	+	+	+
3	.2	6.8	+	+	+	+	+	+	+	+
4	.3	7.0	+	+	−	+	−	+	+	+
5	.4	6.9	+	+	+	+	−	+	+	+
6	.5	7.0	+	+	+	+	−	+	+	+
7	.6	7.1	+	+	+	+	−	+	+	+
8	.7	7.0	+	+	+	+	+	+	+	+
9	.8	7.1	+	+	+	+	+	+	+	+
10	.9	7.1	+	+	+	+	+	+	+	+
11	1.0	7.1	+	+	+	+	+	+	+	+
12	2.0	7.1	+	+	+	+	+	+	+	+
13	3.0	7.2	+	+	+	+	+	+	+	+
14	4.0	7.2	+	+	+	+	+	+	+	+
15	5.0	7.1	+	+	+	+	+	+	+	+

(a) Experiment started 3-12-24.

Six experiments were conducted along somewhat different lines in an effort to ascertain whether the number of *Azotobacter* introduced has any material effect upon the longevity in limed acid soils. In one case the inoculation was excessively heavy while in another only one-hundredth and in the third only one-thousandth as much inoculum was added. The inoculum was prepared by suspending in 600 cc. water the surface films from twelve 300 cc. culture flasks containing 50 cc. of media in which there was a typical *Azotobacter* development. The suspension was then shaken with glass beads in order to bring about the disintegration of the films. Twenty cubic centimeters of this suspension served as the inoculum. It is therefore quite evident that even those flasks receiving the least amount were in reality inoculated rather heavily. The inoculum used in the last three experiments was prepared at a different time and therefore not identical with that in the first three.

The quantity of $CaCO_3$ added was based upon that found necessary to maintain living *Azotobacter* in the corresponding soil in the preceding experiments. For soil "1001" the value appeared to be between 0.1 per cent and 0.2 per cent, for soil "1002" between 0.3 per cent and 0.4 per cent, and for soil "1003" between 0.0 per cent and 0.1 per cent.

The results secured with soil "1001" are given in Tables XIV and XV. These, while not altogether consistent, indicate very strongly

that the heavier the inoculum the longer *Azotobacter* are capable of living under unfavorable conditions. However, even such enormous numbers as were introduced in the heaviest inoculation soon disappeared, when no lime, or quantities insufficient to raise the pH to approximately 6.0 or above were added, which for this soil appeared to be somewhere between 0.1 and 0.15 per cent.

TABLE XIV.—EFFECT UPON THE LONGEVITY OF AZOTOBACTER OF ADDING DIFFERENT QUANTITIES OF INOCULUM TO SOIL "1001"

Sample No.	Grams $CaCO_3$	Inoculum = 1		Inoculum = 1 — 100		Inoculum = 1 — 1000	
		Presence of *Azotobacter*		Presence of *Azotobacter*		Presence of *Azotobacter*	
		12-5-24	1-8-25 (a)	12-5-24	1-8-25	12-5-24	1-8-25
1	000	+ +	+ ?	— —	— —	— —	— —
2	.025	+ +	+ +	.....	.....	— —	— —
3	.050	+ +	+ +	+ ?	— ?	+ ?	— —
4	.075	+ +	+ +	+ +	+ +	+ +	? —
5	.100	+ +	+ +	+ +	+ +	+ +	+ +
6	.125	+ +	— +	+ +	+ +	+ +	+ +
7	.150	+ +	+ +	+ +	+ +	+ +	+ +
8	.175	+ +	+ —	+ +	+ +	+ +	+ +
9	.200	— +	+ +	+ +	+ +	+ —	+ +

(a) Experiment started 10-28-24.

TABLE XV.—EFFECT UPON THE LONGEVITY OF AZOTOBACTER OF ADDING DIFFERENT QUANTITIES OF INOCULUM TO SOIL "1001"

Sample No.	Grams $CaCO_3$	Inoculum = 1			Inoculum = 1 — 100			Inoculum = 1 — 1000	
		Presence of *Azotobacter*			Presence of *Azotobacter*			Presence of *Azotobacter*	
		7-22-25(a)	8-12-25	11-27-25	7-22-25	8-12-25	11-27-25	7-22-25	8-812-25
1	0000	— —	— —	— —	— —	— —	— —	— —	— —
2	.0125	+ —	— —	— —	— —	— —	— —	— —	— —
3	.0250	+ +	+ +	? —	+ —	— —	— —	+ ?	+ ?
4	.0500	+ +	+ —	+ ?	+ +	+ +	— —	+ —	+ ?
5	.0750	+ +	+ +	+ +	+ +	+ +	— —	— —	+ +
6	.1000	+ +	+ +	+ +	+ +	+ +	— —	+ +	+ +
7	.1250	+ +	+ +	+ +	.....	.....	.....	+ +	+ +
8	.1500	+ +	+ +	+ +	+ +	+ +	+ +	+ —	+ +
9	.1750	+ +	+ +	+ +	.....	.....	.....	+ +	+ +

(a) Experiment started 7-2-25.

The results with soil "1002," as given in Tables XVI and XVII, agree with those secured with soil "1001" in that the heavier the inoculum the more persistent the organisms are under unfavorable conditions.

The data presented in Table XII indicate that 0.5 per cent to 0.7 per cent $CaCO_3$ was necessary to raise the pH to 6.0, but that somewhat smaller quantities maintained an *Azotobacter* flora for the duration of the experiment. This is in agreement with the results

secured in the present case with the lighter inocula, but with the very heavy inoculum the organism survived the experimental period in the presence of 0.3 to 0.4 per cent of $CaCO_3$.

TABLE XVI.—EFFECT UPON THE LONGEVITY OF AZOTOBACTER OF ADDING DIFFERENT QUANTITIES OF INOCULUM TO SOIL "1002"

Sample No.	Grams $CaCO_3$	Inoculum = 1			Inoculum = 1 — 100			Inoculum = 1 — 1000		
		Presence of *Azotobacter*			Presence of *Azotobacter*			Presence of *Azotobacter*		
		7-22-25(a)	8-12-25	11-27-25	7-22-25	8-12-25	11-27-25	7-22-25	8-12-25	11-27-25
1	0	— —	— —	— —	— —	— —	— —	— —	— —	— —
2	.1	+ +	+ +	— —	+ ?	— —	— —	— —	— —	— —
3	.2	+ +	+ +	— —	+ +	+ +	— —	+ +	— ?	— —
4	.3	+ +	+ +	+ +	+ +	+ +	— ?	+ +	+ ?	— —
5	.4	+ +	+ +	+ +	+ +	+ +	— ?	+ +	+ +	— —
6	.5	+ —	+ +	+ +	+ —	+ +	+ +	+ —	+ +	+ —
7	.6	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ —
8	.7	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ —

(a) Experiment started 7-2-25.

TABLE XVII.—EFFECT UPON THE LONGEVITY OF AZOTOBACTER OF ADDING DIFFERENT QUANTITIES OF INOCULUM TO SOIL "1002"

Sample No.	Grams $CaCO_3$	Inoculum = 1		Inoculum = 1 — 100		Inoculum = 1 — 1000	
		Presence of *Azotobacter*		Presence of *Azotobacter*		Presence of *Azotobacter*	
		12-5-24(a)	1-8-25	12-5-24	1-8-25	12-5-24	1-8-25
1	0	— —	— —	— —	— —	— —	— —
2	.1	— —	— —	— —	— —	— —	— —
3	.2	— —	— ?	— —	— —	— —	— —
4	.3	+ ?	+ +	— —	— —	— —	— —
5	.4	+ +	+ +	+ +	+ +	— ?	— ?
6	.5	+ +	+ +	+ +	+ +	+ +	+ +
7	.6	+ +	+ +	+ +	+ +	+ +	+ +
8	.7	+ +	+ +	+ +	+ +	— —	+ +

(a) Experiment started 10-28-24.

TABLE XVIII.—EFFECT UPON THE LONGEVITY OF AZOTOBACTER OF ADDING DIFFERENT QUANTITIES OF INOCULUM TO SOIL "1003"

Sample No.	Grams $CaCO_3$	Inoculum = 1		Inoculum = 1 — 100		Inoculum = 1 — 1000	
		Presence of *Azotobacter*		Presence of *Azotobacter*		Presence of *Azotobacter*	
		12-5-24 (a)	1-8-25	12-5-24	1-8-25	12-5-24	1-8-25
1	0000	+ +	+ +	+ —	— —	+ ?	— —
2	.0125	+ +	+ +	+ +	— —	+ ?	— —
3	.0250	+ +	+ +	+ +	+ +	+ ?	— —
4	.0500	+ +	+ +	+ +	+ +	+ +	+ +
5	.0750	+ +	+ +	+ +	+ +	+ +	+ +
6	.1000	+ +	+ +	+ +	+ +	+ +	+ +
7	.1500	+ +	+ +	+ +	+ +	+ +	+ +

(a) Experiment started 10-28-24.

The results secured with soil "1003," as recorded in Tables XVIII and XIX, are in exact accord with those from the other two soils. The quantity of $CaCO_3$ necessary to raise the H^+ to approximately 10^{-6} was found to be probably between 0.05 and 0.1. All quantities in excess of 0.075 per cent maintained the *Azotobacter* for the longest test period. With the heavier inoculations they lived for similar periods in the presence of much smaller quantities of lime.

TABLE XIX.—EFFECT UPON THE LONGEVITY OF AZOTOBACTER OF ADDING DIFFERENT QUANTITIES OF INOCULUM TO SOIL "1003"

Sample No.	Grams $CaCO_3$	Inoculum = 1			Inoculum = 1 — 100			Inoculum = 1 — 1000		
		Presence of *Azotobacter*			Presence of *Azotobacter*			Presence of *Azotobacter*		
		7-22-25 (*a*)	8-12-25	11-27-25	7-22-25	8-12-25	11-27-25	7-22-25	8-12-25	11-27-25
1	0000	+ +	+ +	— —	— —	— —	— —	— —	— —	— —
2	.0125	+ +	+ +	+ +	.....	.....	.....	— —	— —	— —
3	.0250	+ +	+ +	+ +	+ +	+ +	— —	— —	— —	— —
4	.0500	+ +	+ +	+ +	+ —	+ +	+ +	+ +	+ +	— —
5	.0750	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	— ?
6	.1000	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
7	.1500	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +

(*a*) Experiment started 7-2-25.

The results secured in the experiments here recorded may fairly definitely be assumed to show that where *Azotobacter* are introduced into strongly acid soils that normally do not harbor such organisms they will always disappear therefrom in a comparatively short time. The length of their existence appears to depend upon the degree of acidity. The more acid a soil is the more rapidly the *Azotobacter* die, and the larger the number introduced the longer some will live.

The longevity of *Azotobacter* in acid soils can be increased materially by adding $CaCO_3$ and possibly other basic compounds. The period of viability in any given soil increases as the quantity of lime increases until a sufficient quantity to bring the H^+ concentration to near 10^{-6} has been added. In all tests here recorded the longevity was markedly prolonged when this quantity was approached.

THE EFFECT OF MIXING TWO SOILS, ONE CONTAINING, THE OTHER NOT CONTAINING AZOTOBACTER, UPON THE LONGEVITY OF AZOTOBACTER IN THE MIXTURE

Several experiments were carried out in which a soil containing a typical *Azotobacter* flora was mixed in varying proportions with soils that did not contain *Azotobacter* presumably because of their high hydrogen-ion concentration. The mixtures were then incubated and cultured for *Azotobacter* after varying periods of time. The

pH of the mixtures was also determined. Eight different soils, all from near Manhattan, Kan., designated as A, B, C, D, E, F, G, and H were used.

Soil "A" was a fertile garden silt loam with a reaction very close to neutrality. This soil contained the most active nitrogen-fixing flora of any local soil tested and always gave a typical black *Azotobacter* film when cultured in a suitable medium.

Soil "B" has already been described as a strongly acid, highly buffered silt loam that never yielded *Azotobacter* when cultured in the usual manner.

Soil "C" is a silt loam of fair fertility and approximate neutral reaction. This soil is lacking in uniformity partially because of the treatment received and as a result exhibits rather wide variations in reaction.

Soil "D" came from fertile silt loam in blue-grass sod. It is slightly alkaline in reaction and well populated with *Azotobacter*.

Soil "E," a colluvial silt loam, contains large quantities of calcium carbonate from the outcropping limestone just above it. The particular samples used came from uncultivated brush, hence lacked uniformity, but always induced the development of a typical *Azotobacter* film when introduced into a mannite solution.

Soil "F" came from typical uncultivated upland pasture. At the particular location from which it was collected the underlying limestone was perhaps 15 feet below the surface. The pH of this rather unfertile silt loam usually varies from 5.2 to 5.8, and no *Azotobacter* could be detected by culturing.

Soil "G" has already been described as a silt loam of fair productivity but slightly too acid to tolerate *Azotobacter*.

Soil "H" was also from a fairly fertile silt loam field, slightly too acid to support *Azotobacter*.

EXPERMENTAL RESULTS

Three experiments were first, conducted with soils "A" containing, and "B" not containing, *Azotobacter*. Various mixtures were used as indicated in Tables XX, XXI and XXII which follow. It is evident from the results presented in Table XX that *Azotobacter* may be recovered immediately after mixing these two soils in all proportions up to one part of "A" to 299 parts of "B." However, in no subsequent examination, either in this experiment or in those recorded in Tables XXI and XXII, could *Azotobacter* be detected when the proportion of soil "A" was less than half. Even where

equal quantities of the two soils were employed, *Azotobacter* soon disappeared, remaining viable for long periods of time only where the ratio of "A" to "B" was as high as 4 to 1 for the conditions portrayed by Table XX or 3 to 1 for those which existed in the experiments reported in Tables XXI and XXII. Where $CaCO_3$ was added to mixtures of any proportion, *Azotobacter* were recovered at all subsequent examinations.

TABLE XX.—EFFECT UPON THE SUBSEQUENT AZOTOBACTER FLORA OF MIXING IN VARYING PROPORTIONS TWO SOILS, ONLY ONE OF WHICH CONTAINED AZOTOBACTER

Sample No.	Grams soil "A"	Grams soil "B"	Grams $CaCO_3$	Reaction pH	Presence of *Azotobacter*					
					1-30-19 (a)		5-22-19		12-11-19	
1	300	0	0	7.02	+	+	+	+	+	+
2	300	0	0	6.85	+	+	+	+	+	+
3	270	30	0	6.42	+	+	+	+	+	+
4	270	30	0	6.32	+	+	+	−	+	+
5	240	60	0	5.76	+	+	+	+	+	+
6	240	60	0	5.82	+	+	+	+	+	+
7	150	150	0	4.60	+	+	+	−	−	−
8	150	150	0	4.61	+	+	−	−	−	−
9	60	240	0	4.11	+	+	−	−	−	−
10	60	240	0	4.09	+	+	−	−	−	−
11	30	270	0	3.79	+	+	−	−	−	−
12	30	270	0	3.90	+	+	−	−	−	−
13	15	285	0	3.77	+	+	−	−	−	−
14	15	285	0	3.75	+	+	−	−	−	−
15	3	297	0	3.75	+	+	−	−	−	−
16	3	297	0	3.68	+	+	−	−	−	−
17	1	299	0	3.66	+	+	−	−	−	−
18	1	299	0	3.65	+	+	−	−	−	−
19	300	0	3	7.2	+	+	+	+	+	+
20	300	0	3	7.2	+	+	+	+	+	+
21	270	30	3	6.9	+	+	+	+	+	+
22	270	30	3	6.9	+	+	+	+	+	+
23	240	60	3	6.9	+	+	+	+	+	+
24	240	60	3	6.9	+	+	+	+	+	+
25	150	150	3	6.8	+	+	+	+	+	+
26	150	150	3	6.8	+	+	+	+	+	+
27	60	240	3	6.7	+	+	+	+	+	+
28	60	240	3	6.7	+	+	+	+	+	+
29	30	270	3	6.8	+	+	+	+	+	+
30	30	270	3	6.8	+	+	+	+	+	+
31	15	285	3	6.7	+	+	+	+	+	+
32	15	285	3	6.6	+	+	+	+	+	+
33	3	297	3	6.6	+	+	+	+	+	+
34	3	297	3	6.6	+	+	+	+	+	+
35	1	299	3	6.6	+	+	+	+	+	+
36	1	299	3	6.6	+	+	+	+	+	+
37	0	300	3	6.3	−	−	−	−	−	−
38	0	300	3	6.6	−	−	−	−	−	−
39	0	300	0	4.6	−	−	−	−	−	−
40	0	300	0	4.7	−	−	−	−	−	−

(a) Experiment started 1-30-19.

When the survival of *Azotobacter* is compared with the reaction produced in the various mixtures it may be noted that they were capable of surviving for short periods of time in fairly acid conditions, but that no sample with a pH appreciably below 6.0 yielded *Azotobacter* after six months. On the other hand, all samples with a pH of 6.0 or above gave typical cultures as long as they were tested, approximately two years.

TABLE XXI.—EFFECT UPON THE SUBSEQUENT AZOTOBACTER FLORA OF MIXING TWO SOILS, ONLY ONE OF WHICH CONTAINED AZOTOBACTER

Sample No.	Grams soil "A"	Grams soil "B"	Reaction pH	Presence of *Azotobacter*		
				4-21-20(*a*)	5-15-20	6-18-20
1	200	0	7.08	+ +	+ +	+ +
2	199	1	6.96	+ +	+ +	+ +
3	195	5	6.86	+ +	+ +	+ +
4	190	10	6.61	+ +	+ +	+ +
5	175	25	6.10	+ +	+ +	+ +
6	150	50	5.36	+ +	+ +	+ +
7	125	75	5.07	+ +	+ +	— —
8	100	100	4.73	+ +	— —	— —
9	75	125	4.46	+ +	— —	— —
10	50	150	4.06	+ +	— —	— —
11	25	175	3.80	+ +	— —	— —
12	10	190	3.77	+ +	— —	— —
13	5	195	3.70	+ ?	— —	— —
14	1	199	3.68	— —	— —	— —
15	0	200	3.72	— —	— —	— —

(*a*) Experiment started 4-20-20.

TABLE XXII.—EFFECT UPON THE SUBSEQUENT AZOTOBACTER FLORA OF MIXING TWO SOILS, ONLY ONE OF WHICH CONTAINED AZOTOBACTER

Sample No.	Grams soil "A"	Grams soil "B"	Reaction pH	Presence of *Azotobacter*					
				8-29-21 (*a*)	10-3-21	11-21-21	6-19-22	9-8-22	6-18-23
1	200	0	7.05	+ +	+ +	+ +	+ +	+ +	+ +
2	190	10	7.34	+ +	+ +	+ +	+ +	+ +	+ +
3	175	25	6.54	+ +	+ +	+ +	+ +	+ +	+ +
4	150	50	6.14	+ +	+ +	+ +	+ +	+ +	+ +
5	125	75	4.87	+ +	+ ?	+ ?	— —	— —	— —
6	100	100	4.31	+ ?	— —	— —	— —	— —	— —
7	75	125	3.99	— —	— —	— —	— —	— —	— —
8	10	190	3.42	— —	— —	— —	— —	— —	— —
9	0	200	3.42	— —	— —	— —	— —	— —	— —

(*a*) Experiment started 8-13-21.

The results of experiments with soils "A" and "F" are given in Table XXIII. Soil "F" is much less acid than soil "B," hence the ratio of "A" to "F" necessary to produce a reaction favorable to *Azotobacter* was much less than in the preceding experiments.

TABLE XXIII.—EFFECT UPON THE SUBSEQUENT AZOTOBACTER FLORA OF MIXING TWO SOILS, ONLY ONE OF WHICH CONTAINED AZOTOBACTER

Sample No.	Grams soil "A"	Grams soil "F"	Reaction pH	Presence of *Azotobacter*					
				8-30-21 (*a*)	9-28-21	11-30-21	6-13-22	9-8-22	6-18-23
1	200	0	6.93	+ +	+ +	+ +	+ +	+ +	+ +
2	125	75	6.39	+ +	+ +	+ +	+ +	+ +	+ +
3	100	100	6.02	+ +	+ +	+ +	+ +	+ +	+ +
4	75	125	6.04	+ +	+ +	+ +	+ +	+ +	+ +
5	50	150	5.68	+ +	+ +	+ +	+ +	+ ?	+ +
6	25	175	5.75	+ +	+ +	+ +	— —	— —	— ?
7	10	190	5.36	+ +	+ ?	+ ?	+ ?	— —	— —
8	5	195	5.09	+ +	+ —	+ ?	— —	— —	— —
9	0	200	5.29	— —	— —	— —	— —	— —	— —

(*a*) Experiments started 8-17-21.

Again it is evident that only when the mixture was such as to give a reaction approaching pH 6.0 or above did *Azotobacter* survive any appreciable time, whereas they remained viable in sufficient numbers in all mixtures with pH above 6.0 to give typical films as long as the experiment was continued.

Similar experiments involving soil "G" were conducted, the results being presented in Table XXIV. The results so far as the relationship between pH and *Azotobacter* survival are concerned are identical with those of the preceding experiments. However, the sample of soil "G" employed in this and other experiments of approximately the same date gave a higher H^+ concentration than this soil usually exhibits.

TABLE XXIV.—EFFECT UPON THE SUBSEQUENT AZOTOBACTER FLORA OF MIXING TWO SOILS, ONLY ONE OF WHICH CONTAINED AZOTOBACTER

Sample No.	Grams soil "A"	Grams soil "G"	Reaction pH	Presence of *Azotobacter*											
				8-29-21 (a)		10-3-21		11-21-21		7-3-22		9-8-22		6-18-23	
1	200	0	7.17	+	+	+	+	+	+	+	+	+	+	+	+
2	125	75	6.32	+	+	+	+	+	+	+	+	+	+	+	+
3	100	100	6.27	+	+	+	+	+	+	+	+	+	+	+	+
4	75	125	5.31	+	+	+	+	+	+	+	?	+	+	—	—
5	50	150	4.89	+	+	+	+	+	+	—	—	—	—	—	—
6	25	175	4.90	+	+	+	+	+	+	—	—	—	—	—	—
7	10	190	4.35	+	+	—	—	—	—	—	—	—	—	—	—
8	5	195	4.53	—	—	—	—	—	—	—	—	—	—	—	—
9	0	200	4.50	—	—	—	—	—	—	—	—	—	—	—	—

(a) Experiment started 8-13-21.

The data presented in Table XXV, secured by mixing soils "A" and "H," are not quite so uniform as those already given. They, however, lend additional proof to the apparent fact that the *Azotobacter* present in soil "A" cannot survive when introduced into acid soils unless the quantity of "A" is sufficient to lower the H^+ concentration of the mixture to approximately 10-6 or lower.

TABLE XXV.—EFFECT UPON THE SUBSEQUENT AZOTOBACTER FLORA OF MIXING TWO SOILS, ONLY ONE OF WHICH CONTAINED AZOTOBACTER

Sample No.	Grams soil "A"	Grams soil "H"	Reaction pH	Presence of *Azotobacter*									
				8-30-21 (a)		9-26-21		11-25-21		6-9-22		9-8-22	
1	200	0	6.7	+	+	+	+	+	+	+	+	+	?
2	125	75	6.4	+	+	+	+	+	+	+	?	+	?
3	100	100	6.0	+	+	+	+	+	+	—	?	+	?
4	75	125	5.8	+	+	+	+	+	+	—	—	—	—
5	50	150	5.8	+	+	+	+	—	—	—	—	—	—
6	25	175	5.6	+	?	+	+	+	+	—	?	—	—
7	10	190	5.6	+	?	+	+	—	?	—	?	—	—
8	5	195	5.6	—	—	+	?	—	—	—	?	—	—
9	0	200	5.6	—	—	—	—	—	—	—	—	—	—

(a) Experiment strated 8-17-21.

Soil "C" was employed as the source of *Azotobacter* in five experiments. The results of two experiments in which "C" was mixed with "B" are presented in Tables XXVI and XXVII. In the experiment recorded in Table XXVI the ratio of "C" to "B" necessary to give a pH of approximately 6.0 was 190 to 10, whereas a ratio of 175 to 25 was sufficient in the experiment recorded in Table XXVII. A typical *Azotobacter* flora was maintained in both experiments with this latter ratio, indicating the possibility of an error in the pH determinations in the former. This is further indicated by the low pH of soil "C" alone in the first experiment. The two batches of soil "C" were collected at different times and the variation in reaction could be accounted for by the variability of this soil already referred to. In spite of the slight variation in the critical pH exhibited in the two experiments, it is quite evident that *Azotobacter* are incapable of surviving in a mixture of these two soils that is materially more acid than pH 6.0. It is also evident that time is a factor that must be considered in determining the viability of *Azotobacter* in an undesirable medium.

TABLE XXVI.—EFFECT UPON SUBSEQUENT AZOTOBACTER FLORA OF MIXING TWO SOILS, ONLY ONE OF WHICH CONTAINED AZOTOBACTER

Sample No.	Grams soil "C"	Grams soil "B"	Reaction pH	Presence of *Azotobacter*					
				4-22-20 (a)		5-19-20		6-21-21	
1	200	0	6.31	+	+	+	+	+	+
2	199	1	6.24	+	+	+	+	+	+
3	195	5	6.00	+	+	+	+	+	+
4	190	10	5.85	+	+	+	+	+	+
5	175	25	5.43	+	+	+	+	+	+
6	150	50	4.85	+	+	+	?	—	—
7	125	75	4.87	+	+	—	—	—	—
8	100	100	4.55	+	+	—	—	—	—
9	75	125	4.19	+	+	—	—	—	—
10	50	150	3.99	+	—	—	—	—	—
11	25	175	3.74	—	—	—	—	—	—
12	10	190	3.72	—	—	—	—	—	—
13	5	195	3.63	—	—	—	—	—	—
14	1	199	3.60	—	—	—	—	—	—
15	0	200	3.63	—	—	—	—	—	—

(a) Experiment started 4-22-20.

From the data presented in Table XXVIII it may be seen that only very small quantities of soil "C" are necessary to materially alter the reaction of soil "F." The ratio necessary to maintain a vigorous *Azotobacter* flora was apparently somewhat larger than necessary to give a pH of 6.0.

It required approximately equal quantities of soil "C" and "G" to give a pH of 6.0, as indicated in Table XXIX, and similar quantities were necessary to maintain an *Azotobacter* flora for the longer periods of incubation.

TABLE XXVII.—EFFECT UPON THE SUBSEQUENT AZOTOBACTER FLORA OF MIXING TWO SOILS, ONLY ONE OF WHICH CONTAINED AZOTOBACTER

Sample No.	Grams soil "C"	Grams soil "B"	Reaction pH	Presence of *Azotobacter*											
				8-29-21 (a)		10-3-21		11-21-21		7-3-22		9-8-22		6-18-23	
1	200	0	7.13	+	+	+	+	+	+	+	+	+	+	+	+
2	190	10	7.17	+	+	+	+	+	+	+	+	+	+	+	+
3	175	25	6.83	+	+	+	+	+	+	+	+	+	+	+	+
4	150	50	5.09	+	?	+	?	+	?	—	—	—	—	—	—
5	125	75	4.80	+	?	—	?	—	—	—	—	—	—	—	—
6	100	100	4.24	—	—	—	—	—	—	—	—	—	—	—	—
7	50	150	3.77	—	—	—	—	—	—	—	—	—	—	—	—
8	10	190	3.37	—	—	—	—	—	—	—	—	—	—	—	—
9	0	200	3.23	—	—	—	—	—	—	—	—	—	—	—	—

(a) Experiment started 8-13-21.

TABLE XXVIII.—EFFECT UPON THE SUBSEQUENT AZOTOBACTER FLORA OF MIXING TWO SOILS, ONLY ONE OF WHICH CONTAINED AZOTOBACTER

Sample No.	Grams soil "C"	Grams soil "F"	Reaction pH	Presence of *Azotobacter*											
				8-30-21 (a)		9-28-21		11-30-21		6-13-22		9-8-22		6-18-23	
1	200	0	7.60	+	+	+	+	+	+	+	+	+	—	+	+
2	125	75	6.90	+	+	—	+	+	+	+	+	+	+	+	+
3	100	100	6.37	+	+	—	+	+	+	+	+	+	—	+	?
4	75	125	6.76	+	+	+	+	+	—	+	+	+	?	+	?
5	50	150	7.03	+	+	+	+	+	—	+	—	+	?	+	?
6	25	175	6.41	+	+	—	+	+	+	+	?	+	?	+	?
7	10	190	6.35	+	+	—	?	+	+	—	—	—	—	—	—
8	5	195	6.23	+	—	+	?	—	—	—	—	—	—	—	—
9	0	200	5.36	—	?	—	—	—	—	—	—	—	—	—	—

(a) Experiment started 8-17-21.

TABLE XXIX.—EFFECT UPON THE SUBSEQUENT AZOTOBACTER FLORA OF MIXING TWO SOILS, ONLY ONE OF WHICH CONTAINED AZOTOBACTER

Sample No.	Grams soil "C"	Grams soil "G"	Reaction pH	Presence of *Azotobacter*											
				8-29-21 (a)		10-3-21		11-21-21		7-5-22		9-8-22		6-18-23	
1	200	0	6.80	+	+	+	+	+	+	+	+	+	+	+	+
2	125	75	6.22	+	+	+	+	+	+	+	+	+	?	+	—
3	100	100	5.70	+	—	+	+	+	+	—	?	—	—	+	—
4	75	125	5.71	+	?	+	+	+	+	+	—	—	?	+	?
5	50	150	4.95	—	?	+	+	+	?	—	—	—	—	—	—
6	25	175	4.92	—	?	—	—	—	—	—	—	—	—	—	—
7	10	190	4.56	—	—	—	—	—	—	—	—	—	—	—	—
8	5	195	4.24	—	—	—	—	—	—	—	—	—	—	—	—
9	0	200	4.80	—	—	—	—	—	—	—	—	—	—	—	—

(a) Experiment started 8-13-21.

The data contained in Table XXX secured from mixtures of soils "C" and "H" again indicate that slightly higher proportions of "C" are necessary to maintain *Azotobacter* than are required to produce a pH of approximately 6.0.

In a general way the data presented in Tables XXVI to XXX in which soil "C" was used as a sources of *Azotobacter* agree very

well with those recorded in Tables XX to XXV in which soil "A" was used for a similar purpose. However, there is some indication that the *Azotobacter* in soil "C" are not quite so well adapted to the acid soils under study as are those in soil "A." This is not necessarily surprising for there is no reason to believe that soil reaction is the only factor influencing the development of *Azotobacter* or that all strains of *Azotobacter* respond exactly alike to a given reaction.

TABLE XXX.—EFFECT UPON THE SUBSEQUENT AZOTOBACTER FLORA OF MIXING TWO SOILS, ONLY ONE OF WHICH CONTAINED AZOTOBACTER

Sample No.	Grams soil "C"	Grams soil "H"	Reaction pH	Presence of *Azotobacter*									
				8-30-21 (a)		9-26-21		11-25-21		6-9-22		9-27-22	
1	200	0	6.9	+	+	+	+	+	+	+	+	+	+
2	125	75	6.5	+	+	+	+	+	+	+	+	—	?
3	100	100	6.4	+	+	+	+	+	+	—	—	—	—
4	75	125	6.2	+	+	+	+	+	+	+	?	—	—
5	50	150	5.8	+	?	+	+	+	+	—	—	—	—
6	25	175	5.7	+	?	+	?	—	?	—	—	—	—
7	10	190	5.6	+	—	+	?	+	?	—	—	—	—
8	5	195	5.6	—	?	—	—	+	?	—	—	—	—
9	0	200	5.6	—	—	—	—	—	—	—	—	—	—

(a) Experiment started 8-17-21.

Only one experiment was conducted in which soil "D" was used as the source of *Azotobacter,* in which instance it was mixed with soil "B." The data are recorded in Table XXXI. Three parts of the former to one of the latter were necessary to maintain viable *Azotobacter* and likewise to give a pH of approximately 6.0.

TABLE XXXI.—EFFECT UPON THE SUBSEQUENT AZOTOBACTER FLORA OF MIXING TWO SOILS, ONLY ONE OF WHICH CONTAINED AZOTOBACTER

Sample No.	Grams soil "D"	Grams soil "B"	Reaction pH	Presence of *Azotobacter*					
				4-23-20 (a)		5-18-20		5-21-20	
1	200	0	7.18	+	+	+	+	+	+
2	199	1	7.15	+	+	+	+	+	+
3	195	5	7.03	+	+	+	+	+	+
4	190	10	6.66	+	+	+	+	+	+
5	175	25	6.32	+	+	+	+	+	+
6	150	50	5.60	+	+	+	+	+	+
7	125	75	5.10	+	+	+	+	—	—
8	100	100	4.75	+	+	+	?	—	—
9	75	125	4.51	+	+	—	—	—	—
10	50	150	4.23	+	+	—	—	—	—
11	25	175	3.79	+	+	—	—	—	—
12	10	190	3.82	+	+	—	—	—	—
13	5	195	3.67	+	+	—	—	—	—
14	1	199	3.45	—	—	—	—	—	—
15	0	200	3.55	—	—	—	—	—	—

(a) Experiment started 4-23-20.

Two experiments were carried out with soils "E" and "B," the results being recorded in Tables XXXII and XXXIII. Soil "E," it may be recalled, contained a very high per cent of $CaC0_3$, hence a ratio of 1to 3 when mixed even with the highly acid soil "B" was sufficient to furnish a favorable pabulum for *Azotobacter* and incidentally a pH of 6.0 or above.

Soil "E" was mixed with soils "F," "G," and "H," respectively, in three different experiments. The data are recorded in Tables XXXIV, XXXV, and XXXVI. The $CaCO_3$ content of soil "E" was so high that in all cases only five parts to 195 parts of the acid soils, the widest ratio employed, were necessary to give a practically neutral mixture and also to enable *Azotobacter* to survive for the longest periods tested.

TABLE XXXII.—EFFECT UPON THE SUBSEQUENT AZOTOBACTER FLORA OF MIXING TWO SOILS, ONLY ONE OF WHICH CONTAINED AZOTOBACTER

Sample No.	Grams soil "F."	Grams soil "B"	Reaction pH	Presence of *Azotobacter*					
				5-1-20 (a)		5-18-20		6-21-20	
1	200	0	7.73	+	+	+	−	+	+
2	199	1	7.64	+	+	+	+	+	+
3	195	5	7.67	+	+	+	+	+	+
4	190	10	7.67	+	+	+	+	+	+
5	175	25	7.56	+	+	+	+	+	+
6	150	50	7.61	+	+	+	+	+	+
7	125	75	7.52	+	+	+	−	+	+
8	100	100	7.37	+	+	+	+	+	+
9	75	125	7.06	+	+	+	+	+	+
10	50	150	6.02	+	?	+	+	+	+
11	25	175	4.87	+	?	+	+	−	−
12	10	190	3.99	+	?	−	?	−	−
13	5	195	3.94	−	?	+	−	−	−
14	1	199	3.79	−	−	−	−	−	−
15	0	200	3.64	−	−	−	−	−	−

(a) Experiment started 5-1-20.

TABLE XXXIII.—EFFECT UPON THE SUBSEQUENT AZOTOBACTER FLORA OF MIXING TWO SOILS, ONLY ONE OF WHICH CONTAINED AZOTOBACTER

Sample No.	Grams soil "E"	Grams soil "B"	Reaction pH	Presence of *Azotobacter*											
				8-30-21 (a)		9-28-21		12-3-21		6-6-22		9-8-22		6-18-23	
1	200	0	7.47	+	+	+	+	+	+	+	+	+	+	+	+
2	190	10	7.41	+	+	+	+	+	+	+	+	+	+	+	+
3	175	25	7.42	+	+	+	+	+	+	+	+	+	+	+	+
4	150	50	7.25	+	+	+	+	+	+	+	+	+	+	+	+
5	125	75	7.13	+	+	+	+	+	+	+	+	+	+	+	+
6	100	100	7.15	+	+	+	+	+	+	+	+	+	+	+	?
7	50	150	7.07	+	+	+	+	+	+	+	+	+	+	+	+
8	10	190	5.12	+	−	+	−	+	+	−	−	+	?	+	−
9	0	200	3.21	−	−	−	−	−	−	−	−	−	−	−	−

(a) Experiment started 8-17-21.

TABLE XXXIV.—EFFECT UPON THE SUBSEQUENT AZOTOBACTER FLORA OF MIXING TWO SOILS, ONLY ONE OF WHICH CONTAINED AZOTOBACTER

Sample No.	Grams soil "E"	Grams soil "F"	Reaction pH	Presence of *Azotobacter*											
				8-30-21 (a)		9-28-21		11-30-21		6-16-22		9-8-22		6-18-23	
1	200	0	7.62	+	+	+	+	+	+	+	+	+	+	+	+
2	125	75	7.78	+	+	+	+	+	+	+	+	+	+	+	+
3	100	100	7.78	+	+	+	+	+	+	+	+	+	+	+	+
4	75	125	7.56	+	+	+	+	+	+	+	+	+	+	+	+
5	50	150	7.73	+	+	+	?	+	+	+	+	+	?	+	+
6	25	175	7.64	+	+	+	+	+	+	+	+	+	?	+	+
7	10	190	7.02	+	+	+	+	+	+	+	+	+	+	+	+
8	5	195	6.69	+	+	+	+	—	?	+	?	+	+	+	?
9	0	200	5.37	—	—	—	—	—	—	—	—	—	—	—	—

(a) Experiment started 8-17-21.

TABLE XXXV.—EFFECT UPON THE SUBSEQUENT AZOTOBACTER FLORA OF MIXING TWO SOILS, ONLY ONE OF WHICH CONTAINED AZOTOBACTER

Sample No.	Grams soil "E"	Grams soil "G"	Reaction pH	Presence of *Azotobacter*											
				8-30-21 (a)		9-18-21		12-7-21		6-6-22		9-8-22		6-18-23	
1	200	0	7.1	+	+	+	+	+	+	+	+	+	?	+	+
2	125	75	7.1	+	+	+	+	+	+	+	+	+	?	+	+
3	100	100	7.0	+	+	+	+	+	+	+	+	+	?	+	+
4	75	125	7.0	+	+	+	+	+	+	+	—	+	?	+	+
5	50	150	7.0	+	+	+	+	+	+	—	—	+	?	—	?
6	25	175	7.0	+	+	+	+	+	+	—	—	+	?	+	?
7	10	190	6.9	+	+	+	+	+	—	—	—	+	?	+	?
8	5	195	6.7	+	?	+	+	+	—	—	—	+	?	+	?
9	0	200	5.4	—	—	—	—	+	—	—	—	—	—	—	—

(a) Experiment started 8-16-21.

TABLE XXXVI.—EFFECT UPON THE SUBSEQUENT AZOTOBACTER FLORA OF MIXING TWO SOILS, ONLY ONE OF WHICH CONTAINED AZOTOBACTER

Sample No.	Grams soil "E"	Grams soil "H"	Reaction pH	Presence of *Azotobacter*									
				8-30-21 (a)		9-26-21		11-25-21		6-9-22		9-27-22	
1	200	0	7.1	+	+	+	+	+	+	+	+	+	+
2	125	75	7.1	+	+	+	+	+	+	+	+	+	+
3	100	100	7.0	+	+	+	+	+	+	+	+	+	+
4	75	125	7.0	+	+	+	+	+	+	+	+	+	+
5	50	150	7.0	+	+	+	+	+	+	+	+	+	+
6	25	175	7.0	+	+	+	+	+	+	+	+	+	+
7	10	190	6.8	+	+	+	+	+	+	+	+	+	+
8	5	195	6.6	+	+	+	?	+	+	+	—	+	+
9	0	200	5.6	—	—	—	—	—	—	—	—	—	—

(a) Experiment started 8-19-21.

This phase of the work may be summarized by saying that the available evidence indicates that when an acid soil not containing *Azotobacter* is mixed with a neutral of alkaline soil containing *Azotobacter* these organisms are capable of and will survive appreciable lengths of time only in those combinations which result in mixtures in which the concentration of the H^+ approaches very near or is less than 1×10^{-6}.

THE EFFECT OF INCREASING THE H' OF SOILS UPON THEIR AZOTOBACTER FLORA

The effect of increasing the H-ion concentration was studied by adding varying quantities of different acids, both mineral and organic, to soils containing typical *Azotobacter* and culturing at intervals. In some instances $CaCO_3$ was also added in sufficient quantities to neutralize the acid, while to other samples large but not molecular equivalent quantities were thoroughly mixed in the soil with the acid. The reaction of the soil was determined either at the beginning or end of the experiment.

In studying the data presented in the following tables it is highly essential that note be taken as to when the pH determinations were made. If the reaction is tested shortly after an organic acid has been added, the increase in H^+ concentration is proportional to the amount of acid added, provided the quantity added is in excess of that necessary to neutralize all free bases in the soil. However, if the determination is delayed a few weeks, absolutely no change in reaction may be detected; at least such is true with any reasonable quantities of the more common organic acids. In fact in a number of instances in the experiments reported here there is distinct evidence of a decrease in the hydrogen-ion concentration as the quantity of acid is increased.

There are possibly two explanations for this anomalous and apparently contradictory phenomenon, one of which is quite evident to one who has studied the effect of adding organic acids to soils. In the first place there is the possibility of volatilization. This could not be a factor in the case of nonvolatile and is probably of minor importance even with the most volatile acids, because the high adsorptive capacity of most soils would reduce volatilization of dilute solutions to a minimum.

The second, and certainly the most important, of the two factors is the utilization of the acid as food by certain soil organisms. There are present in all soils numerous microörganisms that readily metabolize various organic acids and their salts, reducing them to the end produces CO_2 and water. Where the metallic salt of an organic acid is added to a soil a very marked decrease in H^+ concentration may be brought about by microorganisms utilizing the organic radicle, thereby setting free the metallic ions.

In numerous instances in the experiments here recorded it was noted that a few days after the addition of certain organic acids the soil appeared as a white mass of fungi, every soil particle being so bound up in the network of hyphæ that water poured thereon re-

mained almost perfectly clear. In fact, it was sometimes necessary in preparing a suspension from such soil samples to actually cut the soil mass in pieces before shaking with water in order to obtain even gross disintegration of the mass.

It has been repeatedly pointed out that the time factor is very important in considering the effect various hydrogen-ion concentrations may have upon *Azotobacter;* hence, the unknown rapidity with which organic acids disappear from soils that have been temporarily rendered strongly acid makes it extremely difficult to interpret certain of these data. In certain instances where the pH was determined at the end of the experiment the data indicated that rather large quantities of a particular acid were without effect upon the reaction, yet the *Azotobacter* were destroyed. Or if the pH were run at the beginning the results may indicate a very high H^+ concentration without the destruction of the *Azotobacter.* Evidently in the first instance the concentration of hydrogen ions was great enough for a sufficient length of time to destroy *Azotobacter* followed by complete oxidation of the acid. In the second case the oxidation was brought about before sufficient time had elapsed for complete elimination of *Azotobacter* and after the reduction in H^+ concentration these organisms were able to recover sufficiently to give a typical film when cultured.

There is still another factor that might have been, and apparently was, operating in certain instances. The acid may possess toxic properties toward *Azotobacter* other than by a change in the hydrogen-ion concentration. The writer has recently called attention to the toxic effect of the salts of certain organic acids *(13),* among them being formic, which was employed in some of these experiments.

With these preliminary explanations, the available data will be presented along with such conclusions as the limited information would seem to justify.

EXPERIMENTAL RESULTS

In three different experiments different acids were added in varying concentrations to soil "A" and the soil subsequently cultured for *Azotobacter.* The data recorded in Table XXXVII indicate that small quantities of sulphuric, hydrochloric, acetic, and butyric acids may be added to soil without destroying the *Azotobacter.* Larger quantities of all four acids caused the disappearance of these organisms, even the addition of sufficient, $CaCO_3$ to more than neutralize the acid not preventing the harmful effect in all instances. With

sulphuric and hydrochloric acids quantities sufficient to produce a pH of less than 6.0 were insufficient to destroy *Azotobacter*. In the case of acetic and butyric acids the harmful effect was evidently followed by complete oxidation of the acid since no lasting increase in acidity is evident.

Quantities of the five acids employed in the experiment recorded in Table XXXVIII sufficient to lower the pH below 6.0 were also sufficient to kill *Azotobacter* in all instances except with lactic and the smallest quantity of citric acid. In the case of lactic acid it is quite evident that there was a temporary decrease in either numbers or virulence of the *Azotobacter,* the injury being more marked the higher the concentration of acid. When quantities of $CaCO_3$ sufficient to neutralize the acid were added the toxic effect was eliminated. If the quantity of $CaCO_3$ added were insufficient to neutralize the acid the harmful effect of the acid was not counteracted.

TABLE XXXVII.—EFFECT UPON ITS AZOTOBACTER FLORA OF ADDING VARIOUS ACIDS TO SOIL "A"

Sample No.	Acid added	cc. 2-N acid	Grams $CaCO_3$	pH at end	Presence of *Azotobacter*							
					8-26-20 (*b*)		10-13-20		12-31-20		2-18-21	
1	None			6.6	+	+	+	+	+	+	+	+
2	Sulphuric	1		5.9	+	+	+	+	+	—	+	+
3	do	5		4.5	—	—	—	—	—	—	—	—
4	do	10		3.8	—	—	—	—	—	—	—	—
5	do	25		3.6	+	—	—	—	—	—	—	—
6	do	25	(*a*) 5	7.1	—	—	—	—	+	?	+	?
7	Hydrochloric	1		6.1	+	+	+	+	+	?	+	?
8	do	5		4.6	+	—	—	—	—	—	—	—
9	do	10		4.1	—	—	—	—	—	—	—	—
10	do	25		3.8	—	—	—	—	—	—	—	—
11	do	25	5	6.9	—	—	—	—	—	—	—	—
12	Acetic	1		6.6	+	+	+	+	+	+	+	+
13	do	5		6.6	—	—	—	—	—	—	—	—
14	do	10		6.9	—	—	—	—	—	—	—	—
15	do	25		6.8	—	—	—	—	—	—	—	—
16	do	25	5	7.2	+	+	—	—	—	—	—	—
17	Butyric	1		6.8	+	+	+	+	+	+	+	+
18	do	5		6.7	—	—	+	?	—	—	—	—
19	do	10		6.8	—	—	—	—	—	—	—	—
20	do	25			—	—	—	—				
21	do	25	5	7.4	—	—			—	—	—	—

(*a*) Approximately 2.5 grams $CaCO_3$ required to neutralize acid.
(*b*) Experiment started 8-16-20.

The data presented in Table XXXIX further indicate that quantities of acid sufficient to materially increase the H^+ concentration result in the elimination of *Azotobacter,* the harmful effect in this instance not being neutralized by an excess of $CaCO_3$.

Four experiments were conducted with soil "C." The results are presented in Tables XL to XLII. The data in Table XL show no

destruction of *Azotobacter* where the quantities of acid added were insufficient to lower the pH to below 6.0. In those cases in which the quantities of mineral acids were sufficient to give a H^+ concentration greater than 10^{-6} a harmful effect is evident, although complete elimination of *Azotobacter* had not taken place when the experiment was discontinued. A temporary harmful effect with apparent recovery is evident with the higher application of the organic acids.

The results recorded in Table XLI indicate that the smaller quantities of sulphuric, hydrochloric, acetic, and butyric acids have but slight or questionable effects upon *Azotobacter*. The larger quantities, however, which with the mineral acids resulted in a condition more acid than an H^+ concentration of 10^{-6}, caused complete destruc-

TABLE XXXVIII.—EFFECT UPON ITS AZOTOBACTER FLORA OF ADDING VARIOUS ACIDS TO SOIL "A"

Sample No.	Acid	cc. 2-M acid	Grams $CaCO_3$ added	pH at beginning	8-22-21 (b)		10-10-21		11-26-21		6-9-22		9-9-22	
1	None	0	.....	6.81	+	+	+	+	+	+	+	+	+	+
2	Sulphuric	10	.....	2.55	—	—	—	—	—	—	—	—	—	—
3	....do	20	.....	2.26	—	—	—	—	—	—	—	—	—	—
4	....do	50	.....	2.01	—	—	—	—	—	—	—	—	—	—
5	....do	50	10	1.83	—	—	—	—	—	—	.....		—	—
6	Acetic	10	.....	5.70	+	+	+	+	—	—	.....		—	—
7	....do	20	.....	4.50	—	—	—	—	—	—	—	—	—	—
8	....do	50	.....	4.06	—	—	—	—	—	—	—	—	—	—
9	....do	50	10	7.03	+	+	+	+	+	+	+	+	+	+
10	Lactic	10	.....	6.64	—	—	+	+	+	+	—	—	+	+
11	....do	20	.....	5.09	—	—	+	+	+	+	+	+	+	+
12	....do	50	.....	3.73	—	—	—	—	+	+	+	+	+	+
13	....do	50	10	6.00	+	—	—	—	+	—	+	+	+	+
14	Formic	10	.....	6.20	+	+	+	+	+	+	+	—	—	—
15	....do	20	.....	4.09	—	—	—	—	—	—	—	—	—	—
16	....do	50	.....	3.43	—	—	+	?	—	—	—	—	—	—
17	....do	50	10	7.78	+	+	—	—	+	+	+	+	+	+
18	Citric	10	.....	4.60	+	—	+	+	+	+	+	+	+	+
19	....do	20	.....	2.62	—	—	—	—	—	—	—	—	—	?
20	....do	50	.....	2.10	—	—	—	—	—	—	—	—	—	—
21	....do	50	10	2.55	—	—	—	—	—	—	—	—	—	—

(a) Experiment started 8-6-21.

TABLE XXXIX.—EFFECT UPON THE AZOTOBACTER FLORA OF ADDING ACIDS TO SOIL "A"

Sample No.	Acid	cc. 2-N	Grams $CaCO_3$	pH at end	9-26-21 (b)		11-23-21		6-16-22		9-9-22		7-3-23	
1	None	0	.....	6.8	+	+	+	+	+	+	+	+	+	+
2	Hydrochloric,	5	.....	6.5	+	+	+	+	—	—	—	—	—	—
3	....do	10	.....	5.8	—	—	—	—	—	—	—	—	—	—
4	....do	25	.....	4.6	—	—	—	—	—	—	—	—	—	—
5	....do	25	(a) 5	6.8	+	+	+	+	—	—	—	—	—	—
6	Oxalic	5	.....	6.8	+	+	+	+	+	+	+	?	+	+
7	....do	10	.....	6.8	—	—	—	—	—	—	—	—	—	—
8	....do	25	.....	6.8	—	—	—	—	—	—	—	—	—	—
9	....do	25	(a) 5	7.0	—	—	—	—	—	—	—	—	—	—

(a) Approximately 2.5 grams $CaCO_3$ required to neutralize acid added.
(b) Experiment started 9-1-21.

tion of these organisms. The addition of excess lime protected *Azotobacter* from the toxic effect of sulphuric and acetic acids but not from hydrochloric and butyric.

Table XLII shows the effect upon *Azotobacter* flora of adding various quantities of sulphuric, acetic, lactic, formic, and citric acids to 200 grams of soil "C." It may be noted that quantities of these acids sufficient to produce a condition even temporarily more acid

TABLE XL.—EFFECT UPON THE AZOTOBACTER FLORA OF ADDING VARIOUS ACIDS TO SOIL "C"

Sample No.	Acid added	cc. 1-N acid	pH at end	Presence of *Azotobacter*	
				3-2-20 (*a*)	6-23-20
1	Sulphuric	.2	6.53	+ +	+ +
2	do	1.0	6.73	+ +	+ +
3	do	5.0	5.88	+ +	+ ?
4	do	10.0	5.29	+ +	+ ?
5	do	20.0	4.74	+ −	− ?
6	Acetic	.2	6.42	+ +	+ +
7	do	1.0	6.61	+ +	+ +
8	do	5.0	6.64	+ +	+ +
9	do	10.0	6.74	+ +	+ ?
10	do	20.0	7.10	− −	+ ?
11	Butyric	.2	6.44	+ +	+ +
12	do	1.0	6.58	+ +	+ +
13	do	5.0	6.69	+ +	+ +
14	do	10.0	6.70	− −	+ +
15	do	20.0	7.05	− −	+ ?
16	Hydrochloric	.2	6.41	+ +	+ +
17	do	1.0	6.51	+ +	+ +
18	do	5.0	5.58	+ +	− −
19	do	10.0	5.51	+ ?	− ?
20	do	20.0	4.51	+ +	− ?
21	None	0.0	8.03	+ +	+ +

(*a*) Experiment started 2-16-20.

TABLE XLI.—EFFECT UPON THE AZOTOBACTER FLORA OF ADDING VARIOUS ACIDS TO SOIL "C"

Sample No.	Acid added	cc. 2-N acid	Grams $CaCO_3$	pH at end	Presence of *Azotobacter*			
					8-30-20 (*b*)	10-13-20	1-1-21	2-18-21
1	None			6.5	+ +	+ +	+ +	+ +
2	Sulphuric	1		6.0	+ +	+ +	+ ?	+ ?
3	do	5		4.6	− −	− −	− −	− −
4	do	10		4.1	− −	− −	− −	− −
5	do	25		3.8	− −	− −	− −	− −
6	do	25	5	7.0	+ +	+ +	+ +	+ +
7	Hydrochloric	1		6.4	+ +	+ +	− −	+ ?
8	do	5		4.5	− −	− −	− −	− −
9	do	10			+ −	− −	− −	− −
10	do	25		4.2	− −	− −	− −	− −
11	do	25	5	7.0	+ +	− −	− −	− −
12	Acetic	1		6.7	+ +	+ +	+ +	+ +
13	do	5		6.6	− −	− −	− −	− −
14	do	10		6.7	− −	− −	− −	− −
15	do	25		6.7	− −	− −	− −	− −
16	do	25	5	7.3	+ +	+ +	+ +	+ ?
17	Butyric	1		6.7	+ +	+ +	+ +	+ +
18	do	5		6.7	− −	− −	− −	− −
19	do	10		6.6	− −	− −	− −	− −
20	do	25			− −	− −		
21	do	25	5		− −	− −		

(*a*) Experiment started 8-18-20.

than H^+ concentration of 10^{-6} either partially or completely destroyed *Azotobacter* except with the smallest application of citric acid. In this instance the presence of a visable growth indicated rapid oxidation of the acid. Formic acid eliminated *Azotobacter* even in concentration that had but little effect upon the reaction. In every instance where sufficient $CaCO_3$ was added to neutralize the acid it served as a protection to *Azotobacter*.

TABLE XLII.—EFFECT UPON ITS AZOTOBACTER FLORA OF ADDING VARIOUS ACIDS TO SOIL "C"

Sample No.	Acid	cc. 2-M acid	Grams $CaCO_3$	pH at beginning	Presence of *Azotobacter*									
					8-13-21 (a)		10-10-21		11-28-21		6-9-22		9-9-22	
1	None	0		7.00	+	+	+	+	+	+	+	+	+	+
2	Sulphuric	10		3.90	—	—	—	—	—	—	—	—	—	—
3	do	20		3.25	—	—	—	—	—	—	—	—	—	—
4	do	25		3.09	—	—	—	—	—	—	—	—	—	—
5	do	25	10	7.83	+	+	+	+	+	+	+	+	+	—
6	Acetic	10		6.85	+	+	+	+	+	+	+	—	—	—
7	do	20		4.46	—	—	—	—	—	—	—	—	—	—
8	do	50		4.18	—	—	—	—	—	—	—	—	—	—
9	do	50	10	7.79	+	+	+	+	+	+	+	+	+	+
10	Lactic	10		6.51	—	—	+	+	+	+	+	+	+	+
11	do	20		5.07	—	—	—	—	+	—	+	+	+	—
12	do	50		3.97	—	—	—	—	—	—	—	—	—	—
13	do	50	10	7.47	+	+	+	+	+	+	+	+	+	+
14	Formic	10		6.70	+	+	—	—	—	—	—	—	—	—
15	do	20		6.66	—	—	—	—	—	—	—	—	—	—
16	do	50		3.65	—	—	—	—	—	—	+	—	—	—
17	do	50	10	8.03	+	+	+	+	+	+	+	+	+	+
18	Citric	10		5.14	+	+	+	+	+	+	+	+	+	+
19	do	20		3.18	—	—	—	—	—	—	—	—	—	—
20	do	50		2.16	—	—	—	—	—	—	—	—	—	—
21	do	50	10	3.25	—	—	—	—	—	—	—	—	—	—

(a) Experiment started 8-8-21.

The data recorded in Table XLIII do not differ materially from those in preceding tables except in the destruction of *Azotobacter* by quantities of HCl insufficient to produce a H^+ concentration in excess of 10^{-6}.

TABLE XLIII.—EFFECT UPON THE AZOTOBACTER FLORA OF ADDING ACIDS TO SOIL "C"

Sample No.	Acid added	cc. 2-N acid	Grams $CaCO_3$ added	pH at end	Presence of *Azotobacter*									
					9-26-21 (b)		11-23-21		6-16-22		9-9-22		7-3-23	
1	None	0		6.9	+	+	+	+	+	+	+	+	+	+
2	Hydrochloric,	5		6.8	+	+	+	+	—	+	—	—	—	—
3	do	10		6.0	+	—	+	+	—	—	—	—	—	—
4	do	25		4.6	—	—	—	—	—	—	—	—	—	—
5	do	25	(a) 5	7.0	+	+	+	+	—	+	—	+	—	—
6	Oxalic	5		6.8	+	+	+	+	+	+	+	?	+	+
7	do	10		6.8	—	—	+	—	—	—	—	—	—	—
8	do	25		6.8	—	—	—	—	—	—	—	—	—	—
9	do	25	(a) 5	7.3	+	—	+	+	+	+	+	?	—	?

(a) Approximately 2.5 grams $CaCO_3$ required to neutralize acid added.
(b) Experiment started 9-1-21.

Only one experiment was carried out with soil "D" and the data from it are included in Table XLIV. These data tend to prove that quantities of sulphuric, hydrochloric, acetic, and butyric acids in quantities insufficient to lower the pH of this soil to approximately 6.0 have at most but a temporary harmful effect upon *Azotobacter*.

Soil "E" with its high $CaCO_3$ content was only slightly influenced in reaction by the heaviest applications of the various acids tested. The records in Table XLV indicate that with the possible exception of hydrochloric the seven acids were without effect upon the *Azotobacter*. The apparent toxic effect of HCl, independent of any change in reaction, has already been noted.

TABLE XLIV.—EFFECT UPON THE AZOTOBACTER FLORA OF ADDING VARIOUS ACIDS TO SOIL "D"

Sample No.	Acid added	cc. 1-N acid	pH at end	Presence of *Azotobacter*			
				3-3-20 (*a*)		6-28-20	
1	Sulphuric	0.2	7.41	+	+	+	+
2	do	1.0	7.27	+	+	+	+
3	do	5.0	7.27	+	+	+	+
4	do	10.0	7.05	+	+	+	+
5	do	20.0	6.22	+	−	+	+
6	Acetic	0.2	7.57	+	+	+	+
7	do	1.0	7.52	+	+	+	+
8	do	5.0	7.69	+	+	+	+
9	do	10.0	7.91	+	+	+	+
10	do	20.0	7.86	−	−	+	+
11	Butyric	0.2	7.54	+	+	+	+
12	do	1.0	7.66	+	+	+	+
13	do	5.0	7.81	+	+	+	+
14	do	10.0	7.93	−	−	+	+
15	do	20.0	7.71	−	−	+	+
16	Hydrochloric	0.2	7.47	+	+	+	+
17	do	1.0	7.30	+	+	+	+
18	do	5.0	7.18	+	+	+	+
19	do	10.0	.....	+	+	+	+
20	do	20.0	6.47	−	−	+	+
21	None	.....	.....	+	+	+	+

(*a*) Experiment started 2-18-20.

The adding of acid to soils as a means of studying the effect of reaction upon *Azotobacter* has perhaps been less productive of definite and conclusive results than any other line of attack. It is believed that satisfactory explanations have been offered to account for some of the apparent discrepancies, and in spite of the others, certain fairly definite conclusions in support of the general thesis are, it is believed, justified and are noted in the following summary:

1. The addition of acids experimented with insufficient in quantity to produce a marked increase in the hydrogen-ion concentration had a questionable or no effect upon the *Azotobacter*. The exceptions to this generality were probably due to some toxic property other than reaction.

TABLE XLV.—EFFECT UPON THE AZOTOBACTER FLORA OF ADDING VARIOUS ACIDS TO SOIL "E"

Sample No.	Acid	cc. 2-N acid	Grams $CaCO_3$	pH at end	Presence of *Azotobacter*		
					10-5-21 (a)	11-28-21	9-30-22
1	None	0		7.2	+ +	+ +	+ +
2	Hydrochloric	5		7.1	+ +	+ +	+ +
3	....do	10		7.1	+ +	+ +	+ +
4	....do	25		7.0	+ +	+ +	— —
5	....do	25	5	7.1	+ +	+ +	— —
6	Oxalic	5		7.2	+ +	+ +	+ +
7	....do	10		7.2	+ +	+ +	+ +
8	....do	25		7.2	+ +	+ +	+ —
9	....do	25	5	7.2	+ +	+ +	+ +
10	Sulphuric	5		7.1	+ +	+ +	+ +
11	....do	10		7.0	+ +	+ +	+ +
12	....do	25		7.0	+ +	+ +	+ +
13	....do	25	5	7.0	+ +	+ +	+ —
14	Acetic	5		7.2	+ +	+ +	+ +
15	....do	10		7.2	+ +	+ +	+ +
16	....do	25		7.3	+ +	+ +	+ +
17	....do	25	5	7.3	+ +	+ +	+ +
18	Lactic	5		7.2	+ +	+ +	+ +
19	....do	10		7.2	+ +	+ +	+ +
20	....do	25		7.3	+ +	+ +	+ +
21	....do	25	5	7.3	+ +	+ +	+ +
22	Formic	5			+ +	+ +	+ +
23	....do	10		7.2	+ +	+ +	+ +
24	....do	25		7.2	+ +	+ +	+ +
25	....do	25	5	7.2	+ +	+ +	+ +
26	Citric	5		7.2	+ +	+ +	+ +
27	....do	10		7.2	+ +	+ +	+ +
28	....do	25		7.2	+ +	+ +	+ +
29	....do	25	5	7.3	+ +	+ +	+ +

(a) Experiment started 9-2-21.

2. The addition of sufficient quantities of the acids tested to increase the H^+ concentration of the soil to approximately 10^{-6} or greater, always in the case of mineral and usually in case of organic acids, destroyed the *Azotobacter* present. The exceptions in the case of organic acids were probably due to the very rapid elimination of the acid condition through the oxidation of the acids by microorganisms.

3. The addition of $CaCO_3$ in excess of that required to neutralize the acid usually, though not always, counteracted the toxic effect of the acid. Hydrochloric acid was a striking exception to this rule, quantities of this acid insufficient to effect marked increases in the H^+ concentration sometimes killing *Azotobacter*. It is probable that either the acid as such or some product formed from it is toxic. The same may hold true with such other exceptions as are evident.

FIELD INOCULATION EXPERIMENTS

Field inoculation experiments were carried out by locating small plats on areas of acid *Azotobacter* free soils, treating them in various ways, and at varying intervals thereafter testing for the presence of *Azotobacter*, the nitrogen-fixing ability, and the reaction. The

work was somewhat handicapped because of the lack of suitable areas of sufficiently acid soil. However, five series involving a total of 80 plats on soils "B," "E," and "G" were studied for periods of time varying from a few months to five years. In some instances it became necessary to abandon certain plats before very satisfactory data were secured, while in other instances it was felt that further study would not be justified. For these reasons some of the data in the following tables may appear somewhat incomplete.

EXPERIMENTS WITH SOIL "G"

The reaction of this soil as indicated in Table XLVI varied from pH 4.87 to 5.28. Twenty-four plats, each 10 by 12 feet separated by an alley 2 feet wide, were laid out on an area 82 by 46 feet. The soil from two plats (1 and 6) was removed to a depth of 9 inches and replaced with soil "A" in order to ascertain whether a soil normally well supplied with *Azotobacter* would retain such a flora when placed under the conditions of this experiment. The plats were then treated in various ways as indicated in Table XLVI the week of July 19, 1918, except that the syrup was not added until September 6. The quantities of the different materials added were as follows: Inoculum, 50 pounds of soil "A"; wheat straw, 5 pounds; ground limestone, 5 pounds; and "Karo" corn syrup, 2 pounds. After treatment the soil was spaded to a depth of 7 inches.

The weather remained so hot and dry following the treatment that it was feared inoculation might not be successful, hence the inoculation was repeated on September 6, 1918.

It was found necessary to discontinue these plats after February 1, 1919, hence the data are of little value aside from showing that *Azotobacter* can be introduced into a soil not containing them by the transfer of soil containing an active *Azotobacter* flora.

When it became necessary to discontinue these plats, a second series composed of 22 plats of the same size was located on the same field. In fact plats 6, 12, 18, and 24 of the first experiment became 6A, 12A, 18A, and 24A of the second. There was one marked difference, however, that influenced materially the results. The first plats were located in a small compact, rectangle, whereas it was necessary to arrange the plats in the second case side by side, thereby stretching out over a distance of 262 feet. This spreading out is reflected in a greater variation in the reaction, which for unlimed plats varied from pH 5.07 to 6.15. There was an area near the middle of the series including plats 9 and 10 in which the reaction was favorable to *Azotobacter, i. e.,* a pH of 6.0 or above, when the

TABLE XLVI.—THE EFFECT OF VARIOUS FIELD TREATMENTS UPON THE AZOTOBACTER FLORA OF SOIL "G"

Plat No.	Treatment, July 19, 1918	Presence of *Azotobacter*					Milligrams nitrogen fixed					pH of soil
		7-19-18	7-26-18	7-30-18	8-27-18	1-22-19	7-19-18	7-26-18	7-30-18	8-27-18	1-22-19	1-22-19
1	Soil "A" lime	+ +	.. ..	+ +	+ +	+ +	7.5	.....	8.2	10.9	11 7	7.57
2	Inoculation, lime and straw	— —	.....	+ —	+ ?	+ +	5.7	.....	4.4	7.1	8.4	5.70
3	Inoculation, lime and corn syrup	.....	? ?	— ?	— —	+ +	.....	8.2	5.3	7.7	8 1	5.65
4	Inoculation and lime	.....	.....	+ +	— +	+ +	.....	.....	8.5	7.3	9.1	6.00
5	Inoculation, lime and straw	— ?	.....	+ +	+ +	+ +	7.9	.....	6.8	8.9	7.9	6.61
6	Soil "A"	+ +	.....	+ +	+ +	+ +	7.0	.....	6.3	11.0	8.0	7.12
7	Inoculation and lime	.....	+ —	+ +	+ ?	+ +	.....	7.2	5.3	10.1	9.2	6.09
8	Inoculation	.....	+ +	+ +	+ +	+ +	.....	7.8	3.8	6.9	8.0	5.00
9	Check	.....	— —	+ +	— ?	— —	.....	6.7	5.6	6.3	6.1	4.92
10	Inoculation and corn syrup	.....	.....	+ ?	+ +	+ +	.....	.....	5.6	9.7	10.0	5.28
11	Inoculation and straw	.....	.....	+ +	+ +	+ +	.....	.....	8.8	8.4	7.5	5.14
12	Inoculation	.....	+ +	+ +	+ +	+ +	.....	8.4	6.0	6.6	7.8	5.19
13	Lime	.....	.....	— ?	— —	+ —	.....	.....	3.8	8.4	6.2	6.27
14	Straw	— —	.....	+ +	— —	— —	7.3	.....	6.4	6.9	5.8	5.07
15	Corn syrup	.....	.....	— —	— —	— —	.....	.....	1.5	7.2	7.0	5.24
16	Lime	.....	? ?	— —	— —	— —	.....	5.1	1.1	6.7	5.7	5.24
17	Straw	— —	.....	— —	— —	— —	7.1	.....	1.4	8.4	.....	4.87
18	Check	.....	.....	— —	— —	— —	.....	.....	2 0	7.2	5.8	5.04

experiment was inaugurated. Toward either end the soil became more acid. The effect of this condition is reflected in the persistent appearance of *Azotobacter* in uninoculated plats and their prolonged existence in certain cases when introduced into unlimed soil.

Limestone and straw were added at the rate of 2,500 grams per plat, and syrup at the rate of 1,000 grams per plat. The application of limestone did not seem to effect the desired change in reaction, and in March, 1921, all limed plats were given an additional application of 2,500 grams of commercial $CaCO_3$. The inoculum was 50 pounds of soil "A" as before. The various treatments were applied the week of June 25, 1919. Plats 6A, 12A, 18A, and 24A received no further treatment, except that 24A received an application of straw. All plats were cultivated to a depth of 7 inches each year and the surface was kept cultivated to prevent the growth of weeds. No crops were grown on any plats. Bindweed *(Convolvusus)* was very abundant, necessitating frequent cultivation, and this may have been partially responsible for the occasional appearance of *Azotobacter* in plats where they presumably had not been introduced, in that cultivating inoculated plats along with uninoculated plats would inevitably result in the transfer of organisms from one plat to another.

These plats were tested for *Azotobacter* a total of fourteen different times during the three years they were under observation, and the results secured are recorded in Table XLVII. The reaction was also determined on numerous occasions, a representative example of which is recorded in Table XLVII for the date of July 26, 1919.

At the same time that qualitative data were recorded for the presence of *Azotobacter,* the quantity of nitrogen fixed in inoculated cultural solutions was also determined, and these data are recorded in Table XLVIII.

As previously mentioned, the relatively low H^+ concentration of some of the unlimed plats render an analysis of the data somewhat difficult. In fact, plats 9 and 10 will be left entirely out of consideration. In spite of the difficulty just mentioned, certain points in connection with the data appear rather evident.

In the first place, *Azotobacter* were detected only a few times in plats that were not inoculated or so treated as to lower the H^+ concentration (plats 1, 8, 12, 24A, and 18A). In Table XLVII *Azotobacter* are recorded as present in these five plats only 12 out of a possible 70 times and in only two of these was the growth re-

TABLE XLVII.—THE EFFECT OF VARIOUS FIELD TREATMENTS UPON THE AZOTOBACTER FLORA OF SOIL "G"

Plat No.	Treatment	Presence of *Azotobacter*													pH of soil
		6-28-19 (*a*)	7-26-19	10-9-19	1-15-20	3-10-20	6-16-20	8-12-20	11-19-20	3-22-21	7-26-21	9-6-21	12-28-21	4-15-22	5-5-22	7-26-19
1	Check	− −	− +	− −	− −	+ −	− −	− −	− −	− −	+ +	− −	− −	− −	+ ?	5.58
2	Lime	− −	− −	− −	− −	− −	− −	− −	− −	+ −	+ +	+ −	+ ?	− −	+ ?	6.47
3	Inoculation	− ?	+ +	− −	+ +	+ +	− −	+ −	− −	− −	+ ?	− −	+ −	− −	− −	5.56
4	Inoculation and lime	− −	+ −	+ +	− −	+ −	+ ?	+ +	+ ?	− −	+ +	+ ?	+ −	+ +	+ +	6.83
5	Inoculation and straw	− −	+ +	+ −	+ +	+ −	− −	+ ?	+ ?	+ ?	+ +	− −	− −	− −	+ ?	5.46
6	Lime and straw	− −	− −	− −	− −	− −	+ ?	− −	− ?	− −	+ +	− +	− +	− −	+ −	6.17
7	Inoculation, lime and straw	+ ?	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	7.18
8	Straw	+ −	− −	− −	− −	− −	− −	− −	− −	− −	+ ?	− −	− −	− −	− −	5.75
9	Inoculation and corn syrup	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	6.05
10	Corn syrup	+ −	+ −	+ ?	+ ?	+ ?	+ +	+ +	+ +	+ +	+ +	+ ?	+ ?	+ ?	− ?	6.15
11	Lime and corn syrup	+ +	+ +	+ +	+ +	+ +	+ +	+ −	+ +	+ +	+ +	+ +	+ +	+ +	+ +	6.54
12	Check	− ?	+ ?	− ?	− −	? +	− −	+ −	− −	− −	+ +	− −	− −	− −	− −	5.66
13	Lime	− −	− ?	+ +	+ −	− −	+ −	− −	+ −	− −	− −	+ −	+ +	+ ?	− +	6.86
14	Inoculation	− −	+ +	+ −	+ +	+ −	− −	− −	+ ?	− −	− −	− −	− −	− −	+ ?	5.34
15	Inoculation and lime	− −	+ +	+ +	+ +	+ +	+ −	+ +	+ +	− ?	+ +	+ −	+ ?	− −	+ +	6.78
16	Inoculation and straw	− −	+ +	+ +	+ +	+ +	− −	− −	+ ?	− −	+ −	− −	− −	− −	− −	5.17
17	Inoculation, lime and straw	− −	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	6.49
18	Lime and straw	+ +	− −	− −	− −	− −	− −	− −	− −	+ −	+ +	+ +	+ +	− +	+ +	6.49
24 A	Straw	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −	5.17
18 A	Check	+ ?	− −	− −	− −	− −	− −	− −	− −	− −	+ −	− −	− ?	− −	+ −	5.07
12 A	Inoculation	+ ?	+ −	+ −	− −	− −	− −	− −	+ −	+ −	− −	− −	− −	− −	+ ?	5.22
6 A	Soil	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	7.02

(*a*) Before plats were inoculated.

TABLE XLVIII.—THE EFFECT OF VARIOUS FIELD TREATMENTS UPON THE NITROGEN-FIXING ABILITY OF A SOIL

Plat No.	Treatment	Milligrams nitrogen fixed per culture							
		6-28-19	7-26-19	10-9-19	11-19-20	4-15-21	5-5-22	Average	Relative
1	Check	3.7	5.6	5.9	6 8	5.0	7.2	5.70	100
2	Lime	4.6	2.2	6.4	5.7	4.0	6.1	4.83	93
3	Inoculation	6.6	8.1	6.2	5.9	4.5	3.6	5.82	112
4	Lime and inoculation	3.6	6.5	9.1	9.1	8.2	6.7	7.20	138
5	Inoculation and straw	4.2	8.3	5.8	9.5	5.0	4.7	6.25	120
6	Lime and straw	5.1	1.7	4.0	7.4	5.1	6.4	4.95	95
7	Inoculation, lime and straw	6.0	10.3	8 2	10.8	9.8	8.5	8.93	172
8	Straw	7.8	4 2	6.4	6.7	2.6	4.5	5.37	103
9	Inoculation and corn syrup	12.6	5.8	11.4	11.1	9.5	9.5	9.98	192
10	Corn syrup	3.1	9.4	7.2	10.1	6.9	7.8	7.42	143
11	Lime and corn syrup	7.4	4.8	8.7	11.2	5.6	7.1	7.47	144
12	Check	7.9	4.0	6.3	7.9	4.6	3.4	5.68	100
13	Lime	4.0	3.7	8.9	7.9	4.6	5.7	5.80	112
14	Inoculation	4.4	8.3	8.6	6.7	4 3	5.8	6.35	122
15	Inoculation and lime	6.7	7.0	9.4	10.8	4.4	7.7	7.67	148
16	Inoculation and straw	4.4	8.7	8.1	7.7	6.0	4.2	6.52	125
17	Inoculation, lime and corn syrup	8.3	7.1	10.3	8.7	8.9	8.8	8.68	167
18	Lime and straw	8.5	0.0	7.3	7.2	7.6	8.6	6.53	126
24 A	Straw	3.8	5.4	7.1	6.5	0.0	3.3	4.35	84
18 A	Check	3.4	0.0	7.5	6.2	4.4	3.7	4.20	100
12 A	Inoculation	3.2	7.1	9.0	6.7	4.6	5.7	6.05	116
6 A	Soil	8.9	8.9		10.1	9.8	9.7	9.48	182

corded as typical as indicated by two ++ signs. The average nitrogen fixed in cultures from these plats was only 5.06 milligrams per culture.

In the second place, introducing *Azotobacter* without altering the reaction of the soil failed to establish a flora, with the possible exception of plats 9 and 10, which for reasons already recorded have been dropped from consideration. Thus in plats 3, 5, 14, 16, and 12A *Azotobacter* are recorded as present 33 out of a possible 70 times, 12 of which are recorded as two plus. Twenty of the 33 positive records, and 11 of the 12 two-plus instances, are recorded for the first seven times that tests were run, indicating a marked tendency for *Azotobacter* to disappear from those soils the reaction of which was unaltered. These plats showed some increase in nitrogen fixation, as would be expected, the average per culture being 6.20 milligrams.

In the third place, changing the reaction alone did not result in the establishment of an active *Azotobacter* flora during the course of the experiment, although there is a distinct tendency evidenced in the data from plats 2, 6, 13, and 18 for *Azotobacter* to become more abundant in such treated plats. In 56 possible instances *Azotobacter* were recorded as present 25 times, 20 of which, however, were for the last half of the period. The failure to effect any marked change in the flora is also evident in the quantity of nitrogen fixed per culture which for the four plats averaged 5.53 milligrams.

Finally, altering the reaction, accompanied by inoculation, tended to establish an active *Azotobacter* flora. Plats 4, 7, 15, and 17 were so treated and of 56 examinations for *Azotobacter* only five failed to reveal their presence and three of these were for the first period, *i.*e., before the plats were inoculated. The marked beneficial effect of lime and inoculation upon the nitrogen-fixing flora is reflected in the quantity of nitrogen fixed, which on the average for the four plats was 60 per cent greater than for the check plats.

EXPERIMENTS WITH SOIL "B"

Thirteen plats were located on the same area from which soil "B" was secured. Since this area was thickly covered with pine trees the plats were necessarily small, being only one foot square, and were separated a distance of one foot. Treatment was carried out as indicated in Table XLIX on October 14, 1919. Soil "B," it may be recalled, is very acid and highly buffered, requiring excessive quantities of basic materials to effect an appreciable change in reaction.

The experiment was discontinued after a few months and the data

are of little value except as indicating that the introduction of *Azotobacter* without lowering the H^+ concentration, or that lowering the H^+ concentration without inoculation, are without effect upon the subsequent *Azotobacter* flora, as would be expected. On the other hand, heavy liming accompanied by adequate inoculation may be expected to result in the establishment of an *Azotobacter* flora.

An additional series of plats was located on the same area and only a short distance from those just described. Each plat was 2 by 2 feet, surrounded by an alley 1 foot wide. In preparing the plats the mat of pine needles was removed and the soil from each plat transferred to a metal box, treated as indicated in the following tables, and thoroughly mixed. After mixing, the soil was returned to the original location and the mat of pine needles again spread over the surface. After this the soil was never again disturbed except such as was necessary in securing samples for analyses. The inoculum for each plat where a culture was employed consisted of the films from 50 cc. mannite cultures thoroughly disintegrated by shaking. Where soil was employed as the inoculum it, consisted of 1,000 grams of soil "A" The corn syrup was that sold under the trade name of "Karo," 180 grams being dissolved in 400 cc. water.

The examinations of these plats extended over a period of four years, sixteen examinations being carried out, and the data are recorded in Tables L, LI, and LII. An examination of the data relative to the presence of *Azotobacter* recorded in Table L shows very conclusively that *Azotobacter* is incapable of existing for any appreciable length of time in this soil unless inoculation is accompanied by the application of rather large quantities of a basic material of some kind. If the basic material is limited, as on plat 8, *Azotobacter* soon disappear, whereas with larger quantities of the same material their viability may be prolonged, at least for four years. Inoculating with a crude culture was apparently more effective than inoculation with soil as the two were used in this experiment, as shown, for example, by a comparison of plats 6 and 9. Also the addition of an available food seemed to aid in prolonging their existence, as shown by the data from plats 9 and 11.

A study of the reaction data contained in Table LI reveals the fact that even with the excessive application of 410 grams per 4 square feet, or 90,000 pounds per acre (plats 3, 6, 9, and 13), the pH of the soil soon dropped to approximately 6.0 or lower. With the lighter application (plat 8) the reaction, approximately pH 7.0 to start with, fell almost to pH 5.0 in nine months.

TABLE XLIX.—FIELD EXPERIMENTS WITH SOIL "B"

Plat No.	Treatment, November 14, 1924	Presence of *Azotobacter*					Reaction pH	
		10-24-19	1-16-20	3-10-20	6-11-20	6-15-20	10-24-19	6-15-20
1	Inoculated with 500 grams soil	+ +	— —	— —	— —	— —	4.8	5.2
2	$MgCO_3$, 105 grams. Inoculated with 10 grams soil	— —	— —	+ —	— —	— —	5.8	5.7
3	Inoculated with 10 grams soil	— —	— —	— —	— —	— —	5.2	5.4
4	$CaCO_3$, 105 grams. Inoculated with 10 grams soil	+ +	— —	— —	— —	+ —	6.3	6.6
5	Check	— —	— —	— —	— —	— —	5.0	5.3
6	Check	— —	— —	— —	— —	— —	4.8	5.3
7	$CaCO_3$, 105 grams. Inoculated with 500 grams soil	+ +	+ +	+ +	— —	+ +	6.7	6.2
8	$CaCO_3$, 105 grams	— —	+ —	— —	— —	— —	6.8	6.7
9	$MgCO_3$, 105 grams. Inoculated with 500 grams soil	+ +	+ +	+ +	+ +	+ +	7.1	6.0
10	$MgCO_3$, 105 grams	— —	— —	— —	— —	— —	6.2	5.8
11	NaOH, 10 grams	— —	— —	— —	— —	— —	5.3	5.4
12	NaOH, 10 grams. Inoculated with 10 grams soil	— —	— —	— —	— —	— —	4.9	5.3
13	NaOH, 10 grams. Inoculated with 500 grams soil	— —	+ +	— —	— —	— —	5.4	5.4

TABLE L.—THE EFFECT OF VARIOUS FIELD TREATMENTS IN ESTABLISHING AN AZOTOBACTER FLORA IN SOIL "B"

Plat No.	Treatment, June 24, 1920	Presence of *Azotobacter*																									
		6-24-20		8-10-20		10-6-20		11-20-20		3-12-21		8-1-21		9-7-21		12-29-21		3-23-22		5-25-22		6-26-22		8-11-22		6-9-23		4-3-24		5-26-24		6-26-24	
1	Check	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
2	Corn syrup, 180 grams	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3	$CaCO_3$, 420 grams	−	−	−	−	+	+	+	+	+	+	−	−	+	+	−	−	−	−	−	−	−	−	+	+	−	+	+	−	−	−	−	−
4	Inoculated (soil)	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
5	Inoculated (culture)	+	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
6	$CaCO_3$, 420 grams. Inoculated (culture)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	Corn syrup, 180 grams. Inoculated (soil)	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
8	$CaCO_3$, 210 grams. Inoculated (soil)	+	+	+	+	+	+	+	+	+	+	−	−	+	+	−	−	+	?	−	?	−	−	−	−	−	−	−	−	−	−	−	−
9	$CaCO_3$, 420 grams. Inoculated (soil)	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	−	−	−	−	−	+	?	+	−	+	−	−	−	−	−
10	$CaCO_3$, 840 grams. Inoculated (soil)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	−	+	+	+	+	+	+	+	+	+	?	−	−
11	$CaCO_3$, 420 grams. Corn syrup, 180 grams. Inoculated (soil)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	Check	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

TABLE LI.—THE EFFECT OF VARIOUS FIELD TREATMENTS UPON THE REACTION OF SOIL "B"

Plat No.	Treatment, June 24, 1920	Reaction expressed as pH															
		6-24-20	8-10-20	10-6-20	11-20-20	3-12-21	8-1-21	9-7-21	12-29-21	3-23-22	5-25-22	6-26-22	8-11-22	6-9-23	4-3-24	5-20-24	6-26-24
1	Check	3.73	.....	.....	.....	4.07	3.92	.....	.....	.....	.....	4.02	.....	3.97	.....	3.60	3.23
2	Corn syrup, 180 grams	4.02	.....	.....	.....	4.14	4.09	.....	.....	.....	.....	4.41	.....	4.28	.....	4.19	3.65
3	$CaCO_3$, 420 grams	6.88	.....	.....	.....	7.00	7.27	.....	.....	.....	.....	6.17	.....	6.07	.....	5.70	6.49
4	Inoculated (soil)	3.82	.....	.....	.....	4.14	3.97	.....	.....	.....	.....	4.51	.....	3.99	.....	3.63	3.41
5	Inoculated (culture)	3.94	.....	.....	.....	4.06	4.04	.....	.....	.....	.....	4.26	.....	4.09	.....	3.94	3.80
6	$CaCO_3$, 420 grams. Inoculated (culture)	7.49				6.42	5.63					5.76		4.97		5.51	4.90
7	Corn syrup, 180 grams. Inoculated (soil)	3.84				4.19	4.11					4.41		4.24		4.11	3.41
8	$CaCO_3$, 210 grams. Inoculated (soil)	6.93				5.12	5.11					5.09		4.90		4.82	4.34
9	$CaCO_3$, 420 grams. Inoculated (soil)	6.70	Not run	Not run	Not run	6.70	6.56	Not run	Not run	Not run	Not run	5.98	Not run	5.63	Not run	5.26	4.92
10	$CaCO_3$, 840 grams. Inoculated (soil)	7.02				7.44	7.13					7.03		6.74		6.76	6.54
11	$CaCO_3$, 420 grams. Corn syrup, 180 grams. Inoculated (soil)	7.08				6.91	6.27					6.54		6.09		6.31	5.88
12	Check	4.01				4.26	4.14					4.38		4.23		4.04	3.58

When the reaction data are compared with longevity of *Azotobacter* the failure of the latter to survive in the presence of the light application of lime is easily understood. On the contrary, the question might with propriety be raised as to how the *Azotobacter* could survive as long as they did in this particular plat or in plats 6 and 9, which received twice as much $CaCO_3$ yet were well below pH 6.0 in reaction long before the experiment was discontinued. The ability of *Azotobacter* to survive for a limited time in such strongly acid soils to which lime has recently been added may easily be explained on the assumption that small particles of $CaC0_3$ remain unchanged in the soil regardless of how thoroughly it is mixed with the soil. Efforts were made, of course, to get the $CaCO_3$ thoroughly mixed in the soil, but this was extremely difficult to do, and up until the last examination for *Azotobacter* small masses of the highly insoluble $CaCO_3$ could still be observed in some of the plats. So long as such masses of $CaCO_3$ remain undissolved the reaction in their immediate vicinity will remain favorable for *Azotobacter,* and hence an examination of the soil may be expected to reveal their presence. Sooner or later these lumps will disappear, and the more acid the soil the sooner such will take place, after which all *Azotobacter* may be expected to disappear.

The addition of lime alone, as on plat 3, or syrup alone, as on plat 2, did not result in the establishment of an *Azotobacter* flora.

The comparison of either the absolute (Table LII) or relative (Table LIII) quantities of nitrogen fixed with the development of an *Azotobacter* film shows a very high correlation. The highest quantities of nitrogen fixed were in those plats (numbers 6, 10, and 11) where typical films developed throughout the experiment, in which cases the actual quantities averaged more than twice that fixed in unlimed and uninoculated plats. Inoculation alone, either with a culture or with soil or the addition of syrup without inoculation, was without effect upon the nitrogen-fixing ability of the soil. Lime alone, owing to the occasional development of a film, increased to a limited extent, the fixation of nitrogen.

EXPERIMENTS WITH SOIL "F"

Soil "F" is a typical upland pasture soil that had never been under cultivation. The plats were only one foot square separated from each other by an undisturbed foot of soil. The treatments indicated in the following tables were made and thoroughly mixed with the soil to a depth of eight inches October 16, 1919. During the follow-

TABLE LII.—THE EFFECT OF VARIOUS FIELD TREATMENTS UPON THE NITROGEN-FIXING ABILITY OF SOIL "B"

Plat No.	Treatment, June 24, 1920	Average milligrams nitrogen fixed per culture																	
		6-24-20	8-10-20	10-6-20	11-20-20	3-12-21	8-1-21	9-7-21	12-29-21	3-29-22	5-25-22	6-26-22	8-11-22	6-9-23	4-3-24	5-26-24	6-26-24	Average	Relative
1	Check			3.0	5.5		3.7				3.9	1.9	1.7	4.5	3.7	3.5	4.7	3.61	100
2	Corn syrup 180 grams			5.1	5.8		3.3				3.9	1.8	1.9	3.9	2.4	1.9	5.9	3.59	99
3	$CaCO_3$ 420 grams			7.5	6.6		3.5				4.8	2.5	4.1	6.5	5.7	2.9	5.9	5.00	138
4	Inoculated (soil)			5.2	6.6		3.2				2.3	2.0	3.6	3.7	3.2	4.2	5.5	3.95	109
5	Inoculated (culture)			4.2	5.9		3.5				2.7	1.6	2.7	4.1	1.8	4.0	5.9	3.64	100
6	$CaCO_3$ 420 grams. Inoculated (culture)			8.6	12.6		9.1				9.8	5.7	7.6	7.3	6.6	9.6	8.7	8.56	236
7	Corn syrup 180 grams. Inoculated (soil)	Not run	Not run	4.7	8.1	Not run	3.5	Not run	Not run	Not run	5.0	5.7	1.5	5.7	2.4	5.4	5.5	4.75	131
8	$CaCO_3$ 210 grams. Inoculated (soil)			9.9	11.4		1.2				1.5	3.1	3.2	4.8	3.5	4.4	6.2	4.92	136
9	$CaCO_3$ 420 grams. Inoculated (soil)			8.3	12.6		7.7				4.1	2.8	3.3	6.6	5.0	5.7	6.4	6.25	173
10	$CaCO_3$ 840 grams. Inoculated (soil)			8.9	11.2		8.0				4.1	3.3	5.8	10.8	8.4	6.6	5.8	7.29	201
11	$CaCO_3$ 420 grams. Corn syrup 180 grams. Inoculated (soil)			7.3	10.1		8.6				7.5	7.3	5.5	6.1	8.8	7.9	.....	7.68	212
12	Check			4.3	7.1		1.6				3.4	2.2	3.3	4.2	2.5	4.2	.....	3.64	100

TABLE LIII.—THE EFFECT OF VARIOUS FIELD TREATMENTS UPON THE NITROGEN-FIXING ABILITY OF SOIL "B"

Plat No.	Treatment, June 24, 1920	Relative nitrogen fixation																
		6-24-20	8-10-20	10-6-20	11-20-20	3-12-20	8-1-21	9-7-21	12-29-21	3-23-22	5-25-22	6-26-22	8-11-22	6-9-23	4-3-24	5-26-24	6-26-24	Middle values
1	Check			100	100		100				100	100	100	100	100	100	100	100
2	Corn syrup 180 grams			140	92		125				107	88	76	90	77	49	126	90- 92
3	$CaCO_3$ 420 grams			205	105		132				132	122	164	149	184	75	126	132-132
4	Inoculated (soil)			143	105		121				63	98	144	85	103	109	117	105-109
5	Inoculated (culture)			115	94		132				74	78	108	94	58	104	126	94-104
6	$CaCO_3$ 420 grams. Inoculated (culture)			236	200		343				268	278	304	168	213	249	185	236-249
7	Corn syrup 180 grams. Inoculated (soil)	Not run	Not run	129	129	Not run	132	Not run	Not run	Not run	137	278	60	131	77	140	117	129-131
8	$CaCO_3$ 210 grams. Inoculated (soil)			271	181		45				41	151	128	110	113	114	132	114-128
9	$CaCO_3$ 420 grams. Inoculated (soil)			227	200		291				112	137	132	152	161	148	136	152-148
10	$CaCO_3$ 880 grams. Inoculated (soil)			244	178		302				112	161	232	248	271	171	123	178-232
11	$CaCO_3$ 420 grams. Corn syrup 180 grams. Inoculated (soil)			200	160		325				205	356	220	140	284	205	.....	205-220
12	Check			100	100		100				100	100	100	100	100	100	.....	100

ing five years, eighteen examinations for *Azotobacter* were made and the results are recorded in Table LIV. The applications of $MgCO_3$ and $CaCO_3$ were excessive, but it was desired to make the application so heavy that there would be no question as to maintaining a favorable reaction. Equally large quantities of NaOH could not be applied without injuring the soil.

The data recorded in Table LIV apparently justifies the following deductions. This soil in its natural condition does not contain *Azotobacter* (Plats 1and 9), and when they are introduced into it without in any way altering the soil (plat 2) they will remain viable only a limited period of time. The addition of $CaCO_3$ (plat 6) or $MgCO_3$ (plat 4) alone appeared to result in the gradual establishment of *Azotobacter* flora, since the examination of such treated plats during the first year failed to reveal *Azotobacter,* whereas thereafter they appeared fairly regularly, though not so consistently as when the liming was accompanied by inoculation. In the latter instances (plats 3 and 7) typical films developed on both cultures at every examination, showing the ease with which it is possible to establish an *Azotobacter* flora in an acid soil that does not contain such organisms if inoculation is accompanied with adequate liming.

It is questionable whether the NaOH added had any effect upon the *Azotobacter* since they disappeared from plat 8 within a few months of the time they were no longer evident in the inoculated untreated plat 2.

When the longevity of *Azotobacter* in the variously treated plats is compared with the reaction as recorded in Table LV it is seen that the quantities of $MgCO_3$ and $CaCO_3$ added were sufficient to maintain a favorable reaction, *i. e.,* a pH of 6.0 or above, throughout the entire period, but that the NaOH had but little, if any, effect upon the reaction, thus accounting for the failure to prolong the viability of *Azotobacter.*

The quantities of nitrogen fixed in the nitrogen-fixation experiments as recorded in Table LVI and converted into relative values in Table LVII correlate perfectly with the development of *Azotobacter* films as recorded in Table LV. The average quantity fixed per culture from inoculated and limed plats 3 and 7 was a little more than twice that in cultures from the check plats. Those plats in which *Azotobacter* survived for a while and then disappeared (plats 2 and 8) and those in which they apparently were gaining a foothold (plats 4 and 6) rank approximately halfway between the check plats and those in which *Azotobacter* survived the entire period.

TABLE LIV.—THE EFFECT OF VARIOUS FIELD TREATMENTS ON AZOTOBACTER IN SOIL "F"

Plat No.	Treatment, October 16, 1919	Presence of *Azotobacter*																													
		10-16-19		10-24-19		3-11-20		6-9-20		8-11-20		10-5-20		11-18-20		3-9-21		8-2-21		9-8-21		12-30-21		4-6-22		7-18-22		8-10-22		6-16-23		4-14-24		6-5-24		6-24-24		1-14-20	
1	Check	−	−	−	−	−	−	−	−	−	?	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	−	−
2	Inoculated	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	−	−	−	−	−	−	−	−	+	?	+	?	−	−	−	−	−	−	+	−	+	+
3	Inoculated and $CaCO_3$, 105 grams			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	$MgCO_3$, 105 grams			−	−	−	−	−	−	+	+	−	+	+	+	+	−	+	−	+	−	+	+	−	−	+	?	−	−	+	−	+	+	+	+	−	−	−	−
5	NaOH, 10 grams			−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	+	−	−	−	+	?	−	−	−	−	−	−	−	−	−	−	+	−
6	$CaCO_3$, 105 grams			−	−	−	−	−	−	−	−	−	−	+	+	−	+	−	+	+	−	+	−	+	−	+	?	+	+	−	−	+	+	+	+	−	+	−	−
7	Inoculated and $MgCO_3$, 105 grams			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	Inoculated and NaOH, 10 grams			+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	−	−	−	+	+	−	+	−	−	−	−	−	−	−	+
9	Check			−		−	−	+	?	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−

TABLE LV.—THE EFFECT OF VARIOUS TREATMENTS UNDER FIELD CONDITIONS ON THE REACTION OF SOIL "F"

Plat No.	Treatment, October 16, 1919	Reaction expressed as pH																	
		10-16-19	10-24-19	3-11-20	6-9-20	8-11-20	10-5-20	11-18-20	3-9-21	8-2-21	9-8-21	12-30-21	4-6-22	7-18-22	8-10-22	6-16-23	4-14-24	6-5-24	6-24-24
1	Check		5.39					5.63			5.39				5.41	5.61	5.12		4.85
2	Inoculated		5.46					5.53			5.36				5.43	5.75	5.43		5.41
3	Inoculated and $CaCO_3$, 105 grams		7.78					7.67			7.79				7.62	6.53	6.37		7.15
4	$MgCO_3$, 105 grams		8.10					7.88			7.22				7.05	6.53	6.29		6.42
5	NaOH, 10 grams	Not run	5.97	Not run	Not run	Not run	Not run	5.65	Not run	Not run	5.78	Not run	Not run	Not run	5.63	5.61	5.60	Not run	5.24
6	$CaCO_3$, 105 grams		7.90					7.74			7.45				7.15	7.34	7.68		6.17
7	Inoculated and $MgCO_3$, 105 grams		7.95					7.50			7.44				6.86	6.73	6.32		6.05
8	Inoculated and NaOH, 10 grams		5.82					5.97			5.75				5.65	5.63	5.31		5.46
9	Check		5.53					5.53			5.46				5.36	5.02	5.68		5.38

TABLE LVI.—THE EFFECT OF VARIOUS FIELD TREATMENTS UPON THE NITROGEN-FIXING POWER OF SOIL "F"

Plat No.	Treatment, October 16, 1919	Average milligrams nitrogen fixed per culture																		
		10-16-19	10-24-19	3-11-20	6-9-20	8-11-20	10-5-20	11-18-20	3-9-21	8-2-21	9-8-21	12-30-21	4-6-22	7-18-22	8-10-22	6-16-23	4-14-24	6-5-24	6-24-24	Average	Relative
1	Check	3.5	6.3				4.5	4.2		1.3			0.0	2.3	3.4	3.3	5.0	4.7	3.4	3.4	100
2	Inoculated	8.6	7.8				7.6	10.6		3.1			3.9	5.0	7.6	3.0	4.1	3.4	4.8	5.8	159
3	$CaCO_3$, 105 grams. Inoculated		9.5				7.0	9.5		8.1			8.9	8.3	8.3	6.3	7.0	7.2	7.5	8.0	219
4	$MgCO_3$, 105 grams		3.9				6.6	7.3		9.7			0.8	3.0	3.4	4.6	5.5	7.9	3.7	5.1	140
5	NaOH, 10 grams		5.3	Not run	Not run	Not run	5.6	6.7	Not run	1.7	Not run	Not run	3.4	4.1	3.0	3.7	3.0	3.7	2.5	3.9	107
6	$CaCO_3$, 105 grams		3.1				3.6	7.5		4.2			6.2	5.5	7.9	3.2	5.7	8.5	6.5	5.6	153
7	$MgCO_3$, 105 grams. Inoculated		6.6				6.9	10.4		8.1			6.9	5.7	8.9	6.3	7.0	7.3	7.0	7.4	203
8	NaOH, 10 grams. Inoculated		7.3				6.7	10.1		8.1			3.0	4.8	9.4	4.4	3.0	2.8	2.6	5.7	156
9	Check		3.9				5.1	5.7		2.5			3.4	3.1	4.4	3.7	5.8	2.7	2.9	3.9	100

TABLE LVII.—THE EFFECT OF VARIOUS FIELD TREATMENTS UPON THE RELATIVE NITROGEN-FIXING POWER OF SOIL "F"

Plat No.	Treatment, October 16, 1919	Relative nitrogen fixed																		
		10-16-19	10-24-19	3-11-20	6-9-20	8-11-20	10-5-20	11-18-20	3-9-21	8-2-21	9-8-21	12-3-21	4-6-22	7-18-22	8-10-22	6-16-23	4-14-24	6-5-24	6-24-24	Middle values
1	Check	100	100				100	100		100			100	100	100	100	100	100	100	100
2	Inoculated	246	153				158	214		163			229	193	195	100	76	92	152	160
3	Inoculated and $CaCO_3$, 105 grams		186				146	192		426			524	307	213	210	130	195	238	210
4	$MgCO_3$, 105 grams		76				138	147		511			47	111	87	153	102	214	117	117
5	NaOH, 10 grams	Not run	104	Not run	Not run	Not run	117	135	Not run	89	Not run	Not run	200	152	77	123	56	100	79	104
6	$CaCO_3$, 105 grams		61				75	152		221			365	204	203	107	106	230	206	203
7	Inoculated and $MgCO_3$, 105 grams		129				144	210		426			406	211	228	210	130	197	222	210
8	Inoculated and NaOH, 10 grams		143				140	204		426			176	178	241	147	56	76	83	147
9	Check		100				100	100		100			100	100	100	100	100	100	100	100

The information secured from field inoculation experiments may be summarized as follows: The introduction of *Azotobacter* alone into acid soils did not result in the establishment of a permanent *Azotobacter* flora. In some instances, particularly where the soil was not strongly acid, the introduced organisms could be recovered several months following inoculation, but eventually disappeared if the experiment was of several years' duration.

The addition of lime alone adequate to correct the reaction apparently tended toward the establishment of an *Azotobacter* flora, but in all such instances accidental inoculation was possible from adjoining inoculated plats. The establishment of an *Azotobacter* flora by this means was very slow, the cultures from such plats, even after several years, not being typical though a typical flora existed in adjoining plats within a distance of one or two feet.

The addition of adequate lime to maintain a pH greater than 6.0 accompanied by inoculation always resulted in the establishment of an *Azotobacter* flora which, as far as the experiments go, was permanent. If the quantity of lime was insufficient to maintain a favorable reaction the *Azotobacter* gradually disappeared.

GENERAL SUMMARY

A brief summary of the principal facts indicated by the data has been given following the presentation of the experiments under the four main headings into which they have been divided. In the following brief discussion an effort will be made merely to point out the general conclusions to which the four lines of investigations point.

In the first place, attention is again called to the previously published data relative to the very close relationship that evidently exists between the absolute reaction of the soil solution and the natural distribution of *Azotobacter.* Soils were collected under very widely varying geographic, climatic, and geologic conditions and studied as to their reaction and *Azotobacter* content. When these soils were divided into two groups depending upon reaction, *i. e.,* those with pH above and those with pH below 6.0, and again divided into two groups, one containing, the other not containing *Azotobacter,* the association coefficient between the reaction and the presence of *Azotobacter,* calculated by Yule's formula *(29),* was found to be 0.96. This high value seems especially remarkable in view of the numerous possibilities that exist for soils not containing *Azotobacter* to become contaminated in the processes of

collection, shipment, etc., and also in view of the variations in critical pH that would naturally be expected to exist among the many species and strains of any group of organism as widely distributed and living under as widely varying environmental condit'ionsas are *Azotobacter*. On the basis of the data that have been submitted from this and other laboratories, notably Christensen's *(2), (3), (22), (23), (24), (28)* it is believed that one is justified in concluding that the natural distribution of *Azotobacter* is very closely associated with, if not dependent upon, the absolute reaction of the soil.

The second general conclusion, certainly justified from these data, is that the introduction of *Azotobacter* into soils more acid than expressed by a pH of 6.0, either in the form of crude cultures or soil containing an active flora, will not of itself result in the establishment of an *Azotobacter* flora. This conclusion is based upon thirty separate laboratory experiments recorded in this paper in which *Azotobacter* from a half dozen sources were introduced into eight different acid soils and upon extensive field experiments with three entirely different acid soils. In no case were they capable of surviving an appreciable length of time when the acidity was greater than pH 6.0. In those cases where the H^+ concentration was only slightly greater than 10^{-6} *Azotobacter* survived a short period of time; the more acid the more rapidly the introduced organisms disappeared. Evidently there is something about acid soils that render them incapable of supporting an *Azotobacter* flora.

The third fundamental fact brought out in these experiments is that the addition of a sufficient quantity of basic substances, $CaCO_3$, $MgCO_3$, or neutral or alkaline soil, to neutralize the major portion of the acid present, *i. e.,* reduce the H^+ concentration to less than 10^{-6}, will so alter such soils as to render them capable of supporting an *Azotobacter* flora. The quantities of such basic substances necessary may be small, as in the case of slightly acid, poorly buffered soils such as "1001" and "1003," or it may be very high, as in the case of such soils as "B," "1000," and "1002." No quantity of material that was insufficient to reduce the H^+ concent,ration to approximately 10^{-6} or less prolonged the viability of *Azotobacter* for more than a few months. It is true that in several instances the viability was appreciably prolonged both in laboratory and field by quantities of basic materials too small to effect the indicated necessary change in reaction, but these instances can be explained upon the basis of incomplete mixing of the insoluble substances in the soil, resulting

in the development of limited areas of favorable reaction. Under field conditions a gradual increase in acidity, following a temporary favorable reaction produced by the addition of quantities of $CaC0_3$ insufficient to maintain this favorable reaction, was accompanied by the disappearance of introduced *Azotobacter*.

A fourth fundamental conclusion, perhaps not quite so definitely proved but nevertheless well supported, is that the addition of sufficient quantities of various acids to soils containing *Azotobacter* to effect a *permanent* increase in H^+ concentration in excess of 10^{-6} will cause *Azotobacter* to disappear from such soils. The evidence in support of this conclusion is complicated by two other factors. A permanent increase in acidity is emphasized because the increase secured by such organic acids as were tested was always temporary. The destruction of *Azotobacter* by the addition of such acids would, then, depend upon the intensity of the acid condition temporarily produced as well as the length of time elapsing before the acid condition is entirely eliminated by the utilization of the acid as food by microörganisms. In the second place, some of the acids evidently possessed toxic properties aside from the acid condition produced.

A fifth conclusion suggested by the data is that the maximum H^+ concentration tolerated by *Azotobacter* in soils is very close to 1×10^{-6}. The evidence in support of this conclusion is very strong regardless of the angle of approach from which the data were secured. As might be expected, some slight differences are evident in the data, but it is believed that most of these can be accounted for either by errors in the determinations of the reaction, by insufficient time elapsing between treatment and subsequent tests for *Azotobacter* for a biological equilibrium to have been reached, or that species or strains of *Azotobacter* with different critical pH's were involved. This conclusion is also strongly supported by previously published data from this laboratory in which pure cultures were studied in comparatively simple media and the same critical pH noted. The very extensive studies of field soils previously reported from this and other laboratories almost unanimously point to a critical pH very near 6.0.

Finally it is believed that the data submitted in this paper, coupled with that previously published from various sources, amply justify the unqualified conclusion that the major factor controlling the natural distribution and hence successful artificial inoculation of soils with *Azotobacter* is the reaction of the soil solution. As to whether the harmful effect of a high acid condition should be at-

tributed to a direct toxic effect of the hydrogen ions or to some indirect effect that these ions produce upon some other soil constituent may be questioned. It is rather difficult to separate the various possible factors in a medium as complex as the soil. However, in view of the various angles from which almost identical results have been obtained in soil studies, together with the substantiating data secured in comparatively simple laboratory media inoculated with pure cultures, it seems that one may at least provisionally attribute the toxic effect to a direct action of the high H^+ concentration.

With the information now available relative to conditions necessary for the activity of *Azotobacter* in soils it seems fairly certain that many inoculation experiments have failed primarily because of the unfavorable reaction of the soil into which the *Azotobacter* were introduced. Furthermore, with this information as a basis upon which to plan future experiments, it would seem safe to predict that future *Azotobacter* inoculation experiments will be attended with a greater degree of success than has been experienced in the past.

CONCLUSIONS

Within the limits of the experimental data previously and herewith submitted, the following conclusions appear justified:

1. The natural distribution of *Azotobacter* is very closely associated with, if not dependent upon, the absolute reaction of the soil.

2. When *Azotobacter* are introduced into cultivated acid soils with a pH of less than 6.0 they soon perish, the rapidity of this disappearance depending upon the degree of acidity.

3. The addition of basic substances such as $CaCO_3$, $MgCO_3$ or neutral or basic soil in sufficient quantities to reduce the H^+ concentration to less than 10^{-6} will render acid soils a fit pabulum for the existence of *Azotobacter.*

4. The addition of sufficient quantities of acid to a soil containing *Azotobacter* to maintain permanently a H+concentration greater than 1×10^{-6} in the soil solution will result in the disappearance of *Azotobacter* therefrom.

5. The maximum H^+ concentration in the soil solution compatible with the existence therein of an active *Azotobacter* flora is very near 1×10^{-6}.

6. The major factor controlling the existence of *Azotobacter* in soils, at least as so far determined, is the hydrogen-ion concentration of the soil solution, the hydrogen ions apparently acting directly as a toxic agent, though there is a possibility that they may act indirectly by effecting some other soil constituent.

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