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Inoculation of Alfalfa on Lime-Deficient Sandy Soils

Soil Transfer vs. Use of Cultures

By Frederick J. Alway and George H. Nesom
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SUMMARY

Observations and experiments are reported on the growth of alfalfa on widely scattered sandy fields in Minnesota, all more or less lime deficient toward both alfalfa and sweet clover, and on which the hydrogen-ion concentration of the surface soils, expressed as pH, was between 5.0 and 6.0. On one of the fields the crop had been under observation for eight consecutive seasons and on the others for much shorter periods.

When the land had been limed well in advance of the seeding, the use of artificial cultures was as effective as a heavy application of soil from an established field of alfalfa or sweet clover; but when it had not been limed, the soil transfer method was far the more effective in the case of the first seeding. Increasing the amount of culture to many times the usual rate did not make it as effective as soil transfer. The difference between the two methods appears to increase with the lime deficiency of the land being seeded.

The growing of alfalfa or sweet clover on such unlimed soils, even where the stand was thin and only a few of the plants were vigorous, satisfactorily inoculated a following seeding of alfalfa.

On these soils, when inoculation has been effected by soil transfer or by the previous growth of alfalfa or sweet clover, remunerative crops of alfalfa may be grown without lime. On all except the least lime-deficient fields, however, liming increases the yields more or less, even where satisfactorily inoculated by one of the above methods, but the increase actually due to liming, independent of its influence on inoculation, may be too small to be profitable, and during a drouth the beneficial effect may be entirely masked.

Liming experiments in which the current pure culture methods of inoculation are relied upon, are likely to lead to very erroneous conclusions as to the economy of liming such soils for alfalfa.

INOCULATION OF ALFALFA ON LIME-DEFICIENT SANDY SOILS

SOIL TRANSFER VS. USE OF CULTURES

By

FREDERICK J. ALWAY AND GEORGE H. NESOM

INTRODUCTION

The relative efficiency of the pure-culture method of inoculating legumes as compared with the soil-transfer methods, has been much discussed in this country during the last thirty years. Many soil bacteriologists are of the opinion that the pure cultures of *Bacillus radicicola* now obtainable from agricultural experiment stations and commercial firms are fully as efficient as soil from old, well established fields, but many American agronomists still consider soil transfer the more reliable method for alfalfa.

Löhnis and Fred, in their Textbook of Agricultural Bacteriology (1922), dealing with the inoculation of legumes, state:

"Before pure cultures became available for this purpose, soil was frequently taken as inoculum from fields where a good crop of the special kind of legume had been grown. The practice is still to be recommended when no reliable pure culture can be secured, provided the soil does not contain too many weeds and plant diseases. As a rule, however, efficient pure cultures for legume inoculation can easily be secured at present from Agricultural Experiment Stations, Agricultural Departments, and from many commercial firms" (13, pp. 271-272).

The only objection at present to the use of artificial cultures, according to a recent New Jersey experiment station extension bulletin (5, p. 3), is the expense involved, from 50 cents to $1.00 per acre. Farmers are assured that they may place a high degree of confidence in the product of the two firms which supply almost all the soil inoculants offered for sale in that state, whether they buy directly from these firms or through a retail dealer.

Stewart (17, pp. 138-139), in his recent book, "Alfalfa Growing in the United States and Canada," states that, according to American opinion, "soil inoculation is better than seed treatment with artificial cultures," and Oakley (1922) considers "the surest way (to inoculate alfalfa) is by scattering soil from a successful alfalfa field or from the roots of sweet clover or bur clover plants," mentioning the use of 250 to 500 pounds per acre, or the mixing of equal amounts of soil and seed (14, p. 14). In reply to an inquiry as to the basis of his

statement, Stewart writes that he secured the opinion, in writing or orally, of 37 Americans, most of them agronomists. The others were men who had had experience with alfalfa and who were in a position to have an opinion in this matter. Twenty-six stated definitely that soil inoculation was superior, seven that there was no difference, as far as they could see; and the remaining four that, under their conditions, they considered the use of a culture the better method.

General interest in the United States in the possibility of the use of pure cultures of *Bacillus radicicola* began with the work of J. F. Duggar in Alabama in 1897 (6). For most of the legumes with which he worked, he used Nitragin imported from Germany, comparing the relative advantages and disadvantages of the pure culture and soil transfer methods (6, pp. 480-482). Several common practices in inoculation, frequently described in later years, seem to have been first recommended in their present form by him in 1897, viz: the spreading broadcast of a ton or two per acre of soil from an old field of the leguminous crop in question, the starting of a small plot of the desired legume in order to provide inoculating soil for later years, and the mixing of the seed with inoculated soil where the latter was scarce.

Four years later the Bureau of Plant Industry of the United States Department of Agriculture inaugurated an investigation of the possibility of the use of pure cultures, and in 1905 this was placed in charge of Kellerman, who, six years later, in a careful review of the situation (9), while admitting that the claim of enthusiasts on the subject of pure culture inoculation to the effect that with proper precautions it was at least as certain as the use of soil from fields where similar leguminous crops have been grown for extended periods, "might be valid where the soil is well adapted to a leguminous crop," stated that "in soils not well adapted to a leguminous crop, however, there seems to be no doubt that soil from old fields gives much better promise of successful inoculation." In his publications between 1905 and 1913, Kellerman repeatedly emphasized that in order to obtain success with pure cultures the soil must be adapted to the growth of the leguminous crop in question, and in the case of alfalfa the soil must in many cases be brought into proper condition by more or less heavy liming (10, p. 7). He strongly opposed accepting as conclusive the results obtained on a single type of soil as to the relative efficiency of the pure culture method, compared with that of soil transfer (11, p. 73; 12). He alone, among the investigators, appears to have suspected that the relative efficiency of the two methods in any particular case might depend upon the character of the soil upon which they were being compared.

The most interesting recent contributions on the subject are probably those of the investigators at Rothamsted, where Russell (15, p. 5) considers that Thornton, by the addition of a very small amount of acid calcium phosphate to a suspension of the bacteria in skimmilk (18, pp. 7-8), has now devised a method for alfalfa that overcomes the previous trouble in ensuring inoculation by the use of pure culture.

CHARACTER OF SOILS UNDER OBSERVATION

The University of Minnesota has six widely distributed groups of experimental fields with sandy soil, viz: at Coon Creek, Becker, Crow Wing, Backus, Park Rapids, and Bemidji, all lying north and slightly northwest of St. Paul, at distances, respectively, of 18, 33, 110, 142, 160, and 190 miles. They are being devoted chiefly to a study of their deficiencies in lime and mineral nutrients, with alfalfa and the clovers as the main trial crops. Experiments with these legumes were started at Coon Creek in 1919; at Crow Wing, Backus, and Bemidji in 1922; and at Becker and Park Rapids in 1924. The texture and the hydrogen-ion concentration values of the soil and subsoil of these fields are illustrated in Tables I and II. The moisture equivalents from typical plots on the experimental farm at St. Paul and on two experimental fields with silt loam soils—Marshall silt loam at Kenyon and Tama silt loam at Caledonia—are given for comparison in order to bring out the coarseness of texture on the sand fields. The fractional values of the moisture equivalents are omitted, they having little significance in such a comparison.

TABLE I

MOISTURE EQUIVALENTS OF MINNESOTA SAND EXPERIMENTAL FIELDS IN COMPARISON WITH THOSE OF THE UNIVERSITY FARM AT ST. PAUL AND TWO EXPERIMENTAL FIELDS WITH SILT LOAM SOIL

Depth ft.	Coon Creek South field	Coon Creek North field	Coon Creek Anderson field	Becker	Crow Wing	Backus	Park Rapids	Bemidj	Univ. Farm	Caledonia	Kenyon
1	5	4	4	10	6	8	13	6	24	27	26
2	4	3	3	6	5	6	4	4	24	27	26
3	3	3	2	4	3	4	2	3	23	28	24
4	2	2	2	2	3	4	1	3	16	28	23
5	2	2	1	2	3	4	1	3	2	27	22
6	2	2	1	2	3	4	1	3	1	26	20

During the last eight years determinations have been made of the moisture equivalents of many sets of soil samples from the Coon Creek South and North fields, taken to a depth of 6 feet or more, and of many samples from the surface of these fields. These have shown a general homogeneity of the soil.

TABLE II

HYDROGEN-ION CONCENTRATION OF REPRESENTATIVE PROFILES OF THE MINNESOTA SAND EXPERIMENTAL FIELDS

Depth	Coon Creek			Becker	Crow Wing	Backus	Park Rapids	Bemidji
	North field	South field	Anderson field					
ft.	pH	pH	pH	pH	pH	pH	pH	pH
1*	5.3	5.2	5.5	5.4	5.4	5.6	5.0	5.7
1†	5.7	5.2	5.7	5.5	5.8	5.3	5.1	6.6
2	6.1	5.9	5.9	5.6	5.8	5.3	6.2	6.5
3	6.2	6.1	5.9	5.5	5.9	5.5	6.4	6.3
4	6.4	6.4	..	..	5.9	6.1	8.4	6.3
5	6.4	6.4	..	..	5.7	5.9	8.4	7.0
6	6.4	6.5	..	..	5.6	..	8.4	7.3

* Upper half.
† Lower half.

In the early part of November, 1926, in order to learn the present condition of the unlimed plots, composite samples to a depth of 6 inches were collected from many of the control plots dealt with in this paper (Table III) and the hydrogen-ion concentration was determined. In all cases the gas chain electrometric method was employed, using a bubbling hydrogen electrode vessel and constant mechanical shaking.

TABLE III

HYDROGEN-ION CONCENTRATION, EXPRESSED AS pH, OF SURFACE 6 INCHES OF SOIL FROM UNLIMED, UNFERTILIZED PLOTS ON MINNESOTA SAND EXPERIMENTAL FIELDS

Coon Creek Fields					
South field, Series A 1921 seeding		South field, Series B Unlimed quarter		South field, Series D Unlimed quarter	
Plot	pH	Plot	pH	Plot	pH
1	5.3	1	5.4	1	5.3
6	5.1	2	5.2	2	5.2
11	5.2	6	5.2	6	5.3
15	5.2	12	5.2	12	5.2
20	5.3	13	5.2	13	5.2
25	5.3	17	5.3	17	5.2
West field		Anderson field		Erickson West field	
G- 1 to 20	5.0	A-12	5.5	B- 3	5.4
G-21 to 40	5.1	B- 9	5.6	B-10	5.5
L- 1 to 20	5.1	B-11	5.3	C-15	5.4
L-21 to 40	5.2	B-13	5.4	D- 7	5.7
South field, Series A 1919 and 1924 seeding		Swanson field		Erickson South field	
15	5.3	A- 4	5.5	IV-b-3	5.2
20	5.2	B- 2	5.4	IV-a-3	5.4
25	5.4	B- 7	5.3		
Northern Fields					
Crow Wing, 1925 seeding		Crow Wing, 1926 seeding		Becker, 1924 seeding	
A-3	5.8	B- 1	5.8	1-C	5.4
A-5	5.8	B- 5	5.5	1-F	5.4
B-3	5.6	B-10	5.7	7-C	5.3
B-5	5.7	B-15	5.7	7-F	5.4
Park Rapids 1924 seeding		Backus, 1925 seeding		Bemidji	
F-10	5.8	B- 1	6.0	1-8 in S-1	5.6
F-12	5.5	B- 8	5.6	1-8 in S-4	5.6
F-14	5.6	B-15	5.8	1-8 in S-7	5.9

The data in Tables II and III show that the surface soils on all these sand experimental fields are acid, but not necessarily that they are lime deficient. The lime deficiency of the fields referred to as distinctly lime deficient has been established by alfalfa liming experiments. The Bemidji fields are very slightly, if at all, lime deficient, altho the hydrogen-ion concentration of the surface, as pH, was between 5.6 and 5.8. Soil acidity and lime deficiency, while more or less related, are not synonymous terms (3, p. 5). Where applications of calcium carbonate do not increase the yields of the common clovers, but increase those of more sensitive crops such as alfalfa and sweet clover, the soil is to be regarded as lime deficient toward only the latter.

METHODS OF SEEDING AND INOCULATION

The seed was usually sown with a press drill, but was sometimes broadcast with a wheelbarrow seeder or, more rarely, by hand. A cultipacker was used in preparing the seedbed, the aim being to have the seedbed very firm, as free of weeds as feasible, and as moist as obtainable. In later years most of the seedings were made as soon as possible after a good rain.

Only the Grimm variety of alfalfa was used, except a one-rod strip on the 1919 seeding on Series A at Coon Creek, where seed bought as "common" was sown. This has suffered more winter injury than the Grimm which was sown parallel with it on the same day, and none of the date from it are used in this bulletin.

The liming materials employed include ground limestone, hydrated lime, and marl, the last both dry and wet. In any particular field, plot, or seeding, the choice was determined by the purpose of the experiment or by the convenience of supply.

Before the experiments were started, neither alfalfa nor sweet clover had ever been sown on any of the fields, nor had volunteer plants of either been seen on them.

The method of inoculation used in each seeding is indicated below. Unless it is clearly stated to the contrary, the seed was, in every case, inoculated with artificial cultures secured from two commercial sources, one being referred to as Q and the other as X. The amount used has always been somewhat in excess of that advised in the directions accompanying the package, which will be referred to in this paper as the usual rate. Usually the seed has been sown within a few hours of the treatment, and seldom has more than a day elapsed between the mixing of the culture with the seed and the completion of the seeding. In all seedings from 1919 to 1924, culture Q was used, and during 1925 and 1926 culture X was used exclusively. In a few cases in the earlier years, as a precautionary measure, soil from a well established alfalfa

or sweet clover field was mixed with the seed before sowing, or scattered over the plots and harrowed, in addition to the use of culture on the seed. In some of the experiments in the last three seasons, heavy applications of inoculating soil, 1 to 4 tons per acre, have been worked into the soil at the time of seeding the alfalfa.

OBSERVATIONS ON SEEDINGS OF 1919 TO 1924 AT COON CREEK

The fields of the Coon Creek group (Fig. 1), about eighteen miles northwest of the experiment station, are located on two soil types—Merrimac loamy sand and Hinckley fine sand. The South, North, West, and two Erickson fields are on the former, which occurs on a level outwash plain of the glacial Mississippi River; while the Anderson and Swanson fields are on the other type, which is found on rounded knolls and hills, the material consisting of wind-blown sand from the adjacent outwash plain. Both were originally covered by oaks and both drift severely when bare. The former type is the less drouthy because of its somewhat finer subsoil.

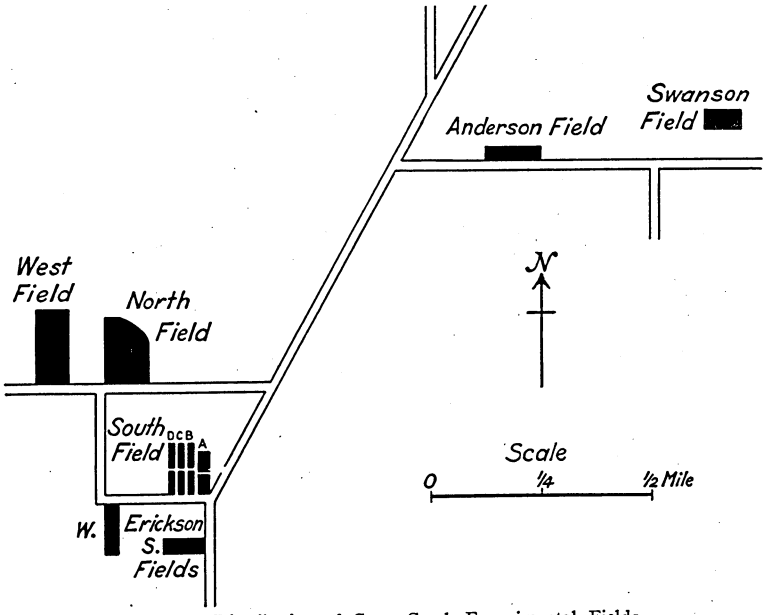

Fig. 1. Distribution of Coon Creek Experimental Fields

The portion of the South field involved in the experiments had been under cultivation about thirty years before the University obtained the lease of it, being cropped to winter rye, corn, and oats. Red clover had often been seeded with the small grains, but seldom, if more than once, had a stand been secured. At the time it was leased it carried a rather thin stand of medium red clover. Very little manure had been applied to this part of the field.

Alfalfa and Sweet Clover Seedings in 1919[1]

As soon as possession had been secured, in April, 1919, four series, A, B, C, and D, were laid out, each in two blocks separated by a roadway one rod wide, the north block to be a duplicate, so far as treatment of the soil is concerned, of the south (Fig. 2). During the second week of May, Series A was plowed and staked out into 28 twentieth-acre plots, separated by 2-foot paths.

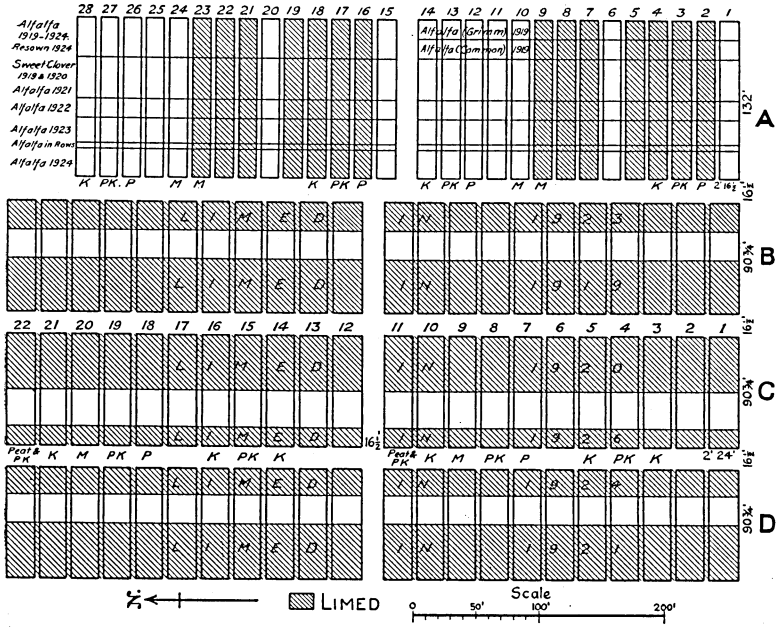

Fig. 2. Portion of South Field at Coon Creek, Included in Experiments Reported
In 1919 the crops seeded on Series A were: from east to west, Grimm alfalfa, 1 rod; common alfalfa, 1 rod; white sweet clover, 1 rod; yellow sweet clover, 1 rod; alsike clover, 1 rod; medium red clover, 1 rod; mammoth clover, 1 rod; white clover, 7½ feet; and soybeans, 9 feet.

Six of the plots were left as controls while the others were given various applications of very finely ground limestone, commercial fertilizer, and stable manure, as indicated in Table IV. Each plot, as soon as the applications to it had been completed, was disked and harrowed, driving the team in the direction of its longer axis in order to minimize the dragging of the applied materials from plot to plot. Control plots were given the same kind and amount of cultivation as the others. During the first week of June the whole series was seeded to nine legume crops, each crossing all 28 plots (Fig. 2). Alfalfa and

[1] James E. Chapman, since 1922 a member of the staff of the North Dakota Agricultural Experiment Station, was in charge of the work at Coon Creek during the seasons of 1919, 1920, and part of 1921.

sweet clover, which were sown with a wheelbarrow seeder, were inoculated with the artificial culture Q, and as a further precaution 400 pounds per acre of soil from an established field of alfalfa on University Farm was at once scattered over this portion of the series and harrowed in with the seed. As a result of very poor stands of alfalfa and the clovers and a dense growth of weeds, all the legumes, except the soybeans, were disked up on July 7. The land was well disked and harrowed from east to west, and a week later, following a rain of 0.9 inch, was reseeded according to the original plan, the same culture being again used on the alfalfa and sweet clover. On all the plots full, uniform stands of alfalfa were obtained and uniform but thin stands of the sweet and common clovers. Few weeds appeared and for the rest of the growing season the weather was very favorable.

TABLE IV

APPLICATIONS OF GROUND LIMESTONE AND FERTILIZERS ON PLOTS OF SERIES A

Plots	Applications and rates per acre
1, 15	None
2, 16	Limestone, 4 tons in 1919; phosphoric acid 88 lbs. in '19, 32 lbs. in '20, 88 lbs. in '24, 88 lbs. in '25, 88 lbs. in '26
3, 17	Limestone, 4 tons in 1919; phosphoric acid 88 lbs. in '19, 32 lbs. in '20, 88 lbs. in '24, 88 lbs. in '25, 88 lbs. in '26; potash 120 lbs. in '19, 112 lbs. in '20, 120 lbs. in '24, 100 lbs. in '25, 100 lbs. in '26
4, 18	Limestone, 4 tons in 1919; potash 120 lbs. in '19, 112 lbs. in '20, 120 lbs. in '24, 100 lbs. in '25, 100 lbs. in '26
5, 19	Limestone, 4 tons in 1919
6, 20	None
7, 21	Limestone, 2 tons in 1919
8, 22	Limestone, 8 tons in 1919
9, 23	Limestone, 4 tons in 1919; manure 10 tons in '19, 10 tons in '24 10 tons in '25, 10 tons in '26
10, 24	Manure 10 tons in 1919, 10 tons in '24, 10 tons in '25, 10 tons in '26
11, 25	None
12, 26	Phosphoric acid 88 lbs. in 1919, 32 lbs. in '20, 88 lbs. in '24, 88 lbs. in '25, 88 lbs. in '26
13, 27	Phosphoric acid 88 lbs. in 1919, 32 lbs. in '20, 88 lbs. in '24, 88 lbs. in '25, 88 lbs. in '26; potash, 120 lbs. in '19, 112 lbs. in '20, 120 lbs. in '24, 100 lbs. in '25, 100 lbs. in '26
14, 28	Potash 120 lbs. in 1919, 112 lbs. in '20, 120 lbs. in '24, 100 lbs. in '25, 100 lbs. in '26

The alfalfa and sweet clover on all plots came through the winter of 1919-20 with apparently uninjured stands, but all four common clovers were so largely killed that the plots were plowed up and reseeded about the middle of May.

Alfalfa of the 1919 seeding.—In the first crop season (1920) the alfalfa, which was mowed twice, showed a striking effect of the lime (Table V and Fig. 3), the average yield on the 6 limed plots being 2.11 tons and that on the 6 unlimed plots only 0.31 ton, an increase of 580 per cent. On all the limed plots the plants were tall

and dark green while on the unlimed plots nearly all were short and yellow. A few scattered green plants were observed in May and the number increased as the season advanced.

During the following winter most of the alfalfa on the unlimed plots died, apparently leaving only the scattered plants which had become green before the close of the growing season. The alfalfa on the limed plots suffered little or no winter injury. In 1921 the three cuttings showed increases from liming of 254, 271, and 383 per cent, respectively (Table V).

In 1922 the beneficial effect of the liming was almost as great. On the north block, Plots 15 to 28, the alfalfa was cut for hay only once, the second growth being left for seed. In 1923 the first growth on these 14 plots was left for seed, no hay being cut from them that season. In the spring of 1924 all the 1919 seeding of alfalfa on the 14 plots, both common and Grimm, was plowed under and the well prepared soil was resown to alfalfa, an experiment dealt with later in this paper.

The yields from 1923 to 1926 have not greatly altered the indication of lime deficiency, altho the difference between the averages for the limed and the unlimed has fallen to only about 100 per cent of the latter (Fig. 3).

On all 7 of the limed plots of the south block a good stand still remains from the original seeding, but on the unlimed plots the stands are thin and irregular.

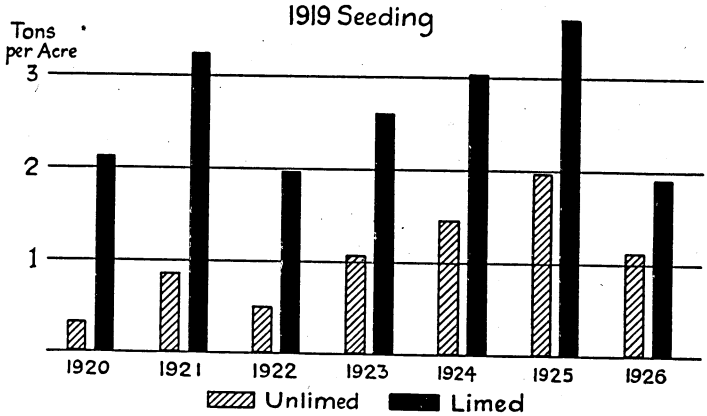

Fig. 3. Effect of Liming on Yields of Alfalfa on 1919 Seeding on Series A at Coon Creek

Sweet clover in 1920.—The effect of the lime was relatively even more marked on the sweet clover, which was cut only once, on June 18, the second growth being very light. The increase averaged 600 per cent with the white and 550 per cent with the yellow variety (Table VI).

TABLE V

EFFECT OF LIMING ON YIELDS OF ALFALFA ON 1919 SEEDING ON SERIES A AT COON CREEK

Plot No.	Application and rate per acre	1920		1921			1922		1823		1924		1925		1926		Total for 7 seasons
		First cutting	Second cutting	First cutting	Second cutting	Third cutting	First cutting	Second cutting	First cutting	Second cutting	First cutting	Second cutting	First cutting	Second cutting	First cutting	Second cutting	
	tons	tons	tons	tons	tons	tons	tons	tons	tons	tons	tons	tons	tons	tons	tons	tons	tons
1	None	0.16	0.08	0.34	0.17	0.08	0.12	0.05	0.20	0.11	0.25	0.14	0.25	0.17	0.26	0.17	2.13
6	do	.20	.16	.54	.36	.14	.40	.20	.77	.56	1.45	.58	1.81	1.07	1.31	.45	7.12
11	do	.20	.18	.97	.60	.25	.49	.27	.99	.59	1.58	.40	1.53	1.13	0.60	.47	7.59
15	do	.18	.08	.23	.14	.05	.28	*	*	*	†	...	....	....	....	...	....
20	do	.20	.08	.40	.23	.12	.54	*	*	*	†	...	....	....	....	...	....
25	do	.22	.16	.28	.16	.08	.13	*	*	*	†	...	....	....	....	...	....
	Average	0.19	0.12	0.46	0.28	0.12	0.33	0.17	0.65	0.42	1.09	0.37	1.20	0.79	0.72	0.36	5.61
4	Limestone 4	0.88	1.20	1.69	0.97	0.48	1.15	0.73	1.57	0.92	2.17	0.66	2.26	1.78	1.29	1.02	19.13
7	do 2	0.96	1.28	1.94	1.21	.54	1.30	.85	1.87	.97	2.47	.93	2.22	1.61	1.16	0.84	20.51
8	do 8	1.12	1.28	1.57	1.09	.36	1.18	.66	1.63	.85	2.30	.57	1.86	1.14	0.77	0.67	17.47
19	do 4	0.76	1.04	1.45	0.97	.65	1.21	*	*	*	†	...	....	....	....	...	.....
21	do 2	0.84	1.12	1.33	0.73	.65	1.08	*	*	*	†	...	....	....	....	...	.....
22	do 8	0.94	1.20	1.81	1.21	.78	1.27	*	*	*	†	...	....	....	....	...	.....
	Average	0.92	1.19	1.63	1.03	0.58	1.20	0.75	1.69	0.91	2.31	0.72	2.11	1.51	1.07	0.84	19.04
Increase from liming, per cent.....		384	892	254	271	383	264	341	160	117	112	95	76	91	49	133	239

* Yields of hay not determined, being left for seed.

† Plowed up and reseeded.

TABLE VI

EFFECT OF LIMING ON YIELD OF SWEET CLOVER IN 1920 ON SERIES A AT COON CREEK
In 1921 the plots were plowed and seeded to alfalfa with no additional application

Plot No.	Application and rate per acre	White variety	Yellow variety
	tons	tons	tons
1	None	0.04	0.05
6	do	.03	.04
11	do	.02	.02
15	do	.07	.03
20	do	.03	.02
25	do	.10	.09
Average		0.05	0.04
5	Limestone 4	0.37	0.37
7	do 2	.23	.20
8	do 8	.52	.52
19	do 4	.33	.15
21	do 2	.11	.11
22	do 8	.57	.23
Average		0.35	0.26
Increase from liming, per cent		600	550

Alfalfa Seeding in 1921 Following Sweet Clover

The portion of Series A (Fig. 2) that had been in sweet clover in 1919 and 1920 was plowed at the end of April, 1921, and seeded to alfalfa on May 14, no culture or soil for inoculation being used. At the end of June the resulting stand was so uneven and weeds were so abundant that the land was disked and kept clean until July 14, when it was reseeded to alfalfa without inoculation. On all the plots an excellent stand was obtained, but on the unlimed ones the plants were yellow and smaller than on the limed. Early in September this seeding provided a very striking demonstration of the benefits of liming. In October the plants on the unlimed plots were much improved in color. During the first week in November, from 60 to 200 plants were collected from each of Plots 1, 5, 6, 8, and 11 and examined for nodules, which were found on the roots of almost all, those from the unlimed as well as those from the limed plots.

All the evidence of the first three seasons, up to the winter of 1921-22, indicated a marked initial lime deficiency on this 33-foot strip on the 28 plots, but in 1922 the results were very different and quite unexpected.

During May and early June, 1922, the plants on all the unlimed portions of this seeding improved rapidly, and after the first cutting showed no very striking difference from those on the adjacent limed plots. Evidently the plants were fully inoculated, had found in the subsoil a supply of lime that was at least almost adequate, and did not suffer seriously from the acidity of the surface soil. With the

first cutting, the average increase from liming (Table VII) was only 64 per cent and with the second only 47 per cent. In the same season, in the 1919 seeding on the adjacent portion of the same plots, the increases were 264 and 341 per cent, respectively, and in the first crop season (1920) they had been 384 and 892 per cent. The differences in yield between the limed and the unlimed plots of the 1921 seeding in the following four seasons became smaller and smaller, falling to 28 per cent in 1923, to 11 per cent in 1924, and to 6 per cent in 1925, until in 1926, a very dry season, it has disappeared (Fig. 4).

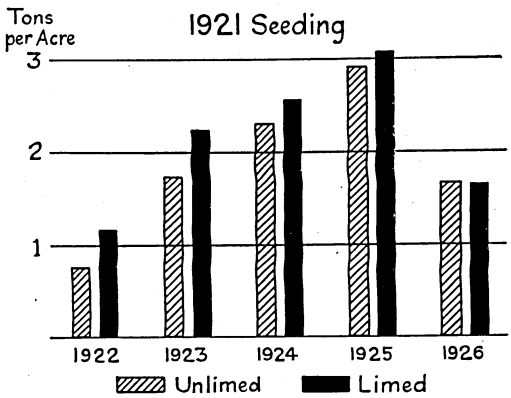

Fig. 4. Effect of Liming on Yields of Alfalfa Sown in 1921 on Plots on Series A at Coon Creek, That Had Been in Sweet Clover During 1919 and 1920

A comparison of Figures 3 and 4 brings out the great difference in behavior between the two seedings. The total yield per acre from the 10 cuttings in the five crop seasons averaged 9.40 tons on the 6 unlimed plots and only 1.36 tons, or 14 per cent, more on the 6 limed plots.

The question of lime deficiency.—At the end of the season of 1926 samples of the surface 6 inches of soil were collected from the portions of the 6 control plots that had been seeded to sweet clover in 1919 and also from the portions of the north 3 control plots seeded to alfalfa at the same time and the hydrogen-ion concentration of each was determined (Table III). The concentration, expressed as pH, varied only between 5.1 and 5.4.

If the only evidence of lime deficiency of the east 66 feet of Series A were the yields of the 1919 seedings of alfalfa and sweet clover (Tables V and VI and Fig. 3) and the present soil reaction, it would certainly be concluded that the soil was initially very lime deficient, and that liming is indispensable for the successful production of alfalfa.

TABLE VII

EFFECT OF LIMING ON YIELD OF ALFALFA ON 1921 SEEDING ON SERIES A AT COON CREEK

All the plots had been in sweet clover in 1919 and 1920

Plot	Application and rate per acre	1922		1923		1924		1925		1926		Total for 5 seasons
		First cutting	Second cutting	First cutting	Second cutting	First cutting	Second cutting	First cutting	Second cutting	First cutting	Second cutting	
	tons	tons	tons	tons	tons	tons	tons	tons	tons	tons	tons	tons
1	None	0.54	0.42	1.45	0.85	1.90	0.46	1.87	1.48	0.80	0.75	10.52
6	do	.48	.30	0.91	.56	1.69	.48	1.80	1.28	0.92	.85	9.27
11	do	.36	.30	0.91	.52	1.37	.55	1.42	0.98	0.82	.74	7.97
15	do	.42	.30	1.21	.93	1.98	.88	1.54	1.34	1.16	.93	10.69
20	do	.42	.27	1.03	.57	1.65	.66	1.60	1.36	0.85	.78	9.19
25	do	.48	.21	0.97	.56	1.77	.48	1.84	1.01	0.74	.69	8.75
	Average	0.45	0.30	1.08	0.67	1.73	0.58	1.68	1.24	0.88	0.79	9.40
5	Limestone 4	0.73	0.42	1.51	0.81	2.06	0.79	1.96	1.48	1.01	0.93	11.70
7	do 2	.79	.45	1.45	.69	1.86	.40	1.66	1.35	0.98	.81	10.44
8	do 8	.79	.48	1.63	.93	2.06	.56	1.81	1.21	0.87	.73	11.07
19	do 4	.66	.42	1.33	.77	1.45	.75	1.55	1.36	0.95	.77	10.11
21	do 2	.79	.45	1.45	.89	2.06	.62	1.82	1.52	0.66	.81	11.07
22	do 8	.66	.42	1.33	.69	2.14	.71	1.74	1.04	0.74	.72	10.19
	Average	0.74	0.44	1.45	0.80	1.94	0.64	1.76	1.33	0.87	0.79	10.76
	Increase from liming, per cent.....	64	47	34	19	12	10	5	7	—1	0	14

On the other hand, if the only evidence on the matter were the yields of alfalfa from the 1921 seeding, it would be concluded that the lime deficiency is slight, the application of lime not necessary and of doubtful economic advantage.

During the following three seasons, 1922 to 1924, the west half of A was gradually seeded to alfalfa, but before considering these seedings, it seems desirable to consider the evidence as to initial lime deficiency furnished by the three series to the west—B, C, and D.

Evidence of Lime Deficiency on Adjacent Land

The 66 plots of Series B, C, and D were devoted for five years to an experiment planned to determine the effect of commercial fertilizers, with and without lime, on the crops in a three-year rotation. The treatments are indicated in Figure 2. Ground limestone, at the rate of 4 tons per acre, was applied to the west half of B in May, 1919, to the east half of C in September, 1920, and to the west half of D in the summer of 1921. The crop history of these series previous to 1919 had been the same as that of A. Since that time it has been as shown in Table VIII.

TABLE VIII

CROPS ON SERIES B, C, AND D AT COON CREEK, 1919 TO 1926

Crop season	Series B	Series C	Series D
1919	Medium red clover	Winter rye	Winter rye
1920	Winter rye	Potatoes	Corn
1921	Medium red clover	Winter rye	Corn and potatoes
1922	Potatoes	Hubam clover	Winter rye
1923	Winter rye	Potatoes	Medium red and alsike clover and sweet clover
1924	Alfalfa and volunteer rye	Winter rye	Corn and potatoes
1925	Alfalfa	Medium red clover, sweet clover and alfalfa	Winter rye and alfalfa
1926	Alfalfa	Alfalfa and sweet clover seeded at different dates	Alfalfa

Series B.—On Series B medium red clover was seeded to winter rye in the spring of 1920. A fair stand was secured but most of the plants died during the following winter. In 1921 there was only one cutting, on June 22. The yield for the 22 plots averaged 0.44 ton per acre on the unlimed half and 0.41 ton on the limed.

The series was planted to potatoes in 1922 and after their removal was seeded to winter rye. Medium red clover sown in this in the spring of 1923 failed to make a stand. After harvesting the rye the stubble was plowed shallow, and on August 11 ground limestone, at the rate of 4 tons per acre, was spread on the east quarter. The object of this was to determine whether the earliness of an application influenced its effectiveness, this quarter being compared with the west

half, which had been limed four years before. The whole series was then disked, harrowed, cultipacked, and two days later seeded to alfalfa, using culture Q at the usual rate. Very few weeds, but much volunteer rye, appeared. At first there was a good stand of alfalfa on all three parts of every plot, but almost from the beginning there were marked differences between the three parts. On the west half, limed in 1919, all the plants were dark green and vigorous, while on the unlimed quarter nearly all were yellow and small, the line of demarcation being sharp on each of the 22 plots. On the east quarter, limed just before seeding, while most of the plants were small and yellow, there were many scattered individuals and groups resembling those on the west half, and the number of these increased as the season advanced.

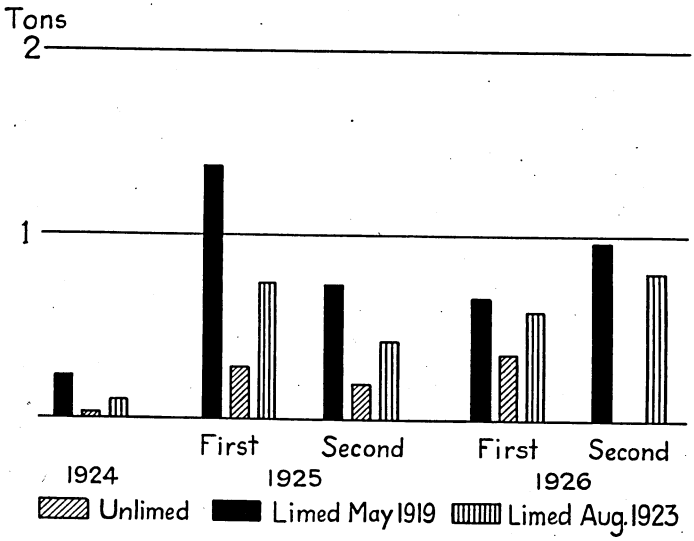

Fig. 5. Effect of Liming on Yields of Alfalfa in Two Cuttings on Series B at Coon Creek

Winter-killing was severe on the late-limed quarter and much more so on the unlimed quarter, but was not observed on the early-limed half. The following May most of the remaining plants on the unlimed portion were still yellow; during the next two months part of these died and the rest became dark green. Evidently about all the dark green plants of the first fall survived and these were rather uniformly distributed. Throughout the early part of the season of 1924 the late-limed quarter showed a mottled appearance, with the proportion of green steadily increasing. The volunteer rye, which was uniform in stand on the different plots, was harvested and the growth of alfalfa that followed was mowed on October 25. The yields were light, even on the best plots, but on the late-limed quarter they were three times as heavy, and on the early-limed half seven times as heavy, as on the unlimed portion (Table IX and Fig. 5). The two cuttings in 1925

showed similar altho less marked differences. With the first cutting in 1926 the unlimed portion gave much the lowest yields. On account of drouth, the thinner stand on the late-limed portion yielded about the same as the thick stand on the early limed. As the unlimed quarter was plowed after the first cutting in 1926, there are no yields on this from a second cutting.

TABLE IX

EFFECT OF LIMING ON YIELD OF ALFALFA ON SERIES B AT COON CREEK SEEDED IN AUGUST, 1923, ON RYE STUBBLE

The series had never been in alfalfa or sweet clover

Plot	West side Limed in 1919	Center Never limed	East side Limed just before seeding
	1924 Only one cutting		
	tons	tons	tons
1	0.23	0.03	0.14
2	.26	.02	.09
6	.22	.04	.09
12	.23	.04	.09
13	.24	.03	.07
17	.20	.04	.12
Average	0.23	0.03	0.10
	1925 First cutting		
1	1.53	0.21	1.11
2	1.15	.21	1.11
6	1.27	.25	0.69
12	1.37	.39	0.87
13	1.42	.31	0.31
17	1.45	.33	0.31
Average	1.36	0.28	0.73
	1925 Second cutting		
1	0.69	0.27	0.48
2	.75	.15	.50
6	.88	.15	.59
12	.71	.24	.46
13	.71	.13	.27
17	.61	.21	.22
Average	0.72	0.19	0.42
	1926 First cutting		
1	0.65	0.31	0.59
2	.68	.29	.66
6	.66	.17	.73
12	.72	.27	.65
13	.62	.35	.44
17	.64	...	.44
Average	0.66	0.28	0.58
	Total in four cuttings		
1	3.10	0.82	2.32
2	2.84	.67	2.36
6	3.03	.61	2.10
12	3.03	.94	2.07
13	2.99	.82	1.09
17	2.90	...	1.09
Average	2.98	0.77	1.84

Series C.—The east half of Series C was limed in September, 1920, and sown to winter rye. The following spring medium red clover was seeded in the rye, but the stand died out before harvest. In May, 1922, the series was plowed and sown to Hubam clover inoculated with culture Q. A fair stand resulted but the growth was very light on all plots, especially on the unlimed half, on which the plants were very small and yellow. On the limed half the yield was more than four times as heavy as on the unlimed (Table X).

TABLE X

EFFECT OF LIMING ON YIELDS OF HUBAM CLOVER IN 1921 AND OF MEDIUM RED CLOVER IN 1925 ON SERIES C AT COON CREEK

Plot	Hubam clover in 1921		Medium red clover in 1925					
			First cutting, June 22		Second cutting, August 4		Two cuttings	
	Unlimed	Limed	Unlimed	Limed	Unlimed	Limed	Unlimed	Limed
	tons	tons	tons	tons	tons	tons	tons	tons
1	0.05	0.36	0.48	0.75	0.22	0.44	0.70	1.19
2	.09	.36	.38	1.29	.13	.78	.51	2.07
6	.05	.30	.11	0.72	.06	.58	.17	1.30
12	.09	.18	.53	1.29	.08	.61	.61	1.90
13	.05	.24	.04	0.90	.00	.40	.04	1.30
17	.05	.24	.30	1.20	.01	.59	.31	1.79
Average	0.06	0.28	0.31	1.02	0.08	0.57	0.39	1.59

In the fall of 1923, following a crop of potatoes, the whole series was sown to rye, and in the spring of 1924 medium red clover was seeded on the west 60 feet, occupying all the unlimed portion of every plot and a third of the limed. Alfalfa and sweet clover were sown on the rest of the limed half. Good stands of all three legumes were at first obtained. On the limed half these remained at the time the rye was harvested and, except red clover on some of the plots, also through the following winter. On the unlimed half, as the rye approached maturity a great many of the red clover plants died and the killing during the following winter was much more severe. In 1925 the rainfall was very favorable until the time of the first cutting, which was followed by an exceptionally severe drouth. The yields (Table X) averaged four times as heavy on the limed as on the unlimed portion—evidence of lime deficiency toward even red clover. There is nothing to indicate any less deficiency of lime on C than on B and the east part of A.

Series D.—The west half of Series D was limed in the summer of 1921 and the whole sown to winter rye. At the end of the next April sweet clover was seeded on the west 15 feet of the limed half and a mixture of medium red and alsike clover on the remainder. Only

thin stands of red clover were obtained and the yields in 1923 were very light, the single cutting averaging only 0.14 ton on the limed portion and 0.09 ton on the unlimed.

In 1924 an experiment somewhat similar to that reported for Series B was started on this series, the east quarter being limed during the first ten days of September, just after a crop of potatoes had been removed. The series was well cultivated and seeded to winter rye. Alfalfa, inoculated with culture Q, was at once sown on Plots 1 to 11 but no legume on Plots 12 to 22. A fair stand appeared but the plants were very small when winter came and practically all died before spring. On April 21, 1925, alfalfa was seeded in the rye with a press drill on all 22 plots, culture X being used. A good stand appeared on all portions of the plots but on the unlimed part most of the plants remained small and yellow, and most of these died before the rye was ready to be harvested. On the early-limed half there was a full stand of dark green plants and these persist to the present time (November, 1926). On the late-limed quarter the alfalfa did better than on the corresponding part of Series B, but many of the plants were yellow and many died before the rye was harvested in July. During the winter of 1925-26 most of the remaining yellow plants died, thus leaving on each of the 22 plots a very thin stand on the center, unlimed portion, and a rather thin but uniform stand on the late-limed portion. In the three cuttings of 1926, the yields on the early-limed portion averaged over 30 times as much as on the unlimed (Table XI and Fig. 6).

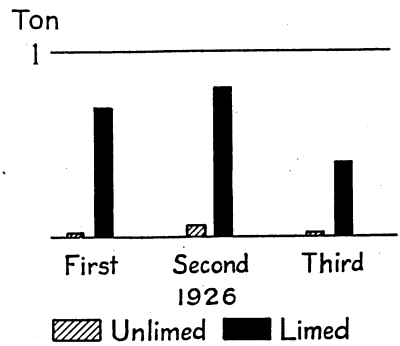

Fig. 6. Effect of Liming on Yields of Three Cuttings of Alfalfa on Series D at Coon Creek

The crops have given evidence of as great an initial lime deficiency on Series B, C, and D as on the east half of Series A. The samples of surface soil from the 12 control plots on B and D, taken at the same time as those from A, show a similar hydrogen-ion concentration, as pH, of 5.2 to 5.4 (Table III).

TABLE XI

EFFECT OF LIMING ON YIELD, IN 1926, OF ALFALFA SEEDED IN RYE IN 1925 ON SERIES D AT COON CREEK

Plot	West side Limed in 1921	Center Never limed	East side Limed Sept. 1924
		First cutting	
	tons	tons	tons
1	0.72	0.01	0.48
2	.79	.01	.43
6	.58	.02	.45
12	.58	.01	.44
13	.76	.03	.51
17	.78	.01	.62
Average	0.70	0.01	0.49
		Second cutting	
1	0.86	0.02	0.91
2	.85	.01	.78
6	.74	.01	.85
12	.70	.03	.62
13	.84	.07	.72
17	.84	.04	.73
Average	0.80	0.03	0.77
		Third cutting	
1	0.36	0.01	0.50
2	.41	.01	.54
6	.43	.01	.42
12	.41	.02	.33
13	.38	.03	.42
17	.43	.01	.33
Average	0.40	0.01	0.42
Total of 3 cuttings	1.90	0.05	1.68

Seedings of 1922, 1923, and 1924 on South Field

During 1922, 1923, and 1924 the remainder of Series A was seeded to alfalfa (Fig. 2). The differences in growth and yield between limed and unlimed plots, with these three seedings, were by no means so striking as with the 1919 seeding nor so slight in the first crop year as was the 1921 seeding, which followed sweet clover.

As stated previously, the four common clovers sown just west of the sweet clover in 1919 killed out during the following winter. In May, 1920, the land was plowed and resown to the same legumes, good stands of all being obtained. There was insufficient growth for a cutting in that season. All came through the winter without serious injury, and by the middle of May the effect of the liming was evident on all four clovers. On the unlimed plots the stand was thin, tho fairly uniform; on all the limed plots the plants were both much more numerous and much larger. Alsike showed less benefit than medium red and mammoth clovers. White clover showed much the same response as alsike but by mistake it was disked up at the end of May. During the growing season the rainfall was so unfavorable for the common

clovers that the yields were light, even on the limed plots. The average yields of cured hay for the same 12 plots dealt with in Table V were:

			Tons per acre		Tons per acre
Medium red,	two cuttings,	unlimed	0.29	limed	—0.45
Mammoth,	do	do	.56	do	.68
Alsike,	one cutting,	do	0.47	do	0.47

1922 Seeding.—On April 29, 1922, alfalfa was seeded on the east 15-foot strip of the above mentioned land and also in three rows, 16 inches apart, 20 feet farther to the west (Fig. 2). The seed was treated with culture Q. On the intervening 20 feet, Hubam clover was sown, inoculated as was the alfalfa. The west 4 feet of Hubam was in rows 16 inches apart and kept free of weeds, like the adjacent rows of alfalfa.

A good stand was secured on all the limed plots and a thinner, but comparatively uniform, stand on the unlimed. The growth of Hubam on the broadcast portion, even on the limed plots, was so poor that the yields were not considered worth determining, and on the unlimed plots it was much poorer still. In the rows there was much less difference, only 42 per cent increase from liming (Table XII).

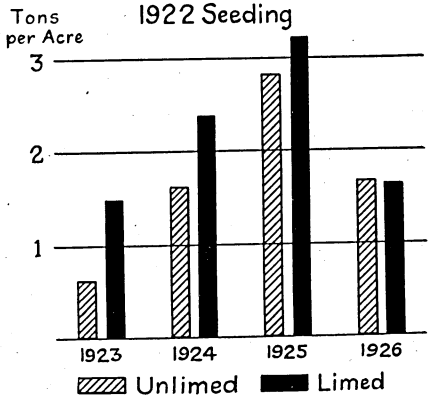

Fig. 7. Effect of Liming on Yields of Alfalfa on 1922 Seeding on Series A at Coon Creek

Throughout that season and the next the difference in appearance and yields of the broadcast alfalfa, in favor of liming, was so pronounced (Table XIII and Fig. 7) that the success of the alfalfa on the unlimed plots of the 1921 seeding, which by that time had become very evident, was still considered exceptional, and was attributed to unusually favorable weather which had allowed the plants to live until their roots reached the calcareous subsoil (3, p. 26).

TABLE XII

EFFECT OF LIMING ON YIELDS OF HUBAM-CLOVER AND ALFALFA SEEDED IN ROWS ON SERIES A AT COON CREEK IN 1922

Plot No.	Application and rate per acre	Hubam clover 1922	Alfalfa 1923 First cutting	Alfalfa 1923 Second cutting	Alfalfa 1924 First cutting	Alfalfa 1924 Second cutting	Alfalfa 1925 First cutting	Alfalfa 1925 Second cutting	Alfalfa 1925 Third cutting	Alfalfa 1926 First cutting	Alfalfa 1926 Second cutting	Total for 4 seasons
	tons	tons	tons	tons	tons	tons	tons	tons	tons	tons	tons	tons
1	None	0.36	0.73	0.73	1.38	0.58	1.46	0.89	0.45	0.43	0.49	7.14
6	do	.46	0.66	.53	1.31	0.52	1.44	0.97	0.46	0.40	.38	6.67
11	do	.49	0.66	.63	1.60	1.09	1.91	2.03	0.76	0.69	.65	10.02
15	do	.45	0.82	.82	2.33	1.29	1.04	1.52	0.86	0.83	.65	10.16
20	do	.52	1.25	.99	2.65	2.00	1.82	1.79	0.95	0.62	.58	12.65
25	do	.52	0.92	.89	3.11	1.76	2.06	1.74	1.03	1.22	.91	13.64
	Average	0.48	0.84	0.76	2.06	1.21	1.62	1.49	0.75	0.70	0.61	10.04
5	Limestone 4	0.82	1.15	1.02	2.21	0.89	1.88	1.37	0.79	0.54	0.47	10.32
7	do 2	.59	0.92	0.73	1.77	0.87	1.39	1.35	0.62	0.36	.39	8.40
8	do 8	.69	1.19	0.99	2.47	1.11	1.87	1.60	0.66	0.45	.43	10.77
19	do 4	.72	1.72	1.52	3.78	1.98	1.95	1.73	1.01	1.15	.71	15.55
21	do 2	.57	1.52	1.45	3.25	2.05	1.88	1.77	1.05	0.66	.74	14.37
22	do 8	.69	1.52	1.32	2.91	1.72	1.92	1.92	1.17	0.97	.78	14.23
	Average	0.68	1.34	1.17	2.73	1.44	1.81	1.62	0.88	0.69	0.59	12.27
Increase from liming, per cent.....		42	59	54	32	19	12	9	17	—1	—3	22

TABLE XIII

EFFECT OF LIMING ON YIELD OF ALFALFA ON 1922 SEEDING ON SERIES A AT COON CREEK

Plot No.	Application and rate per acre	1923 First cutting	1923 Second cutting	1924 First cutting	1924 Second cutting	1925 First cutting	1925 Second cutting	1926 First cutting	1926 Second cutting	Total for 4 seasons
	tons	tons	tons	tons	tons	tons	tons	tons	tons	tons
1	None	0.54	0.21	0.90	0.18	1.41	1.06	0.70	0.62	5.62
6	do	.54	.12	1.21	.38	1.95	1.21	1.08	.86	7.35
11	do	.18	.12	.72	.40	1.27	.60	.97	.75	5.01
15	do	.42	.24	1.33	.57	1.98	1.20	.92	.69	7.35
20	do	.54	.30	1.51	.53	1.87	1.29	.97	.80	7.81
25	do	.52	.18	1.51	.45	1.86	1.21	.91	.78	7.42
	Average	0.46	0.19	1.20	0.42	1.72	1.09	0.92	0.75	6.76
5	Limestone 4	1.09	0.42	1.57	0.27	2.02	1.25	0.91	0.75	8.25
7	do 2	0.85	.30	1.51	.40	1.90	1.24	0.81	.73	7.74
8	do 8	1.09	.60	1.87	.28	1.59	1.42	0.80	.67	8.32
19	do 4	1.03	.42	1.81	.73	1.94	1.42	1.19	.70	9.24
21	do 2	0.97	.60	2.05	.61	1.99	1.39	0.80	.69	9.10
22	do 8	1.21	.36	2.47	.80	1.92	1.23	0.93	.78	9.70
	Average	1.04	0.45	1.88	0.51	1.89	1.32	0.91	0.72	8.72
Increase from liming, per cent		126	137	57	21	10	21	—1	—4	29

In the rows of alfalfa which were kept free of weeds, the proportion of surviving plants was much higher and the marked differences between limed and unlimed plots disappeared sooner (Table XIII).

In 1925 the benefit of liming was not marked on the 1922 seeding, and in 1926, so far as the yields indicate, it disappeared, but there is still a better stand on the limed plots.

1923 Seeding.—On June 22, 1923, the 20-foot strip that had been in Hubam in 1922 was seeded to alfalfa, with no inoculation. The differences between limed and unlimed plots in this seeding have been much like those of the 1922 seedings, becoming less each year (Table XIV and Fig. 8).

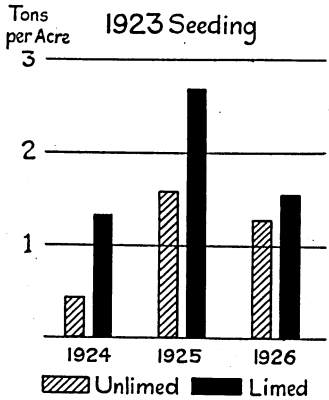

Fig. 8. Effect of Liming on Yields of Alfalfa on 1923 Seeding on Series A at Coon Creek

TABLE XIV

EFFECT OF LIMING ON YIELD OF ALFALFA ON 1923 SEEDING ON SERIES A AT COON CREEK
Hubam clover occupied the plots in 1922

Plot No.	Application and rate per acre	1924 First cutting	1924 Second cutting	1925 First cutting	1925 Second cutting	1926 First cutting	1926 Second cutting	Total 1924-26
	tons	tons	tons	tons	tons	tons	tons	tons
1	None	0.42	0.18	1.15	0.80	0.48	0.57	3.60
6	do	.42	.14	0.99	.82	.60	.66	3.66
11	do	.12	.07	0.36	.12	.30	.30	1.27
15	do	.24	.12	0.89	.57	.84	.61	3.27
20	do	.30	.16	0.80	.94	.94	.56	3.70
25	do	.30	.17	1.35	.83	.93	.94	4.52
	Average	0.30	0.14	0.92	0.68	0.68	0.61	3.34
5	Limestone 4	1.09	0.27	1.56	1.21	0.75	0.73	5.61
7	do 2	1.02	.18	1.54	1.14	0.77	.75	5.40
8	do 8	1.21	.27	1.60	0.97	0.73	.69	5.47
19	do 4	0.98	.23	1.60	1.37	1.29	.69	6.16
21	do 2	1.21	.25	1.68	1.25	0.92	.65	5.96
22	do 8	1.09	.18	1.39	0.93	0.83	.65	5.07
	Average	1.10	0.23	1.56	1.14	0.88	0.69	5.60
Increase from liming, per cent		267	64	70	68	29	13	68

TABLE XV

EFFECT OF LIMING ON YIELDS OF ALFALFA ON TWO 1924 SEEDINGS ON SERIES A AT COON CREEK

Both groups of plots were seeded on the same day. Those on the east had been in alfalfa for the preceding five years, while those on the west had never been in alfalfa or sweet clover.

		West group					East group				
		1925		1926			1925		1926		
Plot No.	Application and rate per acre	First cutting	Second cutting	First cutting	Second cutting	Two seasons	First cutting	Second cutting	First cutting	Second cutting	Two seasons
	Tons	tons	tons	tons	tons	tons	tons	tons	tons	tons	tons
1	None	1.27	0.97	0.57	0.66	3.47	...	...	...	...	...
6	do	0.94	.68	.37	.57	2.56	...	...	...	...	...
11	do	0.36	.42	.40	.53	1.71	...	...	...	...	...
15	do	0.97	.71	.60	.69	2.97	0.83	0.72	0.40	0.52	2.47
20	do	0.75	.66	.63	.63	2.67	0.93	.78	.57	.63	2.91
25	do	0.61	.28	.48	.53	1.90	1.00	.65	.58	.57	2.80
	Average	0.82	0.62	0.51	0.60	2.55	0.92	0.72	0.52	0.57	2.73
5	Limestone 4	1.60	1.07	0.51	0.68	3.86	...	...	...	...	...
7	do 2	1.23	1.05	.45	.62	3.35	...	...	...	...	...
8	do 8	1.26	0.71	.44	.65	3.06	...	...	...	...	...
19	do 4	1.53	1.16	.76	.66	4.11	1.34	0.90	0.59	0.66	3.49
21	do 2	1.38	0.80	.69	.65	3.52	1.20	.78	.46	.64	3.08
22	do 8	1.53	0.78	.78	.75	3.84	1.28	.80	.46	.74	3.28
	Average	1.42	0.93	0.60	0.67	3.62	1.27	0.83	0.50	0.68	3.28
Increase from liming, per cent		73	50	18	12	42	38	15	—4	19	20

1924 Seeding.—On June 16, 1924, the 27-foot strip remaining on the west side of the series, which had been in soybeans and the common clovers during the preceding five years, was seeded to alfalfa, treating the seed with culture Q. The relative yields (Table XV) in 1925 and 1926 have been much like those of the first two years of the 1922 seeding. This may be seen from a comparison of the left half of Figure 9 with Figure 7.

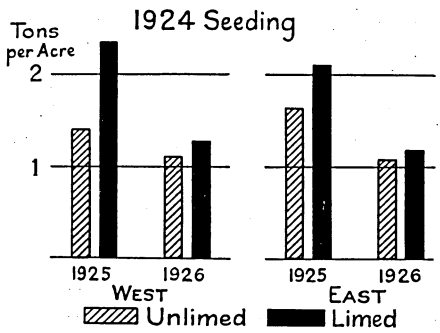

Fig. 9. Effect of Liming on Yields of Alfalfa on Two Seedings in 1924 on Series A at Coon Creek
The east plots had been in alfalfa from 1919 to 1924. while the west plots had not previously been in alfalfa or sweet clover

Adequacy of Mineral Nutrients

The soil of all these series is well supplied with potash, phosphate, and sulphur for alfalfa. Experiments with phosphate and potash alone and in combination, both with and without liming, apply to all seedings on Series A and to most of those on B, C, and D. The results with the first three seedings of alfalfa on A are reported in Table XVI, where the yields of the plots that received only 4 tons of ground limestone in 1919 are compared with those that received the same and in addition phosphate and potash at that time and in four later seasons (Table IV).

The most effect has been shown during the first two crop seasons and even then it was only slightly beneficial. For the seven crop seasons of the 1919 seeding there was no gain, —0.18 ton per acre; for the five crop seasons of the 1921 seeding, only 0.18 ton; and for the four of the third seeding, only 1.27 tons.

Sulphur is abundant in the soil. No sulphur fertilizer has been applied as such, but in 1919 the potash was applied in the form of sulphate of potash and half the phosphoric acid as ordinary acid phosphate. All the phosphate added in 1920 was in the form of acid phosphate and the potash fertilizer used was Nebraska potash salts, which carried a considerable proportion of sulphate.

TABLE XVI

EFFECT OF PHOSPHATE-POTASH APPLICATIONS ON YIELD OF ALFALFA ON LIMED PLOTS OF SERIES A AT COON CREEK

All received 4 tons per acre of limestone in May, 1919, and none afterwards. Phosphate and potash were applied in the spring of 1919, 1920, 1924, 1925, and 1926.

Plot	Fertilization	1920	1921	1922	1923	1924	1925	1926	Total
		Seeding of 1919							
		tons	tons	tons	tons	tons	tons	tons	tons
3	K+P*	2.18	3.02	1.85	2.43	2.95	3.44	2.22	18.09
17	do	2.34	3.51	...	...	...	...	...	...
	Average	2.26	3.26	1.85	2.43	2.95	3.44	2.22	18.41
5	None	2.08	3.14	1.88	2.49	2.83	4.04	2.31	18.77
19	do	1.80	3.07	...	...	...	...	...	...
	Average	1.94	3.10	1.88	2.49	2.83	4.04	2.31	18.59
	Increase with K+P*	0.32	0.16	—0.03	—0.06	0.12	—0.60	—0.09	—0.18
		Seeding of 1921							
3	K+P*	...	...	1.21	2.45	2.46	3.21	1.95	11.28
17	do	...	...	1.21	2.28	2.44	2.92	1.94	10.79
	Average	...	...	1.21	2.36	2.45	3.06	1.94	11.03
5	None	...	...	1.15	2.32	2.85	3.44	1.94	11.70
19	do	...	...	1.08	2.10	2.20	2.91	1.72	10.01
	Average	...	...	1.11	2.21	2.52	3.17	1.83	10.85
	Increase with K+P*	...	...	0.10	0.15	—0.07	—0.11	0.11	0.18
		Seeding of 1922							
3	K+P*	...	...	...	1.81	2.27	3.16	2.20	9.44
17	do	...	...	...	1.75	3.41	3.75	1.71	10.62
	Average	...	...	...	1.78	2.84	3.45	1.95	10.03
5	None	...	...	...	1.51	1.84	3.27	1.66	8.28
19	do	...	...	...	1.45	2.54	3.36	1.89	9.24
	Average	...	...	...	1.48	2.19	3.31	1.77	8.76
	Increase with K+P*	...	...	...	0.30	0.65	0.14	0.18	1.27

* K=potash; P=phosphate.

Experiments with Inoculated Soil in 1924

By the spring of 1924 the conclusion had been reached that the growing of even a very thin stand of alfalfa or sweet clover on these fields contributed something favorable to the growth of a new seeding of alfalfa that was not furnished by the commercial culture and that this was something else than merely a great number of bacteria. To test this, half of the land seeded to alfalfa on Series A in 1919 was plowed up and reseeded to alfalfa; also a heavy application of soil from one of these plowed plots was made to some unlimed plots on a near-by field which was being sown to alfalfa for the first time.

Seeding After Plowing Under Alfalfa

In May, 1924, all the alfalfa of the 1919 seeding, both common and Grimm, on Plots 15 to 28 of Series A was plowed under. Alfalfa seed, treated with culture Q at the usual rate, was sown on the same date (June 16) and in the same manner as on the west 27 feet of the same series.

On all the plots thick stands resulted and still persist. The yellow, stunted plants that had characterized the unlimed plots in previous seedings, at least for a time, were entirely absent. While all the plants on the unlimed plots were of uniformly good color, but somewhat lighter, the growth was somewhat less vigorous during 1924 and 1925 than on the limed plots. On the first cutting of the first crop year there was a difference in yield of only 38 per cent in favor of the limed plots and on the second cutting of only 15 per cent. In 1926 the difference was much less (Table XV and Fig. 9).

Trial of Heavy Application of Soil

The Anderson field, which is on Hinckley fine sand, located on a knoll about a mile from the South field (Fig. 1), had been farmed for many years but had finally become so unproductive that for several years it had been allowed to lie idle. It was rented by the University in 1924 in order to determine whether alfalfa, by means of liming, could be made a successful crop on soil so much inferior to that of the South field. The trial of the heavy application of soil from Series A was limited to three plots, the experiment with the soil being only incidental to the main experiment on this field.

The rather thin growth of weeds occupying the land was plowed under early in July, 1924; 25 plots were laid out, and the hydrogen-ion concentration of the surface 6 inches of soil on each was determined. This was found to be much alike on all. Four plots were left as controls while various applications were made to the others on July 25 to 28 (Table XVII). Ground limestone was used at rates of 2, 4, and 8 tons per acre; and marl, from an adjacent pit and carrying about 35 per cent of water, at 40 tons per acre. On three plots inoculating soil from Plot 22 on Series A in the South field was applied at the rate of 2 tons per acre. That plot in 1919 had been given 8 tons per acre of limestone and seeded to alfalfa, which earlier in 1924 had been plowed up and the plot reseeded. On July 30 all the plots were seeded to alfalfa inoculated with commercial culture Q.

TABLE XVII

EFFECT OF TREATMENTS ON YIELD OF ALFALFA ON ANDERSON FIELD AT COON CREEK
Seeded July 30, 1924. All seed treated with culture

Plot	Application and rate per acre	1925 First cutting	1925 Second cutting	1925 Two cuttings	1926 First cutting	1926 Second cutting	1926 Two cuttings	Total for two seasons
		tons	tons	tons	tons	tons	tons	tons
A-7	None	0.25	0.25	...	0.40	0.62		
A-12	do	.46	.20	...	.44	.76		
B-4	do	.83	.44	...	.44	.80		
B-9	do	.34	.38	...	.48	.60		
	Average	0.47	0.32	0.63	0.44	0.69	1.13	1.76
A-3	Limestone, 2 tons	1.02	0.58	....	0.68	0.73		
A-8	do	1.13	.59	...	.67	.92		
B-5	do	1.08	.60	....	.48	.79		
	Average	1.08	0.59	1.67	0.61	0.81	1.42	3.09
A-1	Limestone, 4 tons	1.28	0.60	...	0.63	0.69		
A-4	do	1.03	.45	...	.57	.72		
A-9	do	1.27	.48	...	.60	.69		
A-13	do	1.37	.43	...	.63	.82		
B-6	do	1.18	.62	...	.48	.81		
	Average	1.23	0.52	1.75	0.58	0.75	1.33	3.08
A-5	Limestone, 8 tons	1.09	0.50	...	0.50	0.75		
A-10	do	1.19	.52	...	.62	.74		
B-7	do	1.16	.70	...	.62	.80		
	Average	1.15	0.57	1.72	0.58	076	1.34	3.06
A-6	Marl, 40 tons	1.20	0.56	...	0.53	0.70		
A-11	do	1.25	.48	...	.53	.74		
B-8	do	1.11	.68	...	.56	.79		
	Average	1.19	0.57	1.76	0.54	0.74	1.28	3.04
A-2	Soil, 2 tons	0.94	0.46	...	0.52	0.66		
B-11	do	.93	.52	...	.59	.75		
B-13	do	.70	.44	...	.40	.56		
	Average	0.86	0.47	1.33	0.50	0.66	1.16	2.49
B-3	Limestone, 4 tons	1.46	0.64	...	0.42	0.77		
B-10	and manure, 10 tons	1.23	.79	...	.66	.82		
B-12	do	1.39	.70	...	.68	.76		
	Average	1.36	0.71	2.07	0.59	0.78	1.37	3.44

A thick stand of plants appeared on all the plots, and on all that had received limestone, marl, or inoculating soil the plants were dark green and vigorous. On the control plots most of them showed the usual characteristic stunted growth and yellow color, and during the following winter and summer most of those on three of these plots died, leaving a thin, rather uniform stand of green plants. On one control plot, B-4, there was a much better growth and fewer plants died. There was no appreciable winter injury on any of the plots treated with limestone, marl, or soil. In the fall of 1924, on the three plots to which soil was applied, the plants were not quite so large or

quite so dark green as those on the limed or marled plots, but they still were so thrifty in appearance that if there had been no adjacent limed plots for comparison their condition would have been considered entirely satisfactory. In 1925 the plants on these three plots appeared similar to those on the limed plots until in June, as they approached bloom, when they fell behind a little. On all the limed, marled, and soil-treated plots, during the last two dry seasons, the thick stands have been a disadvantage, causing injury from lack of moisture, and in every cutting most of the plants dried up without coming into bloom, while on the control plots the much smaller number of plants have kept green, grown much taller, and bloomed. On all the treated plots better yields would doubtless have been obtained if the stands had been much thinner. Wet weather followed the second cutting in 1926, made on July 31, and the resulting growth was far behind on all the treated plots than on the controls, being thicker but not taller. On the soil-treated plots this growth was similar to that on the limed and marled plots. On all it has been left for winter protection.

Trial of Inoculated Soil in Addition to Limestone

At Becker.—Alfalfa, treated with culture Q at the usual rate, was sown on a block of fortieth-acre plots on the Becker experimental field on June 18, 1924. These included four to which inoculating soil from Coon Creek had been applied the day before at the rate of 800 pounds per acre. These four, with many others, had received 3 tons per acre of ground limestone during the first week of the preceding November, while some had been left with no application. The reaction of the surface soil (Table III) on the Becker fields is very similar to that on the Coon Creek fields.

The addition of inoculating soil to the lime has been without effect upon either the appearance of the plants in their early stages of growth or the yields of hay (Table XVIII). In the first season the lime was distinctly beneficial, the control plots carrying a shorter and yellower growth and yielding less. During the winters of both 1924-25 and 1925-26 many plants on the unlimed plots died.

TABLE XVIII

EFFECT OF LIME AND INOCULATING SOIL ON YIELDS OF ALFALFA ON BECKER EXPERIMENTAL FIELD

Seed all treated with commercial culture and sown June 18, 1924

Plot	1925 First cutting	1925 Second cutting	1926 First cutting	1926 Second cutting	Total of 4 cuttings
	tons	tons	tons	tons	tons
A. No application of lime or inoculating soil					
A-7	0.63	0.57	0.44	0.45	2.09
C-7	.57	.51	.35	.39	1.82
F-7	.53	.47	.39	.39	1.78
H-7	.83	.79	.54	.58	2.74
Average	0.64	0.59	0.43	0.45	2.11
B. Application of ground limestone, 3 tons per acre in October, 1923					
A-2	0.72	0.65	0.37	0.51	2.25
A-4	.86	.71	.40	.51	2.48
D-2	.86	.68	.45	.57	2.56
D-4	.83	.61	.38	.44	2.26
Average	0.82	0.66	0.40	0.51	2.39
C. Application of ground limestone, 2 tons per acre in October, 1923, and 800 pounds per acre of inoculating soil on June 17, 1924					
A-3	0.81	0.65	0.36	0.44	2.26
A-5	.89	.96	.54	.63	3.02
D-3	.91	.62	.41	.42	2.36
D-5	.80	.48	.41	.45	2.14
Average	0.85	0.68	0.43	0.48	2.44

Experiments with Inoculated Soil in 1925

Following the encouraging results obtained on the Anderson field, experiments on the use of inoculating soil were started at Park Rapids, Backus, and Crow Wing, and a rather elaborate experiment was laid out at Coon Creek. The results from the first three support the conclusions of the earlier experiments at Coon Creek, while those from the last are not readily explainable.

Alfalfa at Park Rapids

The soil of the Park Rapids field is decidedly lime deficient toward alfalfa. The first season's seeding, 1924, using culture Q, included 24 plots of alfalfa with lime and the same number without. On all the unlimed plots in 1925 there was a large proportion of short yellow plants, while on all the others the plants were uniformly green. The relative yields in 1925 were as follows: controls, 100; with lime only, 180; with potash only, 170; with lime and potash, 300; with lime and phosphate, 170; with lime, phosphate, and potash, 300. In the winter of 1925-26 many plants died on the unlimed plots but few or none on the limed plots. The lime deficiency of the soil appeared to be established.

As the ground was freezing in November, 1924, wet, sticky marl, at a rate equivalent to about 2.5 tons of calcium carbonate per acre, was spread and disked in on the odd-numbered series from 19 to 26. On April 23, 1925, inoculating soil at the rate of 2 tons per acre was applied to all the most easterly (A) and the most westerly (H) plots on these 8 series. This soil was taken from a part of the field that had been marled in the fall of 1923 and sown to alfalfa the following May. The next day alfalfa was sown on A and B and sweet clover on H and D-E, culture X at the usual rate being used on both.

TABLE XIX

EFFECT OF INOCULATING SOIL AND MARL ON YIELDS OF ALFALFA AND SWEET CLOVER ON PARK RAPIDS EXPERIMENTAL FIELD

Seed all treated with commercial culture and sown on April 24, 1925

Application and rate per acre	Alfalfa				Sweet clover			
	Plot	First cutting	Second cutting	Two cuttings	Plot	First cutting	Second cutting	Two cuttings
		tons	tons	tons		tons	tons	tons
No marl or soil	20-B	0.10	0.33	0.43	20-E	0.13	0.08	0.21
do	22-B	.08	.05	.13	22-E	.13	.05	.18
do	24-B	.04	.04	.08	24-E	.09	.05	.14
do	26-B	.08	.19	.27	26-E	.14	.16	.30
Average		0.07	0.15	0.22		0.12	0.08	0.20
Soil, 2 tons	20-A	0.41	0.40	0.81	20-H	0.40	0.22	0.62
do	22-A	.32	.42	.74	22-H	.50	.32	.82
do	24-A	.13	.27	.40	24-H	.53	.26	.79
do	26-A	.20	.31	.51	26-H	.40	.31	.71
Average		0.26	0.35	0.61		0.46	0.28	0.74
Marl, 3 tons in 1925	19-B	0.18	0.26	0.44	19-E	0.29	0.20	0.49
do	21-B	.22	.42	.64	21-E	.40	.31	.71
do	23-B	.16	.15	.31	23-E	.22	.18	.40
do	25-B	.13	.14	.27	25-E	.18	.24	.42
Average		0.17	0.24	0.41		0.27	0.23	0.50
Marl, 3 tons and soil, 2 tons	19-A	0.38	0.44	0.82	19-H	0.47	0.42	0.89
do	21-A	.44	.49	.93	21-H	.76	.51	1.27
do	23-A	.31	.46	.77	23-H	.53	.43	.96
do	25-A	.17	.30	.47	25-H	.44	.35	.79
Average		0.32	0.42	0.75		0.55	0.43	0.98
None	Average of 4 plots	0.07	0.15	0.22		0.12	0.08	0.20
Marl	do	.17	.24	.41		.27	.23	.50
Soil	do	.26	.35	.61		.46	.28	.74
Marl and soil	do	.032	0.42	0.75		0.55	0.43	0.98

In 1926 the effect of both lime and soil was very evident on both legumes. The stand of alfalfa was good on every marled plot and almost as good on the unmarled plots that had been given soil, while on all plots that had received neither marl nor soil it was very thin. Owing to a prolonged drouth the yields were light, even on the best plots, but with each cutting they were heaviest on the plots that received both soil and marl, next on those that received soil alone, and lightest

on the untreated plots (Table XIX). At the close of the season of 1926, on the plots which had received no marl but had been given an application of soil there was a satisfactory stand of plants and they were dark green. On the marled plots there was a still thicker stand, there being far more plants than needed to give maximum yields, while on the untreated plots the stand was far too thin to justify leaving.

On the plots of sweet clover, the stands were thinner but the response to the various treatments was similar.

The beneficial effect of the soil on the marled plots at Park Rapids differs from our observations on the other fields. This may be attributed to the incomplete mixing of the marl with the soil, owing to the wet and frozen condition of the former, and to there being so little opportunity for interaction of marl and soil between the time of application and the time of seeding.

Alfalfa at Backus

An elaborate experiment started in 1922 has shown the lime deficiency of the Backus fields toward alfalfa to be about as distinct as that at Park Rapids. In 1925, on a field immediately adjacent to this experiment and with similar soil, an experiment was started with inoculating soil. The land had been in corn in 1923 and 1924 and for both crops had been liberally manured—in the second year with part of the winter's accumulation at a barn where alfalfa hay had been fed regularly and liberally. Early in June, 3 tons per acre of dry marl was applied and disked in on the west 33 feet of the tract, which is 850 feet from north to south and 56 feet from east to west. On July 10 the field was staked out into 32 plots, half on the marled portion and half on the unmarled. On each half four plots were left as controls and the others were treated with soil from a near-by plot limed in 1921 and sown to alfalfa in 1922, four rates—¼ ton, ½ ton, 1 ton, and 2 tons per acre—being used on the duplicate plots.

The soil was at once disked in and five days later the field was sown with alfalfa treated with culture X at the usual rate. A good stand resulted on all plots. During the rest of that season no beneficial effect of the inoculating soil was observed on the marled plots but on the others it was distinct and varied with the rate.

In 1926 the plots were carefully inspected by one of the authors on four dates—May 6, June 12, July 5, and August 30. The hay was cut twice—on July 13 and August 31. At the time of the first inspection the alfalfa on all the marled plots gave no evidence of winter killing, was very vigorous with 3 or 4 inches of new growth, and showed no effect of the inoculating soil. On the unmarled plots marked differences were observable. On those that had received no soil there was almost no growth and many of the plants had winter killed. On

those given 2 tons per acre of soil they were doing almost as well as on the adjacent marled plots and there was no winter killing. On the unlimed half, the 1-ton plots were inferior to the 2-ton, and the ¼-ton plots were superior to the control plots, with the ½-ton plots intermediate. The most marked difference in the series—0-, ¼-, ½-, 1- and 2-ton plots—was between 0 and ¼ ton. There was no doubt at that time as to the benefit of the soil application on the unmarled land but there was no evidence of any benefit on the marled.

The season was exceptionally dry, with resulting low yields on the best plots. From January 1 to March 31 the precipitation amounted to 2.57 inches; from April 1 to June 16 to only 0.9; with 2.95 inches between the last date and July 10, and 3.44 inches between that and the date of the second cutting, August 31.

The influence of a gravelly subsoil, which is very irregularly distributed over the field, became evident by June 12, at which time on the marled half the alfalfa was found to be dry and brittle on many entire plots and on parts of other plots. A similar condition existed on parts of a few of the unmarled plots. The thick stands on the former were at a great disadvantage, as is usual on such soils in periods of drouth. As a result, even the averages of the yields fail to indicate the effectiveness of the applications. The total for the two cuttings averaged only 1.6 tons per acre of hay on the marled portion, both on the plots that had received no soil and on those that were given 2 tons per acre, 1.3 tons on the unmarled plots given 2 tons per acre of soil, and 0.7 ton on those given neither soil nor marl.

By the time of the second cutting all evidence of uninoculated plants had disappeared except on the four untreated plots. On the soil-treated but unlimed plots the thickness of stand varied in general with the rate of application.

From the four detailed inspections it was evident that the inoculating soil had no distinct effect on the marled plots but was beneficial on the unmarled land, this benefit increasing with the rate of application. Even with the heaviest application, however, the alfalfa did not do nearly as well on the unmarled portion as it did on the marled portion that had received no soil.

Alfalfa at Crow Wing

The experiment started at Crow Wing included only 10 plots, three of which were left as controls, three given 3 tons per acre of inoculating soil from a near-by plot that had been treated with 3 tons per acre of limestone in 1921 and seeded to alfalfa the next summer, two given 3 tons per acre of dry marl, and the remaining two given both marl and inoculating soil. The seed, inoculated with culture X, was sown

on June 13. On all the plots a thick stand resulted and on all except the controls there was a vigorous growth of dark green plants, the color and growth being as good on the plots receiving only soil as on those receiving marl. On August 15 and 27, when careful examinations were made of every plot, the controls stood out sharply, with their smaller yellow plants.

During the following winter little or no killing occurred except on the controls and even on these enough plants survived to make a satisfactory stand. On May 9, by which time growth was well started, the stand on the controls was thinner and the growth smaller than on the marled plots; on all given either soil alone, or soil and marl, the stand and growth were alike and better than on those given only marl. On June 12, after a month of very dry weather, while the color was alike on all the plots and the relative stands the same as a month before, the plants on the controls were taller and showed fewer drying leaves. From that time on no distinct superiority was observed on the treated plots.

The analysis of the first cutting, July 10, gave no indication of any lack of inoculation, the nitrogen content being as high on the controls as on the other plots (Table XX).

TABLE XX

EFFECT OF MARL AND INOCULATING SOIL ON YIELDS AND NITROGEN CONTENT OF 1925 SEEDING OF ALFALFA AT CROW WING

Plot	Application and rate per acre	Yields per acre			Nitrogen content of first cutting
		First cutting	Second cutting	Two cuttings	
		tons	tons	tons	per cent
B-1	None	0.22	0.67	0.89	2.90
B-3	do	.30	.76	1.06	2.12
B-5	do	.31	.64	.95	2.80
	Average	0.28	0.69	0.97	2.94
A-1	Soil, 3 tons	0.33	0.72	1.05	2.66
A-3	do	.31	.88	1.19	2.83
A-5	do	.30	.68	.98	2.81
	Average	0.31	0.76	1.07	2.77
B-2	Marl, 3 tons	0.28	0.76	1.04	3.16
B-4	do	.29	.74	1.03	2.88
	Average	0.28	0.75	1.03	3.02
A-2	Marl, 3 tons and soil, 3 tons	0.29	0.67	0.96	2.70
A-4	do	.32	.70	1.02	2.94
	Average	0.30	0.68	0.98	2.87

After the second cutting, samples of the surface 6 inches of soil were taken from two controls and two soil treated plots and all were much alike in hydrogen-ion concentration, as pH, 5.6 to 5.8 (Table III).

West Field at Coon Creek

The most elaborate experiment with inoculating soil is that laid out on the West field (Fig. 1) at Coon Creek in 1925. Two cuttings of hay were removed from the plots in 1926. The results seem entirely out of harmony with all our other experiments and observations, and for this reason the experiment is given a fuller description.

A rectangle 800×156 feet was subdivided into seven series, G to M, each 20 feet wide. Six-foot alleys were left between H and I, and also between J and K, in order to prevent the dragging of limestone from the plots on I and J, which were to be given both limestone and inoculating soil, to those on H and K, which were to be left unlimed. On May 22 ground limestone at the rate of 3 tons per acre was applied to the whole of Series I, J, and M. Seven days later Series H, I, J, and K were subdivided, each into 40 plots 20×20 feet, giving 160 plots on the four series. On the same four series 96 of the plots were treated with inoculating soil taken from eight different sources, each a well established field or plot of alfalfa or sweet clover at Coon Creek or University Farm. Two of these sources had not been limed. Each soil was used at three rates—sandy soil from Coon Creek at ½, 1, and 2 tons; and silt loam from University Farm, ¼, ½, and 1 ton. With each rate of each of these eight inoculants there were four plots, two treated with ground limestone at 3 tons per acre and two unlimed. At the same time there was given to 12 plots, 6 on the limed series (I and J), and 6 on the unlimed series (H and K) a very heavy application of culture X mixed with surface soil taken from an unlimed plot at Coon Creek, on which neither alfalfa nor sweet clover had grown. The contents of three bottles of the culture, each enough for a bushel of seed, were well mixed with 250 pounds of the soil and one twelfth of this was scattered over each plot and at once worked in. This was about 100 times as much culture as, at the usual rate, would be mixed with the seed for this area.

On June 2 occurred a thunderstorm of tornadic proportions, blocking roads with uprooted trees, and wrecking barns. The wind was accompanied by rain, 1.8 inches falling in less than half an hour. In the slight depressions on the field the water collected as ponds, overflowed from these and swept over many of the plots, thus transferring both soluble and insoluble material from one plot to another. This storm was followed by a week of high winds which caused much drifting of sand on the field, notwithstanding the frequent use of disk and cultipacker to check it. On June 11 the whole field was sown to alfalfa, all the seed except that on Series G being treated with culture X at the usual rate. That for G was treated with neither culture nor soil, but was not sterilized.

A good stand resulted on all parts of the field. During the second week of October, in order to permit a careful study of the relation of the condition of the plants to the soil applications, the plots were staked out and paths cut around each. There was much variation from plot to plot and from one part to another on the unlimed plots but these differences were not associated with differences in the applications of soil. The series were crossed, almost at right angles and at irregular intervals, by strips 6 to 12 feet wide, on which the alfalfa showed a better color and growth, suggesting that these were where manure had been applied with a spreader.

During the winter of 1925-26 there was little killing on any of the plots. A detailed examination was made at the end of June, 1926, just before cutting the first crop of hay. At that time the alfalfa on all the plots was suffering from drouth, and the thicker the stand the more it showed the lack of moisture. In general, the stand on the limed series was thicker and the growth more uniform, altho shorter, than on the others. Wherever lime had been applied the plants were dark green, while elsewhere part of them were yellow and the others were a lighter green than on the limed plots. The soil applications showed no effect upon stand or growth on either the limed or the unlimed plots.

For the purpose of determining yields, G, L, and M were subdivided into 120 plots of the same size as those on the other series and the yield was determined on each of the 280 plots for both cuttings—July 1 and September 20.

The summary (Table XXI) indicates that the lime was beneficial but the soil applications and the culture were without effect upon the yield. At the close of the season there was a satisfactory stand of inoculated plants on all except 16 of the 280 plots, and these 16 formed five scattered groups on the unlimed series G, H, and K. No explanation of the partial failure of the stand on these is suggested.

The anomalous behavior of this field, including the successful stand on the unlimed series treated with neither lime nor soil and sown with untreated seed, is not to be attributed to a lesser acidity of the surface soil. In four samples collected from the unlimed plots after the winter of 1926-27 had set in, the hydrogen-ion concentration, as pH, was found to be 5.0, 5.1, 5.1, and 5.2. A block parallel with this rectangle and lying only 100 feet east of it had been laid out in 120 fortieth-acre plots in the summer of 1924 and the hydrogen-ion concentration of the surface soil of every plot was determined. In nearly all cases the value, expressed as pH, fell between 4.8 and 5.0.

TABLE XXI

EFFECT OF LIME AND INOCULATING SOIL ON YIELDS OF ALFALFA ON WEST FIELD AT COON CREEK IN 1926

Treatment of seed	Treatment of plot and rate per acre		No. of plots	Yield per acre		
	Limestone	Soil		First cutting	Second cutting	Two cuttings
	tons	tons		tons	tons	tons
None	None	None	40	0.48	0.63	1.11
Culture, at usual rate	do	do	66	.47	.61	1.08
do	do	0.25	4	.32	.48	0.80
do	do	0.50	16	.49	.58	1.07
do	do	1.00	16	.47	.51	1.02
do	do	2.00	12	.51	.60	1.11
do	3	None	66	.60	.73	1.33
do	3	0.25	4	.60	.69	1.29
do	3	0.50	16	.68	.71	1.39
do	3	1.00	16	,67	.70	1.37
do	3	2.00	12	.70	.72	1.42
None	None	*	6	.34	.46	0.80
do	3	*	6	0.58	0.67	1.25

* Soil treated very heavily with culture was applied in advance of seeding.

The explanation may lie in the manuring the land had received in the four seasons preceding the leasing of it by the University in 1924. For five or six years, at least, it had been in cultivated crops. Each year, in preparation for these, manure had been applied rather heavily, and probably not at all uniformly. It is only about 200 yards from the cow stables where all the alfalfa hay that had been produced on the Coon Creek experimental fields had been fed, beginning with the fall of 1920. Definite information regarding the manuring is not obtainable, as the man who had farmed the land died before the anomalous behavior of the field became evident.

The previous manuring was recognized as an objection at the time the experiment was started, but the block in question was the only available land conveniently located with respect to the other experimental fields at Coon Creek.

Inoculation Experiments in 1926

By the close of 1925 there was much evidence that soil upon which alfalfa or sweet clover had grown carried something that exerted a beneficial effect upon new seedings of alfalfa on such unlimed soils as these. In 1926 five additional experiments on the use of inoculating soil were started, one at Bemidji, one at Crow Wing, and three at Coon Creek—Erickson South field, Erickson West field, and Swanson field.

In order to secure more information during the first season as to the effect of the different treatments, plants were dug up from representative areas on more than 80 of the plots at the approach of winter, the early advent of which prevented the completion of the work. On each of these plots all the alfalfa plants from four square feet were

removed and separated at once into two groups—inoculated (carrying one or more nodules) and uninoculated—then thoroly washed, counted, oven-dried, and those of each group were weighed without separating the tops from the roots. Nitrogen determinations were made on many of these samples. On every plot sampled, a square, 12×12 inches, was marked out along the diagonals, 2 yards from each of the four corners. The four square blocks of soil, taken to the depth of the spade, were removed, placed on a screen, and the plants carefully separated from the soil, an operation which was greatly facilitated by the coarseness of the soil, its very moist condition, and the general absence of weeds. In some cases, probably, nodules became detached from the roots of inoculated plants. On a considerable number of large green plants no nodules were found, but it was very frequently observed, as these exceptional plants were being examined for nodules, that their roots had grown into distinct lumps of organic residues, which might account for their vigorous appearance.

Experiment at Bemidji

The soil on the Bemidji experimental field is regarded at most as only *very slightly* lime deficient toward alfalfa. An elaborate experiment, under way since 1922, has revealed no beneficial effect of liming except in the first summer, during which the plants on the limed plots made a somewhat better growth. There has been no significant difference in yield between controls and marled plots.

In the spring of 1926, a field that had never been in alfalfa or sweet clover or received much manure, was plowed and kept fallow until August in order to rid it of quack grass, with which it was badly infested. During the last week in July it was subdivided into 90 fortieth-acre plots and various applications were made of inoculated soil, marl, stable manure, and green manures. Alfalfa seed, treated with culture X at the usual rate, was sown immediately after a rain on August 4. A good stand was obtained on all the plots but the growth and color appeared slightly inferior on the controls. The hydrogen-ion concentrations of the surface 6 inches of the control plots, as pH, were between 5.6 and 5.9. During the second week of October the plants from four square feet on each of 42 plots were removed and examined as described above.

On all the plots there was a thick stand and the number of plants (30 to 50 per square foot) showed no relation to the treatment, but the proportion of plants with nodules was distinctly lower on the controls than on the plots treated with inoculating soil, marl, or the combination of these. On 10 of the 11 controls examined the percentage varied from 37 to 63, with an average of 48, but on the eleventh it was 80; with 10 tons per acre of stable manure, the percentages were 65, 71, and 72.

Freshly cut alfalfa, at the rate of 10 tons per acre, was plowed under on three plots and equal amounts of freshly cut sweet clover and oats on two sets of triplicate plots. One plot of each was sampled. On the plot with alfalfa, 68 per cent of the plants had nodules; on the one with sweet clover 45 per cent; and on that with oats 65 per cent. With one ton of marl the percentages were 64, 72, and 76, with an average of 71; with 2 tons of marl 70, 73, and 73, with an average of 72. With inoculating soil it was as follows:

	½ ton soil, per cent		1 ton soil per cent		2 tons soil per cent		4 tons soil per cent	
Soil from unlimed plot of sweet clover.....	76	83	73	80	70	70	88	92
Soil from limed plot of sweet clover.....	87	89	94	97	89	92	94	99

The proportion was high and much alike on all. At each rate it was higher with the soil from the limed plot. The use of marl in addition to inoculating soil from the limed plot gave 82 and 90 per cent, no more than the soil alone.

On 36 plots the inoculated plants averaged about half as heavy again as the uninoculated, while on the other six there was no distinct difference, the ratio for the 42 plots averaging 1.6. There was no distinct connection between the treatment of the plot and the average weight of either inoculated or uninoculated plants.

Nitrogen determinations were made of both inoculated and uninoculated plants from 19 of the plots. In one case there was practically no difference, but in all others the nitrogen in the inoculated was from 0.15 to 0.77 per cent the higher.

Experiment at Crow Wing

A field at Crow Wing which had been under cultivation about 40 years without manuring or the growing of any legume and had been seeded to rye in the fall of 1925, was plowed in June and the land kept fallow until laid out in 40 tenth-acre plots. At the end of the first week of August most of these were given inoculating soil at the rates of ½, 1, 2, or 4 tons per acre and at once sown to alfalfa treated with culture X. On 8 plots no inoculating soil was used. Four of the untreated plots were divided each into four squares and three of the squares on each were sown with seed treated with the usual amount of culture, while the fourth was sown with seed given culture at 30 times the usual rate.

An inspection on October 20 showed no distinct difference in the stand from plot to plot, but there was a better color and growth and a higher proportion of inoculated plants on the soil-treated plots. On the plots left without a soil application there was no marked difference in either appearance or nodulation of the plants, between the quarter that

was sown with very heavily treated seed and the remainder. The early arrival of winter prevented a detailed study like that made on the Bemidji field.

Use of Soil on Erickson South Field

The rectangular tract used for the experiment on the Erickson field, containing about two and a half acres, lies only 150 yards south of the South field (Fig. 1). It had been in corn in 1925, and is part of a well managed private farm, which had for some years received regular applications of stable manure. No alfalfa or sweet clover had ever been sown on the part used for the experiment nor had manure been applied to this after the feeding of alfalfa hay was begun.

The experiment was planned to furnish information along three lines: (1) the extent to which inoculating soil could take the place of limestone, (2) the advantage on limed land of using inoculating soil in addition to an artificial culture, (3) the effect on the alfalfa of delaying the application of marl or ground limestone until just before seeding.

The field was divided into six series, I to VI, running east and west. On April 30, 1926, ground limestone was spread on Series I at the rate of 2 tons per acre and on Series II at 1 ton. The whole tract was then thoroly cultivated and divided by north and south lines into five equal blocks, *a, b, c, d,* and *e,* which on successive dates, each following a rain, were to be subdivided, given certain applications, and seeded. On *a,* the block to be seeded first, applications of ground limestone and marl were made on May 3 to the portions on Series III, V, and VI. Then the block was further subdivided into four north and south rectangles (Nos. 1 to 4) and inoculating soil, ½ ton per acre, was applied to No. 1; 1 ton to No. 2, and 2 tons to No. 4, while none was applied to No. 3 (Fig. 10). The whole block was then thoroly cultivated and next day was sown to alfalfa inoculated with culture X.

The four later seedings—of June 12, July 12, 22, and 31—were carried out in the same way. In each case, after a rain had well moistened the soil, the applications of inoculating soil were made on Nos. 1, 2, and 4, and of ground limestone and marl on Series III, V, and VI. These were thoroly worked in and alfalfa inoculated with culture X was seeded at once. Good stands were secured in each of these seedings but that of June 12 suffered some injury from drouth. The hydrogen-ion concentrations on two control plots, as pH, were 5.2 and 5.4 (Table III).

About the middle of October the plots were carefully compared. In blocks *a, b,* and *c,* all plots on each that had received limestone or marl, appeared alike in stand, color, and growth. On *d* and *e,* seeded on July 22 and July 31, the plants on III-3, which was not limed until just before seeding, were yellower and less vigorous. On V-3, given

limestone at the same time but at twice the rate, the growth appeared as vigorous as on the plots limed in April.

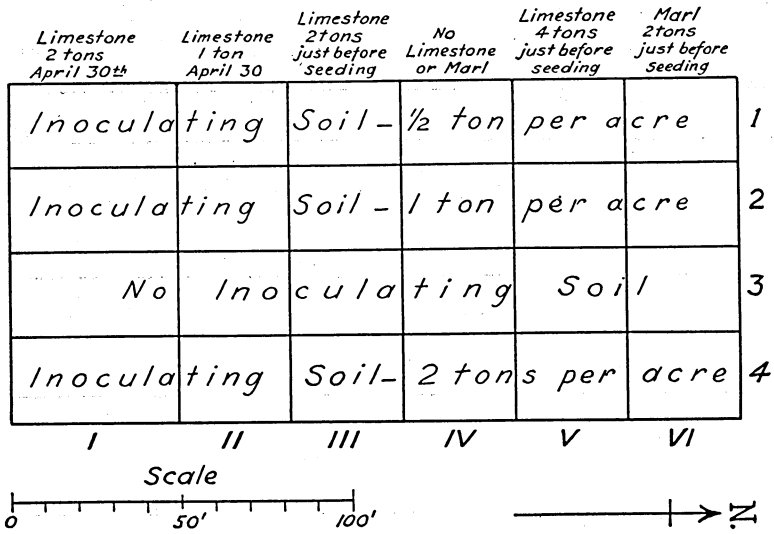

Fig. 10. A Representative Block on Erickson South Field
This shows time and rate of applications of limestone and marl and rate of application of inoculating soil, which was applied just before seeding.

Plants were gathered from the 16 plots of the last four seedings on the unlimed series, IV. On the part of the July 31 seeding that received no inoculating soil the five other plots were sampled. The number of plants on the unlimed plots (Table XXII) had not been increased by the application of soil, and on every plot, when winter set in, enough remained to constitute a full stand, provided they survive the winter and become inoculated early enough in 1927. The soil applications on every date greatly increased the proportion of plants with nodules, but the 2-ton application was not distinctly more effective than the ½-ton. On the untreated plots the percentage varied between 8 and 33, with an average of only 17; while on the soil-treated plots of corresponding dates it was in every case much higher, varying between 24 and 92 per cent, with an average for all of 56. On both untreated and treated plots the proportion was highest with the last seeding.

The average weight of the uninoculated plants, in each seeding, was much alike on all four plots. That of the inoculated plants was lowest on the untreated plot, and the later the seeding the more marked was the difference. This might be interpreted as indicating that most of the plants on the control plots on which nodules were found at the end of October, had not become inoculated as early as those on the treated plots. Increasing the rate of soil application did not increase the weight of either the inoculated or the uninoculated plants.

TABLE XXII

STAND, NODULATION, WEIGHT, AND NITROGEN CONTENT OF ALFALFA ON PLOTS OF THE UNLIMED SERIES, IV, ON THE ERICKSON SOUTH FIELD TO WHICH DIFFERENT AMOUNTS OF INOCULATING SOIL HAD BEEN APPLIED AT TIME OF SEEDING

Seed on all treated with culture at usual rate. All seedings in 1926. Plants collected during second half of October.

Plot	Rate of application per acre	June 12 seeding	July 12 seeding	July 22 seeding	July 31 seeding	Average
	tons					
		Number of plants from 4 square feet				
3	0.0	77	70	142	139	107
1	0.5	87	75	145	172	120
2	1.0	67	92	170	112	110
4	2.0	49	101	125	112	97
		Percentage of plants found inoculated				
3	0.0	8	14	12	33	17
1	0.5	51	27	62	77	54
2	1.0	44	24	59	92	55
4	2.0	53	35	76	73	59
		Average weight of inoculated plants, grams				
3	0.0	0.30	0.17	0.14	0.08	
1	0.5	.39	.49	.29	.22	
2	1.0	.43	.33	.22	.20	
4	2.0	0.40	0.30	0.22	0.19	
		Average weight of uninoculated plants, grams				
3	0.0	0.08	0.13	0.09	0.06	
1	0.5	.08	.20	.12	.07	
2	1.0	.12	.13	.08	.06	
4	2.0	0.08	0.09	0.07	0.09	
		Ratio of average weight of inoculated plants to that of uninoculated plants				
3	0.0	3.7	1.3	1.5	1.3	
1	0.5	4.9	2.4	2.4	3.1	
2	1.0	3.6	2.5	2.5	3.3	
4	2.0	2.7	3.3	3.1	2.1	
		Per cent nitrogen in inoculated plants				
3	0.0	3.58	2.59	2.75	3.05	
1	0.5	3.25	2.94	3.55	3.42	
2	1.0	3.41	2.95	2.98	3.69	
4	2.0	3.19	3.25	...	3.33	
		Per cent nitrogen in uninoculated plants				
3	0.0	2.20	2.45	2.23	2.42	
1	0.5	2.81	1.51	2.72	...	
2	1.0	2.64	1.83	2.47	2.35	
4	2.0	2.30	2.21	2.87	2.99	
		Excess of nitrogen in inoculated plants, per cent				
3	0.0	1.38	0.14	0.52	0.63	
1	0.5	0.44	1.43	.83	...	
2	1.0	0.77	1.12	0.51	1.34	
4	2.0	0.89	1.04	...	0.34	

The nitrogen content of the inoculated plants from each of the untreated plots was distinctly higher than that of the uninoculated plants from the same plot and not distinctly different from that of the inoculated plants from the soil-treated plots seeded on the same day.

TABLE XXIII

EFFECT ON ALFALFA OF TIME AND RATE OF LIMING AND OF RATE OF APPLICATION OF INOCULATING SOIL

Plots of July 31, 1926, seeding on Erickson South field. The seed had been treated with culture at the usual rate.

	No marl or limestone				No application of soil				
						Limestone			
	Soil 2 tons	Soil 1 ton	Soil 0.5 ton	No soil	Marl 2 tons July 30	2 tons July 30	4 tons July 30	1 ton April 30	2 tons April 30
Number of plants from 4 sq. ft.	112	112	172	139	118	107	117	131	115
Percentage of plants with nodules	73	92	77	33	95	78	77	70	94
Average weight of plants with nodules, grams	0.19	0.20	0.22	0.08	0.35	0.17	0.26	0.19	0.36
Average weight of plants without nodules, grams	0.09	0.06	0.07	0.06	0.13	0.12	0.09	0.10	0.10
Ratio of above average weights	2.10	3.30	3.10	1.30	2.70	1.40	2.90	1.90	3.60
Nitrogen in plants with nodules, per cent	3.33	3.69	3.42	3.05	3.33	3.63	3.72	3.84	3.60
Nitrogen in plants without nodules, per cent	2.99	2.35	...	2.42	2.79	2.99	2.82	3.41	2.62
Excess of nitrogen in plants with nodules, per cent	0.34	1.34	...	0.63	0.54	0.64	0.90	0.43	0.98

The data from the latest seeding (Table XXIII) indicate that the liming had not affected the thickness of stand but had more than doubled the proportion of inoculated plants and at least doubled the average weight of these. The increase in the average weight of the uninoculated plants may be due to the liming. In each plot the inoculated plants show a distinctly higher nitrogen content than the uninoculated. The soil, lime, and marl applications appear to have been about equally effective.

It seems safe to conclude that on this field the use of inoculating soil has greatly increased the proportion and growth of inoculated plants, has not affected the growth of the uninoculated, or the number of plants surviving at the end of the first season. Half a ton of soil per acre has been as effective as 2 tons and the effectiveness of this application has varied from seeding to seeding, altho all the soil was taken from the same plot of alfalfa.

Trial of Heavy Applications of Culture

Culture X, at 5, 10, and 30 times the usual rate, in comparison with the usual rate, was tried on unlimed plots on the West Erickson field and the Swanson field, both sown during the second week in August, 1926.

The West Erickson field is on the same farm as the South Erickson field and has a similar history, including the absence of alfalfa and sweet clover. It produced a crop of winter rye in 1926 and was plowed on August 10 and laid out in 60 plots, each 33×33 feet. Two days later marl was applied, at the rate of 3 tons per acre, to 14 plots; and inoculating soil, at 2 tons per acre, to 7 plots. The remaining 39 were given no inoculating soil or any form of lime. All were then well cultivated and the next day all were seeded to alfalfa as follows:

(1) 7 untreated plots with untreated but not sterilized seed.
(2) 7 " " " seed treated with culture at usual rate.
(3) 7 " " " " " " " " 5 times usual rate.
(4) 7 " " " " " " " " 10 " " "
(5) 7 " " " " " " " " 30 " " "
(6) 4 " " " " mixed with 4 times its weight of moist inoculating soil.
(7) 10 marled plots with seed treated with culture at usual rate.
(8) 4 " " " " mixed with 4 times its weight of moist inoculating soil.
(9) 7 plots, to which 3 tons per acre of inoculating soil had been applied, with untreated seed.

The Swanson field lies about half a mile east of the Anderson field (Fig. 1) and has a soil naturally similar. In 1926 it had been planted to corn, but on August 10, for the purpose of this experiment, the corn was pulled and removed from about an acre, the land was disked, and

24 plots, each 33×33 feet, were laid out. The next day marl was applied to 4 of these at the rate of 4 tons per acre, and inoculating soil to 3 at 2 tons per acre and to one at 4 tons per acre. The applications were at once well worked in and the following day all the plots were seeded to alfalfa as follows:

(1) 4 untreated plots with untreated but not sterilized seed.
(2) 3 " " " seed treated with culture at usual rate.
(3) 3 " " " " " " " " 5 times the usual rate.
(4) 3 " " " " " " " " 10 " " " "
(5) 3 " " " " " " " " 30 " " " "
(6) 4 marled plots with seed treated with culture at the usual rate.
(7) 4 plots, to which inoculating soil had been applied, with untreated seed.

On both fields a good stand was secured on all plots, but on account of continuous cool weather growth was slow and at the close of the season the plants were all very small; and on the Erickson field the differences between very differently treated plots were comparatively small. On the plots on the Swanson field that were treated with marl or soil the growth was much like that on the Erickson field, but on the other plots it was distinctly poorer. The hydrogen-ion concentrations, expressed as pH, on three control plots on the former, sampled in November, were 5.5, 5.4, and 5.3; and on four on the latter, 5.7, 5.5, 5.4, and 5.4 (Table III).

At the end of October samples were taken from more than half the plots on the two fields. The data are reported in Tables XXIV and XXV. On neither field was the number of plants distinctly increased by any of the treatments, but the proportion of those with nodules was much influenced, and in the same way and to much the same extent on both fields (Table XXVI). On the Erickson field the average weight per plant showed no distinct effect of the treatment on either the inoculated or the uninoculated plants, which were similar, but on the less productive Swanson field the uninoculated plants were only about half as heavy as the inoculated, which were alike with all the treatments.

TABLE XXIV

EFFECT OF TREATMENT OF SOIL AND SEED ON STAND, NODULATION, AND WEIGHT OF ALFALFA

Erickson West field at Coon Creek. Sown August 13, 1926; plants examined during latter half of October.

Plot	No. of plants			Nodulation	Weight of plants		Average weight per plant		
	Inoculated	Uninoculated	Total		Inoculated	Uninoculated	Inoculated	Uninoculated	Ratio inoculated to uninoculated
				per cent	grams	grams	grams	grams	
No application to plot; seed not treated.									
B-3	1	87	88	1	0.02	3.97	0.020	0.046	0.4
B-10	3	90	93	3	.12	3.80	.040	.042	1.0
C-15	10	107	117	9	.45	7.05	0.45	.066	0.7
D-7	7	54	61	11	.40	3.22	.057	.060	1.0
Average	5	84	89	6	0.25	4.51	0.041	0.053	0.8
No application to plot; seed treated with culture at usual rate.									
B-4	2	80	82	2	0.09	3.77	0.045	0.047	1.0
B-11	7	100	107	7	.58	5.68	0.83	.057	1.4
C-16	1	67	68	1	.10	4.59	.100	.068	1.5
Average	3	82	86	3	0.26	4.68	0.076	0.057	1.3
No application to plot; seed treated with same culture at 30 times the usual rate.									
B-14	4	59	63	6	0.18	4.25	0.045	0.072	0.6
C-12	7	48	55	13	...	2.95	...	.061	..
D-3	21	61	82	25	...	3.20	...	.052	..
Average	11	56	67	16	...	3.17	...	0.062	..
No application to plot; seed mixed with inoculating soil (1:4)									
C-7	33	86	119	28	2.43	4.30	0.074	0.050	1.5
B-9	21	73	94	22	1.34	4.74	.064	.065	1.0
D-5	40	60	100	40	2.10	3.09	.052	.051	1.0
B-16	16	70	86	19	1.23	4.40	.077	.063	1.2
Average	27	72	100	27	1.77	4.13	0.067	0.057	1.2

TABLE XXIV—*Continued*

Plot	No. of plants			Nodulation	Weight of plants		Average weight per plant		
	Inoculated	Uninoculated	Total		Inoculated	Uninoculated	Inoculated	Uninoculated	Ratio inoculated
				per cent	grams	grams	grams	grams	to uninoculated
Two tons per acre inoculating soil; seed not treated.									
B-15	65	15	80	81	4.85	1.38	0.075	0.092	0.7
C-13	55	21	76	72	5.19	1.24	.094	.059	1.6
D-4	82	15	97	85	6.66	0.74	.081	.050	1.6
D-12	109	36	145	75	8.34	1.99	.076	.055	1.2
Average	78	22	100	78	6.26	1.34	0.082	0.064	1.3
Three tons per acre marl; seed treated with culture at usual rate.									
D-19	80	5	85	94	5.53	0.26	0.069	0.052	1.3
B-2	112	6	118	95	9.30	.18	.083	.030	2.8
B-19	70	1	71	99	7.26	.09	.104	.090	1.1
Average	87	4	91	96	7.36	0.18	0.085	0.057	1.7
Three tons per acre marl; seed mixed with inoculating soil (1:4).									
D-14	104	41	145	72	6.62	2.46	0.064	0.060	1.1
D-6	116	17	133	87	10.91	0.82	.094	.048	1.9
C-2	75	3	78	96	6.55	0.18	.087	.060	1.5
Average	98	20	119	85	8.03	1.15	0.082	0.056	1.5

TABLE XXV

EFFECT OF TREATMENT OF SOIL AND SEED ON STAND, NODULATION, AND WEIGHT OF ALFALFA

Swanson Field at Coon Creek. Sown August 12, 1926; plants examined during latter half of October.

Plot	No. of plants			Nodulation	Weight of plants		Average weight per plant		
	Inoculated	Uninoculated	Total		Inoculated	Uninoculated	Inoculated	Uninoculated	Ratio inoculated to uninoculated
				per cent	grams	grams	grams	grams	
No application to plot; seed not treated.									
2-B	9	101	110	8	0.35	2.40	.039	.024	1.6
4-A	6	94	100	6	.39	4.23	.065	.045	1.4
4-C	4	61	65	6	.20	1.72	.050	.028	1.8
7-B	16	75	91	17	.65	1.87	.040	.025	1.6
Average	9	83	92	9	0.40	2.50	0.049	0.030	1.6
No application to plot; seed treated with culture at usual rate.									
2-C	5	75	80	6	0.29	3.11	0.058	0.041	1.4
5-A	6	100	106	6	.17	3.20	.028	.032	0.7
8-B	8	105	113	7	.23	2.92	.029	.028	1.0
Average	6	93	99	6	0.23	3.11	0.038	0.033	1.1
No application to plot; seed treated with same culture at 30 times the usual rate.									
3-C	3	80	83	4	0.14	2.45	0.046	0.030	1.5
7-A	21	70	91	23	0.84	1.50	.040	.020	2.0
9-B	17	60	77	22	1.01	1.60	.060	.027	2.2
Average	14	70	84	16	0.66	1.85	0.049	0.026	1.9
Two tons per acre of inoculating soil; seed not treated.									
2-A	110	17	127	87	5.35	0.53	0.048	0.031	1.5
4-B	54	20	74	73	3.35	.75	.062	.037	1.7
7-C	95	26	121	78	4.63	.80	.049	.030	1.6
Average	86	21	107	79	4.44	0.69	0.053	0.038	1.6
Four tons per acre of inoculating soil; seed not treated.									
9-C	120	12	132	91	7.95	0.38	0.066	0.031	2.1
Three tons per acre of marl; seed treated with culture at usual rate.									
6-A	66	7	73	90	3.70	0.51	0.056	0.073	0.8
8-A	52	20	72	72	2.64	.47	.051	.023	2.2
6-C	68	20	88	77	3.81	.50	.056	.025	2.2
Average	62	16	78	80	3.38	0.49	0.054	0.040	1.7

TABLE XXVI

EFFECT OF TREATMENT OF SOIL AND SEED ON PROPORTION OF ALFALFA PLANTS WITH NODULES

Application to soil and rate per acre	Treatment of seed	Percentage of plants with nodules		
		Erickson field	Swanson field	Average
None	None	1, 3, 9, 11	8, 6, 6, 17	8
None	Culture at usual rate	2, 7, 1	6, 6, 7	5
None	Culture at 30 times usual rate	6, 13, 25	3, 23, 22	15
None	Four times its weight of soil	19, 22, 28, 40	.....	27
Marl, 3 tons	do	72, 87, 96	.....	85
Marl, 3 to 4 tons	Culture at usual rate	94, 95, 99	90, 72, 77	88
Soil, 2 tons	None	72, 75, 81, 85	73, 78, 87	79

The nitrogen content was determined in the two groups of plants from each of the different treatments, there not being enough from some of the individual plots for a satisfactory determination (Table XXVII). With each treatment the nitrogen was higher in the inoculated plants but the difference was negligible on the plots given neither soil nor marl. When the nitrogen content of the plants from the different treatments is compared, taking the weighted average of both inoculated and uninoculated, it will be seen that it was markedly higher on the plots given marl or inoculating soil than on the others and also higher on the Erickson field than on the less productive Swanson field. On both fields the rank in nitrogen content of the plants from a treatment corresponds with the rank on the basis of the proportion of plants with nodules.

TABLE XXVII

NITROGEN CONTENT OF ALFALFA PLANTS, TOPS, AND ROOTS TOGETHER, FROM FIELDS AT COON CREEK SEEDED ON AUGUST 12 AND 13

Plants gathered in October after growth had ceased.

Treatment and rate per acre	Nitrogen content of: Nodulation	Nitrogen content of: Inoculated plants	Nitrogen content of: Uninoculated plants	Nitrogen content of: All plants	Excess of nitrogen in inoculated plants	Rank of plot in order of: Percentage of nitrogen in: Nodulation	Rank of plot in order of: Percentage of nitrogen in: Inoculated plants	Rank of plot in order of: Percentage of nitrogen in: Uninoculated plants	Rank of plot in order of: Percentage of nitrogen in: All plants	Rank of plot in order of: Difference in nitrogen between inoculated and uninoculated plants
	per cent	per cent	per cent	per cent	per cent					
				Erickson West field						
None	6	3.27	3.21	3.21	0.06	4	5	5	5	4
Culture at usual rate	3	3.28	3.25	3.25	.03	5	4	3	4	5
Culture at 30 times usual rate	16	3.48	3.24	3.29	.24	3	3	4	3	3
Soil, 2 tons per acre	78	3.89	3.30	3.78	.59	2	1	2	2	1
Marl, 3 tons per acre and culture at usual rate	96	3.88	3.48	3.87	0.40	1	2	1	1	2
				Swanson field						
None	9	3.01	2.64	2.69	0.37	4	3	4	4	3
Culture at usual rate	6	...	2.51	...	...	5	..	5	..	..
Culture at 30 times usual rate	16	2.76	2.73	2.74	.03	3	4	2	3	4
Soil, 2 tons per acre	79	3.41	2.73	3.32	0.68	2	2	2	1	2
Marl, 3 tons per acre and culture at usual rate	80	3.64	2.81	3.25	.83	1	1	1	2	1

DISCUSSION

Disturbing Effect of Drouth on Interpretation of Data

The disturbing effect of periods of very dry weather in such experiments on soils of low water-retaining capacity is very evident, hence the yields considered alone may often be insufficient for reliable deductions. When moisture has become such a limiting factor that the plants on plots with full stands begin to dry up before having made normal growth, the plots with poor stands may give heavier yields, either because more moisture was left in the subsoil at the time of the previous cutting, or because, on account of the much smaller number of plants, a smaller proportion of the rain falling was required for maintenance of life. In addition to the yields, a record of the appearance of the crop on the different plots is desirable, as was well illustrated in 1925 and 1926 by the plots on the Anderson field.

Value of Nitrogen Determinations

The determination of the nitrogen content of alfalfa plants is often worth while when there is doubt as to the inoculation, for instance when the investigator can not personally examine the roots, when it is suspected that the nodules may have become detached while the roots were being freed of soil, or when the absence of nodules from inoculated plants might be due to hot, dry weather (7, p. 16). Some possibilities of this analytical procedure were pointed out by one of the authors seventeen years ago (2, p. 4).

The analyses of the hay from the Crow Wing plots (Table XX), even in the absence of any observations on the plants, would leave little doubt as to the inoculation having been as complete on the three control plots as on those given both marl and a heavy application of soil. The lower percentages of nitrogen on the small plants from the 1926 seedings at Bemidji and Coon Creek that carried no nodules, make it evident that there had been no serious detachment of nodules during the process of removing and washing the roots (Tables XXII to XXV).

Distribution of Nodule Bacteria in Manure

It has been suggested above that the anomalous behavior of the alfalfa on the West field at Coon Creek might be due to the previous application of manure from stables where alfalfa hay had been fed freely. The use of similar manure at Backus was mentioned, it appearing to have had little effect there.

There seems to be no definite information as to the extent to which manure from alfalfa-fed animals may supply root-nodule bacteria. Fred, Whiting, and Hastings mention that while the "idea is common that if the hay from a well inoculated field is fed to stock and the

manure distributed on other fields the root nodule bacteria will be carried in this manure," experiments of one of them show that the bacteria in the hay that passes the digestive tract are destroyed (7, p. 37). They recognize, however, that some of the small particles of soil mixed with the hay will become mixed with the manure without passing through the animals and so cause a slight distribution of the desired bacteria. The more liberal is the feeding of the hay the more chance there is of the manure thus becoming well supplied with the bacteria.

In 1903 one of the authors examined a newly sown field of alfalfa on the crest of a ridge near Kearney, Nebraska, in which no uninoculated plants were found. The only explanation that appeared reasonable was that the bacteria had been distributed along with the manure taken from stables where the cattle were fed very liberally with alfalfa hay from large fields in the valley in which all the plants examined were found well inoculated (1, p. 14). A light application of the manure had been made in advance of seeding for this very purpose. The full inoculation later found in fields in other parts of Nebraska seemed best explained in the same manner and the matter was discussed briefly in 1910 (1, p. 19). The same explanation appears to apply on some Minnesota fine-textured soils that are not lime deficient.

In drawing conclusions from Nebraska observations to apply to sandy areas in Minnesota, it should be pointed out that the soils of the important Nebraska alfalfa districts are naturally well supplied with lime and that the alfalfa hay was usually fed so liberally that a considerable part of it did not pass through the animals. There are neither observations nor experiments contributing satisfactory evidence in support of such a practice as a means of inoculation on the lime-deficient sands of Minnesota.

Relative Effectiveness of Different Methods of Inoculation

The quality of the two commercial cultures used appears above criticism, no evidence of incomplete inoculation having been observed when the alfalfa or sweet clover was sown on land to which ground limestone, hydrated lime, or marl had been applied some time in advance. In the few cases where it was evident that inoculation was incomplete, the seed had been sown very soon after an application of ground limestone. On the sand experimental fields alone, during the eight seasons under consideration, more than 200 seedings of alfalfa, with the one or the other of these cultures as the only inoculant, were made on land that had never before been seeded to alfalfa or sweet clover, and to which there had been no application of manure from stables where either of these legumes was being fed. These seedings were either on limed plots only or on groups of plots the most of which had been limed.

The effectiveness of the previous growing of alfalfa or sweet clover on such lime-deficient soil is well shown in the case of two of the seedings on Series A at Coon Creek—the 1921 seeding after sweet clover and the 1924 seeding after plowing under alfalfa (Figs. 4 and 9). The experiments furnish no evidence as to whether sweet clover is more effective than alfalfa, as is quite commonly believed. Russell mentions "a recent indication that the growth of one leguminous crop may intensify the infection of a subsequent crop; the inoculation of lucerne is said to be rendered more secure by first growing *Melilotus alba* and the plowing it under before the lucerne is sown" (16, p. 78).

The plowing under of both sweet clover and alfalfa in fresh condition on the Bemidji field failed to increase the proportion of inoculated plants. Evidently the beneficial action of these crops is due to something developed in or imparted to the soil while they are growing, and it might be as great if both roots and tops were entirely removed before the second seeding. Such being the case the advantage of plowing would lie simply in the preparation of a better seedbed and the destruction of weeds.

Some American farmers have been practicing what is known as the "gradual method" of inoculation. On fields on which they wish to make sure of a stand of alfalfa, they seed sweet clover with the grain crops, or add a pound or two of alfalfa seed to the ordinary grass mixtures.

Inoculating soil was found far more effective than cultures on unlimed land and observations indicate that the application of soil from a well established alfalfa or sweet clover field is as effective on new seedings as the previous growth of one of these legumes. The amount of soil required for the maximum effect is uncertain, the experiment on the Backus field indicating that with the soil used there the benefit increased with the amount of soil applied, up to 2 tons per acre, while in the experiment on the Erickson South field at Coon Creek ½ ton appeared as effective as 2 tons. The results on the Erickson West field suggest that a small amount of the soil is much more effective when mixed with the seed than when worked into the seedbed, but that for full inoculation, four times as much soil as seed is insufficient on such lime-deficient soils.

The experiments furnish no evidence as to whether inoculating soil loses in effectiveness when applied some time in advance of seeding, or as to whether soil from a sweet clover field is more effective than that from an alfalfa field. Soil from unlimed land on which one of these legumes is well established might be expected to be preferable if the superiority of the soil were due to more virulent strains of bacteria, but the results of the Bemidji experiment rather favor the use of soil from limed land.

What little experimental evidence there is suggests that the difference in efficiency between the soil transfer and pure culture methods increases with the acidity of the soil. On none of the experimental fields under consideration does the unlimed soil appear too acid to permit of the establishment of successful stands of alfalfa, without liming, provided sufficient inoculating soil be used. It is not improbable that the transfer of soil without liming would prove inadequate on most sandy soils having a higher hydrogen-ion concentration than the most acid of those dealt with here.

In some of the Coon Creek fields there is, within a few feet of the surface, a substratum much less acid than the surface soil (Table II). This does not appear necessary in order that the use of inoculating soil may suffice for the production of satisfactory yields of alfalfa on fields in which the surface soil is lime deficient. The recovery of alfalfa on unlimed control plots on one of the Minnesota experimental fields with clay loam soil and subsoil has recently been described in detail and attributed to the roots getting down to the calcareous subsoil (3, p. 26).

If a beneficial effect of the soil, when used in addition to a pure culture, were found only when it was taken from limed land it might be suggested that the small lumps, granules, or grains of this limed soil, mixed with the surface few inches of a field just sown, would furnish just so many favorable *loci* for both the nodule bacteria that the soil particles might carry and those adhering to such of the treated seeds as came into contact with them. If the benefit of inoculating soil were thus due simply to its favorable reaction, a similar beneficial effect might be expected when the soil applied was taken from limed land upon which neither alfalfa nor sweet clover had been grown. Also, a different explanation would be required for the effect of the previous growing of alfalfa or sweet clover on unlimed land. It is of interest in this connection that in the Bemidji experiment, soil taken from unlimed land on which sweet clover was growing showed a markedly beneficial effect.

The superiority of the soil applications does not appear due to their supplying a greater number of bacteria per seed, because, if such were the case, the proportion of plants with nodules should have been greatly increased on the plots on the Swanson and West Erickson fields on which the culture was used at 30 times the usual rate. On those fields the proportion of plants on which nodules were found nine weeks after seeding was no higher where 2 tons per acre of inoculating soil had been applied than where a culture at only the usual rate was used on marled plots. On the latter it was many times as high as on the unlimed plots on which the culture had been used at 30 times the usual

rate. From this it appears that with a favorable soil reaction a culture used at the usual rate furnishes enough organisms fully to inoculate the plants.

Probable Cause of Superiority of Soil

Russell mentions the failure, hitherto, of attempts to "isolate and use more virulent strains (of alfalfa nodule bacteria) than those normally occurring in the soils" (16, p. 78). The recent work of Fred, Whiting, and Hastings (7, p. 20-21) appears to increase the probability of the greater potency of the soils being due to their furnishing more virulent strains of the organism, altho these authors do not mention any superiority of soils as inoculants. Using eight strains of nodule bacteria of the alfalfa group, four from each of two serological groups, A and B, they compared their behavior, in jars of quartz sand, in relation to the total amount of nitrogen assimilated, number of nodules, and position of the nodules on the roots. The plants treated with the strains of Group A were darker, taller, and contained much more nitrogen than the plants treated with the strains of Group B. With the former the nodules were large and located chiefly within the first few inches of sand, while with the latter they were much smaller, much more numerous, and located in all parts of the root system.

From the Rothamsted investigations referred to above, however, it seems not improbable that the superiority of the soil may be due to its presenting the organism to the alfalfa rootlet in a different form. Thornton emphasizes the importance of the exact method used in applying the bacteria to the seed. "During certain stages in their life cycle they are actively motile and, in this condition, are able to migrate through the soil. When inoculated seed is sown the bacteria have to pass through the soil to reach the various parts of the root system where the nodules are produced. The timely appearance of the motile forms in the soil is thus of vital importance. The nature of the fluid used to convey the bacteria to the soil influences both the time of appearance and the relative numbers of these motile stages. It produces a corresponding influence on the migration of the bacteria through the soil. For example, when a suspension of the bacteria in water was added to the soil it took three days before they began to spread away from the point of inoculation. When a suspension in milk was added, the initial rest-period was reduced to forty-eight hours, while from a suspension in milk and calcium phosphate the bacteria began to spread after twelve hours. The addition of calcium phosphate to the milk thus stimulates the bacteria to migrate through the soil and increases the volume of soil infected by them in a given time" (18, p. 7-8).

No reports of other experiments with this method have so far appeared, but it was used by English farmers last season, papers with the

proper amounts of calcium phosphate being sent out by the Rothamsted Experimental Station with the tubes of pure culture. This method has not been tried on the Minnesota fields, it having come to the attention of the authors only in October, 1926, and Thornton and Gangulee's paper was not available until after the ground was solidly frozen.

Bewley and Hutchinson (1920) found, in comparisons of pure cultures with soils as inoculants, that the latter were almost invariably the more effective and suggested that part of this superiority may be due to the form in which the organism is presented to the host plant, their investigations having shown "that soil is instrumental in the formation of the pre-swarmer form, and this might be expected to make for an earlier or more effective infection of the plant" (4, p. 160). Finding that a small amount of calcium phosphate was very effective in the production of motile forms, they suggested that "the effect of phosphate in converting pre-swarmers into swarmers might be expected to facilitate infection" (4, p. 159).

Studying the effect of various liquid media on the predominance of the different forms of the alfalfa nodule organism, Gangulee observed that soil extract induced the motile stage earlier than any of the other liquid media tested in the experiment (8, p. 363). Following up the work of Bewley and Hutchinson, and using pots filled with a mixture of soil and sand, Thornton and Gangulee found that the addition of as little as 0.1 per cent $CaH_4(PO_4)_2+H_2O$ to the milk used to bring the pure culture into suspension greatly affected the nodule formation for at least 16 weeks after seeding. "The addition of calcium phosphate produces no increase in nodules in the top four inches of the root, near the point of inoculation. There is consequently no effect from the phosphate until the sixth week, when the root system begins to develop to this depth. From this time onwards the phosphate produces an increase in nodule numbers, which, however, is entirely confined to the distal region of the root system. The effect of the phosphate consequently becomes more marked as the plant grows older and the lower roots develop" (19, p. 447-8). While they do not report similar experiments with soil or soil extract, it might be expected from the earlier observations of Gangulee that these would have a similar effect.

It does not appear that the addition of phosphate to the soil in advance of seeding alfalfa induces any such effect as its addition to the medium in which the bacteria are suspended. It is of interest to note that on none of the various seedings on Series A, B, C, and D on the South field at Coon Creek has the stand or growth of alfalfa on the phosphate-fertilized plots (Fig. 2) been superior to that on the controls. On the other sand experimental fields, phosphate fertilizers have been used only on limed plots.

It is an open question whether the superiority of the inoculating soil on these lime-deficient sandy fields is due to its carrying strains of bacteria more tolerant of the acid soils than are those of the pure cultures; or to its presenting the organisms in a more active form; or to the presence in it of a minute quantity of a very potent chemical compound which either has a profound and prolonged effect upon the bacteria or serves to counteract some toxic substance in the soil or to still some other cause.

The difficulty of securing inoculation of alfalfa or sweet clover on such unlimed soils, when pure cultures are used, leads to confusion in interpreting results of liming experiments, where these legumes are employed as the trial crops. The beneficial effect of the liming may be due much more to its influence on the early inoculation, and hence on the survival of a sufficient proportion of the plants, than upon their subsequent growth. Such confusion can be avoided by the use of a liberal application of inoculating soil on all the plots under comparison until more potent pure cultures or improved methods of using the present ones render this no longer necessary.

LITERATURE CITED

1. Alway, F. J. The importance of the inoculation of alfalfa on Nebraska upland soils. Ann. Rept. Neb. Agr. Exp. Sta., 1909:3-20. 1910.
2. ——— and Pinckney, R. M. The nitrogen content of inoculated and uninoculated alfalfa plants. Ann. Rept. Neb. Agr. Exp. Sta., 1909; 33-34. 1910.
3. ——— and Rost, C. O. Liming for alfalfa in southeastern Minnesota. Minn. Agr. Ext. Special Bull. 107. 32 pp. 1926.
4. Bewley, W. F., and Hutchinson, H. B. On the changes through which the nodule organism (*Ps. Radicicola*) passes under cultural conditions. Jour. Agr. Sci. 10:144-162. 1920.
5. Cox, H. A. How to inoculate legumes. N. J. Agr. Exp. Sta. Ext. Bull. 32, 4 pp. 1923.
6. Duggar, J. F. Soil inoculation for leguminous plants. Ala. Agr. Exp. Sta. Bull. 87, 30 p. 1897.
7. Fred, E. B., Whiting, A. L., and Hastings, E. G. Root nodule bacteria of *Leguminosae*. Wis. Agr. Exp. Sta. Research Bull. 72, 43 pp. 1926.
8. Gangulee, N. Studies on the lucerne nodule organism (*B. Radicicola*) under laboratory conditions. Ann. App. Biol. 13:360-373. 1926.
9. Kellerman, R. F. The present status of soil inoculation. Centralb. für Bakt. 34:42-46. 1912.
10. ——— and Robinson, T. R. Inoculation of legumes. U. S. Dept. Agr. Farm. Bull. 240, 7 pp. 1908.
11. ——— and ———. Conditions affecting legume inoculation. U. S. Dept. Agr. Bur. of Pl. Indust. Bull. 100:73 80. 1907.
12. ——— and ———. Progress in legume inoculation. U. S. Dept. Agr. Farm. Bull. 315, 20 pp. 1908.

13. Löhnis and Fred, E. B. Textbook of agricultural bacteriology. 283 pp. New York. 1923.
14. Oakley, R. A. How to grow alfalfa. U. S. Dept. Agr. Farm. Bull. 1283. 35 pp. 1922.
15. Russell, E. J. The spread of lucerne growing. The growing of lucerne, being the report of a conference held at Rothamsted on January 27, 1926. 32 pp. London. 1926.
16. ———. Plant nutrition and crop production. 115 pp. Berkeley, Calif. 1926.
17. Stewart, G. Alfalfa growing in the United States and Canada. 517 pp. New York. 1926.
18. Thornton, W. G. Lucerne inoculation trials. pp. 7-10. The growing of lucerne. 32 pp. London. 1926.
19. ——— and Gangulee, N. The life cycle of the nodule organism *Bacillus radicicola* (Beij.) in soil and its relation to the infection of the host plant. Proc. Roy. Soc., B.99:427-451. 1926.