centage if heated to 250°-260° C., which will give a coloration with Halphen's reagent not more intense than that obtained with lard from cottonseed meal-fed hogs. Under such conditions the value of the test for diagnostic purposes is somewhat questionable, especially in its application to the analysis of lard. It is claimed by lard manufacturers and cottonseed oil refiners that the oil is never heated to such a high temperature. This may be true, but it is nevertheless desirable that chemists should be on their guard, and not place too much reliance upon this comparatively new test. For normal, unheated oil, its value is unquestioned; but in view of the facts above discussed, its limitations should not be overlooked.

The analytical work in this paper was performed by Mr. R. W. Thatcher, assistant chemist of the Washington Experiment Station, to whom our thanks are due.

FIXATION OF ATMOSPHERIC NITROGEN BY ALFALFA ON ORDINARY PRAIRIE SOIL UNDER VARIOUS TREATMENTS.[1]

BY CYRIL G. HOPKINS.

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MANY different farmers have tried to grow alfalfa in various sections of Illinois and adjoining states, but, in most cases, it has been pronounced a failure. Where alfalfa has been grown with success in Illinois, it has usually been necessary to sow it on very rich ground or to keep it well manured.

In theory, alfalfa ought to grow and do well on these prairie soils, and it ought not to require heavy and frequent applications of manures, because alfalfa is a very deep rooting crop and is thus capable of drawing upon the soil to great depths for the necessary mineral elements of plant food, and, being a leguminous plant, it has the power of "gathering" nitrogen from the inexhaustible supply of the air, by means of bacteria which inhabit its roots.

Numerous observations made on several fields of alfalfa in different sections of the country during the past few years led the

[1] From advanced sheets of Bulletin No. 76 of the University of Illinois Agricultural Experiment Station.

writer to question whether alfalfa has the power to secure nitrogen from the atmosphere, when grown on these prairie soils. First, because, in nearly every field examined, the plants presented the same peculiar appearance which plants show when grown under artificial conditions *with an insufficient supply of nitrogen.* Second, because, in none of the fields where alfalfa presented this appearance could there be found any tubercles, or nodules, upon the roots of the alfalfa. Third, because liberal applications of barnyard manure produced a vigorous growth and a natural and healthy appearance.

In order to investigate the question, why Illinois soil does not more generally produce good crops of alfalfa, a series of experiments was begun about a year ago. These experiments comprehended the application of various elements of fertility to ordinary black prairie soil, both singly and in combinations, and both with and without the inoculation of the soil with the alfalfa bacteria; that is, with the bacteria which are known to live upon the alfalfa root in other sections of the country. These experiments have been carried on in the pot culture laboratory under controlled conditions and also on plots of ground under field conditions.

POT CULTURE EXPERIMENTS.

The pot culture experiments were planned with a twofold object; first, to test the effect of applying different elements of plant food to the soil to determine the value of such applications for the growing of alfalfa, and, second, to determine the effect upon the growth of alfalfa of inoculating the soil with the bacteria which are able to live upon the roots of alfalfa and gather nitrogen from the air for use of the growing plant.

Applications of Different Elements of Plant Food.

These pot cultures comprise a double set of our regular series of experiments adopted for investigating soils by pot cultures, which is as follows:

Twelve pots are all filled with the soil to be investigated and they are then treated with the following applications:

Pot No. 1—Check. (Nothing applied).

Pot No. 2—Lime.

Pot No. 3—Lime and nitrogen.

Pot No. 4—Lime and phosphorus.
Pot No. 5—Lime and potassium.
Pot No. 6—Lime, nitrogen and phosphorus.
Pot No. 7—Lime, nitrogen and potassium.
Pot No. 8—Lime, phosphorus and potassium.
Pot No. 9—Lime, nitrogen, phosphorus and potassium.
Pot No. 10—Nitrogen, phosphorus and potassium.
Pot No. 11—Check.
Pot No. 12—Check.

The first ten pots really make the complete series. Pots 11 and 12 are extras, or additional checks.

There is a double trial as to the value of an application of lime. First, by comparison between 1 and 2, which shows the effect of applying lime alone. Second, by comparison between 9 and 10, which shows the effect of applying lime after insuring a sufficient supply of each of the elements, nitrogen, phosphorus, and potassium.

Pots 3, 4, 5, 6, 7, 8 and 9 will show the effect of applying to the soil, nitrogen, phosphorus, and potassium, singly and in all possible combinations, and in all cases, after a sufficient quantity of lime has been added to neutralize acidity.

In the following tables and in the photographs, 0 (zero) means no fertilizer; L means lime; N means nitrogen; P means phosphorus; K means potassium (kalium).

Inoculation with Alfalfa Bacteria.

As stated above, two series of pot cultures were made with alfalfa, each of which received the applications of the different elements of plant food as described above. One of these two series of twelve pots each was inoculated[1] with the alfalfa bacteria; the other series was not. In all other respects the two series were treated exactly alike. All of the pots were filled with ordinary Illinois, black, prairie soil, and twenty-five alfalfa seeds were planted in each pot. They were kept in the glass house and were watered with very clear rain-water which was practically free from nitrogen, but which contained a trace of lime dissolved from a new cistern in which it was stored.

[1] The inoculating solution was made by shaking 500 grams of soil (obtained from an old alfalfa field in Kansas) with 1000 cc. of water, and allowing it to settle for a few minutes. One cc. of the liquid was used for each alfalfa seed planted.

The pots were all planted in June, 1901. The seeds germinated quite well and a fairly uniform stand was secured in all of the pots. The small plants grew slowly and the different pots showed no very marked differences for several months. A small crop was cut from all the pots in the fall, but they all seemed very much alike and no weights of the cuttings were taken. During the winter the pots[1] showed some marked differences in the growth of the alfalfa and the weights of the cuttings of each pot were taken on March 14, 1902, but they are not reported in this paper.

Another crop was cut from all of the pots on April 23rd and the weights of the alfalfa secured from each pot are given in Table I.

TABLE I.—ALFALFA POT CULTURES; CUT APRIL 23, 1902. WEIGHTS IN GRAMS PER POT AND POUNDS PER ACRE.

Serial No.	Pot No.	Treatment applied.	Green alfalfa. Grams per pot.	Air-dry hay. Grams per pot.	Air-dry hay. Pounds per acre.
1	25	o :	9	2	320
1	37	o : Bacteria	37	9	1440
2	26	L :	12	2	320
2	38	L : Bacteria	34	7	1120
3	27	LN :	38	9	1440
3	39	LN : Bacteria	44	10	1600
4	28	LP :	7	1	160
4	40	LP : Bacteria	44	10	1600
5	29	LK :	7	1	160
5	41	LK : Bacteria	32	7	1120
6	30	LNP :	66	16	2560
6	42	LNP : Bacteria	79	19	3040
7	31	LNK :	55	11	1760
7	43	LNK : Bacteria	59	13	2080
8	32	LPK :	7	1	160
8	44	LPK : Bacteria	67	17	2720
9	33	LNPK :	65	16	2560
9	45	LNPK : Bacteria	85	22	3520
10	34	NPK :	68	16½	2640
10	46	NPK : Bacteria	100	20	3200
11	35	o :	10	2	320
11	47	o : Bacteria	24	5	800
12	36	o :	10	2	320
12	48	o : Bacteria	34	7	1120

[1] The pots used were 10.5 inches in diameter, so that one gram of produce per pot corresponds to one pound per square rod or to 160 pounds per acre. While the exact yield per pot is given in grams in the tabular statement, the computed rate of yield in pounds per acre is also given and this rate of yield is used in the discussion in the text. This is relatively accurate and it is used because we are accustomed to the basis of pounds per acre. Another advantage is that the results from the pot cultures thus become more easily comparable with the actual field results which are given in the following pages.

Effect of Inoculation.

The seven uninoculated pots receiving no nitrogen made comparatively small growth[1] and showed uniformly throughout the period of nearly six weeks the characteristic pale yellowish green color indicative of an insufficient supply of nitrogen. The yields from these seven pots are very small, in no case exceeding the rate of 320 pounds per acre, while 1650 pounds per acre is the average yield of the corresponding inoculated pots. The yield from the inoculated pots ranges from two and a half to seventeen times the yield from the uninoculated pots. For example, the most favored pot receiving no nitrogen (serial No. 8), to which applications of lime, phosphorus, and potassium were made, yielded, when uninoculated, only 160 pounds of hay per acre, while 2,720 pounds per acre was the yield of the corresponding inoculated pot.

The crops produced on the seven uninoculated pots receiving no nitrogen would certainly be pronounced a failure, but the inoculated pots which produced yields from 0.75 to 1.75 tons of alfalfa hay per acre in less than six weeks from the previous cutting give evidence of being a very decided success, considering that this is the third cutting and corresponds to the third clipping in the field, which is frequently too light to pay for saving.

Effect of Nitrogen and Bacteria.

The applications of nitrogen produced a very marked increase in yields, but it is interesting to note that even these artificial supplies of nitrogen had evidently become somewhat depleted by the removal of the previous crops and were no longer sufficient for the greatest possible growth of the alfalfa; and, consequently, in every case where pots receiving nitrogen were also inoculated, a notable increase in growth and yield occurred. In the most favored pots (serial No. 9), to which lime, phosphorus, and potassium were supplied with the nitrogen, this increased yield produced by the bacteria amounted to nearly one-half ton per acre, the uninoculated pot yielding at the rate of 2,560 pounds per acre, while 3,520

[1] It may be observed that the uninoculated pots receiving phosphorus or potassium, or both, with lime, but without nitrogen (4, 5, 8) yielded even less than the uninoculated pots which received lime only or no treatment whatever (1, 2, 11, 12). I know of no explanation for this unless it be found in the fact that the yield of the previous crops from these fertilized pots was larger than from the unfertilized pots and the supply of available nitrogen had been correspondingly reduced.

pounds was the rate of yield of the corresponding inoculated pot.

Effect of Phosphorus.

Applications of phosphorus (or potassium) without bacteria or nitrogen (4, 5, 8) are of no value to the alfalfa.

Phosphorus applied to the inoculated pots or to the uninoculated pots receiving nitrogen produced a very marked increase in yield in every instance. These results confirm the indications observed in the previous crops and prove conclusively that, after provision has been made for a sufficient supply of nitrogen, applications of phosphorus to this soil were greatly to the advantage of the alfalfa crop. For instance where lime and nitrogen alone were applied (3) 1,440 pounds of hay per acre were produced, while 2,560 pounds was the rate of yield where phosphorus was added (6). The treatment: lime, nitrogen, bacteria (3), produced a yield of 1,600 pounds, which was increased to 3,040 pounds by the addition of phosphorus (6). Under the most favorable conditions without phosphorus; that is, with lime, nitrogen, and potassium (7), the yields in pounds per acre were 1,760 and 2,080 without and with bacteria, respectively, and these yields were increased to 2,560 and 3,520 respectively, by the addition of phosphorus (9). The inoculated pot receiving lime and potassium (5) yielded at the rate of 1,120 pounds per acre, but, where phosphorus was added to this combination (8), the yield became 2,720 pounds.

Effect of Potassium.

Applications of the element potassium produced a slight increase in yield when added after sufficient nitrogen was provided (3, 7), but the increase becomes more marked when the potassium is added after both nitrogen and phosphorus have been supplied. For example, with both lime and phosphorus added, and nitrogen accumulated by the alfalfa bacteria, the yield was 1,600 pounds without potassium (4) and 2,720 pounds with potassium (8); and, when an inoculated pot was also given an application of nitrogen (6), it yielded at the rate of 3,040 pounds without potassium and 3,520 pounds with potassium.

It should be remembered that no conclusions can be drawn as to the relative value of applying lime to the soil used because of the fact that the water used in all of the pots contained a trace of lime.

There is evidence that both phosphorus and potassium have an indirect value aside from their direct value as plant food for the alfalfa. This is the value of these elements to the bacteria. Bacteria themselves are living plants, and while they are microscopic in size they are almost infinite in number, and their multiplication and development are largely dependent upon the supply of available mineral elements of plant food. It will be observed that a yield of 2,080 pounds per acre (7) was secured without the addition of phosphorus. The yield became 3,520 pounds when phosphorus was applied (9). This increase of 1,440 pounds per acre may be due in part to the direct value of the phosphorus to the alfalfa and in part to its value in promoting the development of the bacteria and thus increasing the supply of nitrogen which the bacteria secure from the air and furnish to the growing alfalfa. Again, the addition of phosphorus to the combination, lime, potassium, bacteria, increased the yield from 1,120 (5) to 2,720 (8), an increase of 1,600 pounds per acre. The fact that the soil itself contained sufficient phosphorus to produce a yield of 2,080 pounds (7) tends to prove that the first 960 pounds of this 1,600 pounds' increase was due to the increased development of the bacteria resulting from the additional supply of available phosphorus; the remainder of the increase, 640 pounds, is probably due to both the direct and the indirect value of the phosphorus.

Without addition of potassium the maximum yield was 3,040 pounds (6); consequently the increase in yield from 1,600 pounds (4) to 2,720 pounds (8), resulting from the addition of potassium to the combination, lime, phosphorus, bacteria, was probably largely due to the increased development of the bacteria in the presence of a larger supply of available potassium.

The field experiments, which are described further on, give abundant evidence of the value of applications of lime in promoting the development of the alfalfa bacteria.

The photographic reproduction of the six pots receiving no artificial fertilizer, three of which were inoculated and three uninoculated, as they appeared on May 11th, less than three weeks after cutting off the crops discussed above, may be of interest (see Fig. 1).

Cuttings made from these six pots on May 21st just four weeks after the previous cuttings, gave the results shown in Table II.

TABLE II.—ALFALFA POT CULTURES ; CUT MAY 21, 1902. WEIGHTS IN GRAMS PER POT AND POUNDS PER ACRE.

Serial No.	Pot No.	Treatment applied.	Green alfalfa. Grams per pot.	Air-dry hay. Grams per pot.	Air-dry hay. Pounds per acre.
1	25	0 :	7	1	160
1	37	0 : Bacteria	42	12	1920
11	35	0 :	8	2	320
11	47	0 : Bacteria	33	8	1280
12	36	0 :	6	2	320
12	48	0 : Bacteria	33	10	1600

It will be observed that the highest yield of the three uninoculated pots was 320 pounds per acre, while the average yield of the three inoculated pots was 1,600 pounds,—five times as great. These results only serve to confirm those secured from the preceding crops, and to show the value of the inoculation in a most conclusive manner.

When we remember that the twenty-four pots used in this series of experiments were all filled with the same kind of well-mixed soil, and that this soil is fairly representative of thousands of square miles of black prairie land in Illinois and adjoining states, that both series, of twelve pots each, were kept on the same table in the greenhouse, watered at the same times with exactly the same kind of water, and in every way treated exactly alike, except that one series was inoculated with alfalfa bacteria while the other series was not inoculated, then these most positive and conclusive results, as shown by the records of the experiment, including the color of the foliage, the height of the growing plants, the photographic reproductions, and the absolute yields per pot and rate of yield per acre, seem truly remarkable and appear to be of tremendous importance in solving the question, Why is alfalfa so commonly an unsuccessful crop on ordinary prairie soils?

FIELD EXPERIMENTS WITH ALFALFA.

An acre of ordinary slightly rolling black prairie land, capable of yielding 70 bushels of corn to the acre, was seeded with alfalfa in June, 1901. The soil was considerably better than ordinary cultivated soil (such as was used in the pot culture experiments).

Previous to 1895, it had been in pasture for at least eighteen years, and since 1897 it had been in meadow; thus, only three grain crops (corn in 1895, 1896, and 1897) were grown on this soil during the past twenty-five years. The field, which was 8 rods wide east and west, and 20 rods long north and south, exclusive of some border and division strips, was divided into two parts by a line running north and south, and into five parts by lines running east and west. The west part of the acre was inoculated with soil taken from an old alfalfa field in Kansas. The five divisions from north to south were fertilized as follows:

Plot No. 1—Check (nothing applied).

Plot No. 2—Lime.

Plot No. 3—Lime and phosphorus.

Plot No. 4—Lime and potassium.

Plot No. 5—Lime, phosphorus, and potassium.

The rates applied per acre were: 320 pounds of air-slaked lime, 320 pounds of bone-meal (containing 30 per cent. phosphoric oxide), and 160 pounds of crude potassium sulphate.

The infected alfalfa soil was applied at different rates of seeding on narrow strips running north and south on the west half of the field, the lightest application (320 pounds) being on the west side, and, on successive strips eastward, the rates of application were 640, 960, 1,280, 1,600, and 1,920 pounds, respectively. Each of these strips was about one-half rod wide. A good stand of young plants was secured, but a very heavy rain storm, which occurred on July 2nd, washed the soil somewhat, and, as the west side of the field was somewhat higher than the east side, it was feared that the bacteria might be carried over the east plots and thus inoculate the whole field to some extent, which afterward proved to be the case, particularly along the east side where the water stood for a short time. The southeast quarter of the field was the lowest part, and, although it was tile-drained, the water stood on it long enough to kill most of the alfalfa plants. Because of these occurrences, the results of the experiment are probably not so marked as they would otherwise have been.

During the summer of 1901, the alfalfa was clipped three times, the clippings being left lying on the field. During midsummer, the weeds seemed to grow faster than the alfalfa, but with each clipping the alfalfa improved, and in the fall the stand was good where it had not been injured by the water standing on it.

No marked differences were noted among the different plots, excepting that the alfalfa made a much more vigorous growth wherever phosphorus had been applied, the line being very noticeable where the application of phosphorus began.

In the fall, tubercles were found in abundance upon the plants growing in the strips of land where the heaviest applications of infected soil were made, but none were found on plants in the uninoculated soil.

In the spring of 1902 the alfalfa began to grow vigorously and was entirely free from weeds, but within a short time some very marked differences appeared among the different plots. The effect of the inoculation became very apparent, all of the inoculated soil producing a much more vigorous growth than occurred on uninoculated soil, and the more vigorous growth was accompanied by a dark green, healthy-looking color in the growing alfalfa, while the plants on uninoculated soil took on a pale green color indicative of an insufficient supply of nitrogen. This difference in growth and color between inoculated and uninoculated plants was very marked even where no fertilizer was applied, but it was more marked where lime was applied and still more marked where both lime and phosphorus were applied.

Fig. 2 shows the effect of inoculating soil to which both lime and phosphorus had been applied, the inoculated soil producing a markedly increased growth of alfalfa over the uninoculated soil. The label stakes are *two feet* high and serve as a measure of the height of the alfalfa. The difference in thickness of stand can be seen but the difference in color between the inoculated and uninoculated alfalfa is not brought out in the figure. To the southeast may be seen the patch of ground where the alfalfa was destroyed by water, on account of which no results were secured from plots 4 and 5.

Table III gives the yield per acre of air-dry hay from one-thousandth acre plots, the inoculated plots being measured off on the strip where the heaviest application of infected soil was made.

It will be seen that the inoculated plots yielded about twice as much hay as the uninoculated plots.

TABLE III.—ALFALFA FIELD EXPERIMENTS; FIRST CUTTING 1902, MAY 28.
WEIGHT IN POUNDS PER PLOT AND PER ACRE.

Plot No.	Treatment applied.	Green alfalfa. Pounds per plot.	Air-dry hay Pounds per plot.	Air-dry hay. Pounds per acre.
1	O :	$5\frac{1}{2}$	$1\frac{5}{16}$	1313
1	O : Bacteria	11	$2\frac{9}{16}$	2563
2	L :	6	$1\frac{7}{16}$	1438
2	L : Bacteria	12	$2\frac{7}{8}$	2875
3	LP :	$7\frac{1}{4}$	$1\frac{15}{16}$	1938
3	LP : Bacteria	15	$3\frac{5}{8}$	3625

QUANTITATIVE DETERMINATIONS OF THE FIXATION OF ATMOSPHERIC NITROGEN BY ALFALFA WHEN GROWN ON ORDINARY SOIL.

The investigations of Atwater in America, Boussingault and Ville in France, Hellriegel, Willfarth, and Nobbe in Germany, Lawes and Gilbert in England, et al., have fully established the scientific facts: (1) that leguminous plants, as the clovers, peas, beans, vetches, alfalfa, etc., have the power to gather, or accumulate, free nitrogen from the atmosphere; (2) that this fixation of free nitrogen is actually accomplished by microscopic organisms called bacteria which live in little nodules, or tubercles, upon the roots of the legumes; and (3) that, for different species of leguminous plants, there are also different species of "nitrogen-gathering" bacteria. Many investigations have also been conducted to determine the amounts of nitrogen which can be fixed by different leguminous plants, but these experiments have actually been carried on in pure sand cultures under conditions which necessitate that all nitrogen which the legume secures *must* be obtained from the air.

There is abundance of evidence that leguminous plants secure some nitrogen from the air when grown in ordinary soil, if they are provided with the bacteria. Indeed, the presence of the tubercles upon the roots is one of the evidences that free nitrogen is being fixed, and another evidence of that fact is found in the beneficial effects of clover and other legumes in crop rotations. It is a simple matter to determine how much nitrogen is contained in a ton of clover; but, after the amount is determined, it still remains a question as to how much of the nitrogen was taken directly from the soil and how much was secured from the air, and a question very frequently asked is, How much of their nitrogen do leguminous crops obtain from the air and how much do they actually take from the soil?

The pot culture and field experiments described in the preceding pages were conducted on ordinary soil and in such a manner that it can be determined with a high degree of accuracy how much nitrogen was secured from the air by the alfalfa. This is due to the fact that, in all cases, alfalfa was grown not only with bacteria present but also in exactly similar duplicate pots or plots with bacteria absent and the difference between the amounts of nitrogen contained in the crop from the inoculated soil, on the one hand, and in the crop from the uninoculated soil, on the other hand, represents the amount of nitrogen which was secured from the atmosphere by the bacteria. Probably in no case will this amount be larger than the actual truth[1]; but, if the soil which was not intentionally inoculated had nevertheless to some extent become infected with alfalfa bacteria by cross inoculation, then the amount of nitrogen actually secured from the air would be even larger than represented by these determinations.

Fixation of Nitrogen by Pot Cultures.

Table IV gives in pounds per acre the amounts of dry matter and of nitrogen in the crops cut from the pot cultures on April 23, 1902; also the percentage of nitrogen in the dry matter and the

TABLE IV.—FIXATION OF NITROGEN BY ALFALFA IN POT CULTURES; CROPS CUT APRIL 23, 1902.

Serial No	Pot No.	Treatment applied.	Dry matter in crop. Pounds per acre.	Nitrogen in dry matter. Per cent.	Nitrogen in crop. Pounds per acre.	Nitrogen fixed. by bacteria. Pounds per acre.
1	25	0 :	280	2.61	7.31	....
1	37	0 : Bacteria	1300	4.09	53.17	45.86
2	26	L :	280	3.47	9.72	...
2	38	L : Bacteria	1010	4.24	42.82	33.10
3	27	LN :	1280	4.48	57.31	....
3	39	LN : Bacteria	1450	4.48	64.96	7.65
4	28	LP :	140	2.78	3.89	....
4	40	LP : Bacteria	1440	4.08	58.76	54.87
5	29	LK :	140	3.59	5.03	....
5	41	LK : Bacteria	1010	4.20	42.42	37.39

[1] It is possible, of course, that the inoculated plants might secure somewhat larger supplies of nitrogen from the soil because of their more fully developed root system; but it should still be borne in mind that any such increased root development is due to the inoculation.

Serial No.	Pot No.	Treatment applied.	Dry matter in crop. Pounds per acre.	Nitrogen in dry matter. Per cent.	Nitrogen in crop. Pounds per acre.	Nitrogen fixed by bacteria. Pounds per acre.
6	30	LNP :	2280	4.53	103.19	....
6	42	LNP : Bacteria	2780	4.06	112.87	9.32
7	31	LNK :	1570	4.70	73.79	...
7	43	LNK : Bacteria	1890	4.38	82.78	8.99
8	32	LPK :	140	3.15	4.41	....
8	44	LPK : Bacteria	2480	3.82	94.74	90.33
9	33	LNPK :	2300	4.09	94.07	....
9	45	LNPK : Bacteria	3230	4.00	129.20	25.13
10	34	NPK :	2370	4.14	98.12	....
10	46	NPK : Bacteria	2940	4.36	128.18	30.06
11	35	o :	280	2.63	7.36	....
11	47	o : Bacteria	730	4.34	31.68	24.32
12	36	o :	280	2.64	7.39	....
12	48	o : Bacteria	1020	4.18	42.65	35.26

amounts of nitrogen per acre obtained from the atmosphere by the alfalfa bacteria in the inoculated pots.

Where neither nitrogen nor bacteria were added to the soil, all of the nitrogen removed in the crops[1] must have been derived from the original soil. In the three series of pots receiving no fertilizers (1, 11, and 12) the crops from the uninoculated pots contained 7.31, 7.36, and 7.39 pounds of nitrogen per acre, respectively, while 53.17, 31.68, and 42.65 pounds of nitrogen in the crop are the respective rates per acre of the corresponding inoculated pots. The average of these is 42.50 pounds for the three inoculated pots and 7.35 pounds for the three uninoculated pots, making an average difference of 35.15 pounds in favor of inoculation. In other words, as an average of three separate determinations, with no application of plant food, the bacteria "gathered" and "fixed" and furnished to the growing alfalfa more than 35 pounds of nitrogen per acre. At the present average price for nitrogen in commercial fertilizers (15 cents a pound) these 35 pounds of nitrogen are worth $5.25.

The addition of potassium without phosphorus (5) gave no in-

[1] The amounts of nitrogen given may possibly be too high for some of the uninoculated pots, because of some cross inoculation. The inoculated and uninoculated series of pots stood side by side on the same table, and either spattering of water or more likely the carrying of infected soil by ants (which were frequently found in the pots) or other insects, finally transferred some bacteria to the uninoculated series, which fact became evident, later in the season of 1902, by the development of tubercles, and the markedly increased growth of an occasional plant in the uninoculated pots.

crease in the amount of nitrogen fixed. With phosphorus added to the soil (4), the nitrogen in the crop was increased by the presence of the bacteria from 3.89 pounds to 58.76 pounds per acre, 54.87 pounds of nitrogen per acre having been fixed by the bacteria.

Under the most favorable conditions, when both phosphorus and potassium were applied (8), still more marked results were obtained, the nitrogen in the crop having been increased from 4.41 to 94.74 pounds per acre by the bacteria; that is, the bacteria gathered nitrogen from the air at the rate of more than 90 pounds per acre, which was utilized by the growing alfalfa. At market prices, the nitrogen gathered is worth $13.50 per acre.

It is of interest to observe that even in the pots to which applications of nitrogen had been made (3, 6, 7, 9, 10) some nitrogen was fixed when the soil was inoculated, ranging from 7.65 pounds per acre (3), with no addition of phosphorus or potassium, to 25.13 and 30.06 (9 and 10) pounds per acre under the most favorable conditions, with applications of all mineral elements.

Usually small immature plants contain much higher percentages of nitrogen than do more fully developed plants, but it will be seen from Table IV that, without exception, the heavy crops of alfalfa contained much higher percentages of nitrogen than the lighter crops. Triplicate determinations of nitrogen in the crops from unfertilized pots (1, 11 and 12) showed 2.61 2.63 and 2.64 per cent. of nitrogen in the dry matter from uninoculated pots yielding only 280 pounds of dry matter per acre, and 4.09, 4.34, and 4.18 per cent. of nitrogen in the dry matter from the inoculated pots with an average yield of more than 1,000 pounds per acre. A similar effect was produced in all cases, whether nitrogen was added as a nitrogenous fertilizer or gathered by the bacteria,—all of which tends to prove that, with insufficient nitrogen, the plants make as much growth as possible until the fixation of carbon is practically stopped by the lack of nitrogen, as indicated by the pale yellowish green color of the foliage.

Table V shows the data relating to the fixation of nitrogen from the next cutting (May 21st) of the six unfertilized pots (1, 11, 12), three of which were inoculated.

TABLE V.—FIXATION OF NITROGEN BY ALFALFA IN POT CULTURES; CROPS CUT MAY 21, 1902.

Serial No.	Pot No.	Treatment applied.	Dry matter in crop. Pounds per acre.	Nitrogen in dry matter. Per cent.	Nitrogen in crop. Pounds per acre.	Nitrogen fixed by bacteria. Pounds per acre.
1	25	0 :	140	2.69	3.77	...
1	37	0 : Bacteria	1720	3.52	60.60	56.83
11	35	0 :	280	2.47	6.91	....
11	47	0 : Bacteria	1170	3.69	43.18	36.27
12	36	0 :	280	2.61	7.31	....
12	48	0 : Bacteria	1460	3.66	53.48	46.17

These results only confirm those of the previous cutting and show that on ordinary unfertilized Illinois soil the alfalfa bacteria were capable of fixing 46.42 pounds of nitrogen per acre, as the average of the three separate determinations.

The average percentage of nitrogen in the dry matter of the crops from the three uninoculated pots was 2.59, while 3.62 is the average percentage from the three inoculated pots.

Fixation of Nitrogen in Field Experiments.

Table VI gives the same data for the field experiments as are given in Tables IV and V for the pot cultures. These determinations were made on the crops cut from the exact thousandth-acre plots on May 28, 1902.

TABLE VI.—FIXATION OF NITROGEN BY ALFALFA IN FIELD EXPERIMENTS; CROPS CUT MAY 28, 1902.

Plot No.	Treatment applied.	Dry matter in crop. Pounds per acre.	Nitrogen in dry matter. Per cent.	Nitrogen in crop. Pounds per acre.	Nitrogen fixed by bacteria. Pounds per acre.
1	0 :	1180	1.85	21.81	....
1	0 : Bacteria	2300	2.70	62.04	40.23
2	L :	1300	2.02	26.20	....
2	L : Bacteria	2570	2.65	68.02	41.82
3	LP :	1740	2.03	35.40	....
3	LP : Bacteria	3290	2.71	89.05	53.65

These results secured under field conditions on good black prairie soil are in perfect agreement with the results from the pot culture experiments, the amount of atmospheric nitrogen fixed by the alfalfa bacteria being 40.23 pounds per acre on the unfertilized plot, 41.82 pounds on the limed plot, and 53.65 pounds per acre on the plot receiving both lime and phosphorus. Almost two-thirds of the total nitrogen contained in the crop from the inoculated unfertilized plot (1) was secured from the atmosphere by the alfalfa bacteria. It should be borne in mind that nitrogen is required for root growth as well as for growth above ground and also that these amounts were obtained from a single crop of alfalfa, and that three or four crops will be cut during the season. From the data already given, it will be seen that on the unfertilized soil four such crops as that cut on May 28th from the inoculated plot would mean at least 160 pounds of atmospheric nitrogen fixed by an acre of alfalfa during a single year, and this would require a total yield of only about five tons of alfalfa hay for the season, which is by no means a maximum yield for alfalfa on Illinois soil under the most favorable conditions, as has been shown in the preceding pages.

The percentage of nitrogen is much higher in the crops from the inoculated plots, the average being 1.97 per cent. in the dry matter for the uninoculated plots and 2.69 for the inoculated plots. This means, of course, that the hay produced on the inoculated plots is not only more in quantity, but it is also much better in quality, the percentage of protein averaging only 12.29 in the dry matter of the uninoculated crops, while 16.84 is the average percentage for the inoculated plots.

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THE IDENTIFICATION AND COMPOSITION OF MALT LIQUORS.[1]

BY CHARLES LATHROP PARSONS.

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THREE of our northeastern states have laws which, if enforced, strictly prohibit the sale of all intoxicating liquors and of some which are, perhaps, not intoxicating. In two of these States the statutes use the phrase "Malt Liquors" of any kind, and prosecutions have been brought in large numbers under this section. To obtain convictions, therefore, it has been necessary to convince the judge or jury that the beer in question was brewed

[1] Read at the Pittsburg Meeting of the American Chemical Society.