

# THE EFFECT OF PHOSPHORUS ON ALFALFA AND ALFALFA BACTERIA<sup>1</sup>

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## INTRODUCTION

While it is generally agreed that, under average conditions, phosphorus treatment has a marked beneficial effect upon the growth of all plants, it has been observed that in the case of some legumes such as alfalfa this increase is especially great. Wing (24), who had great opportunity to observe field conditions, said, "Alfalfa especially revels in phosphorus, and, from our experience and observation on many farms, we know that it pays richly to go over unproductive meadows with an application of basic slag or acid phosphate. We apply about 400 pounds to the acre for top dressing. It is marvellous how this treatment with phosphorus will cause the alfalfa to spring into new life with such a vigor that it gets ahead of annual grasses and over-shadows the weeds."

Recent investigators do not believe the benefit resulting from phosphorus treatment to be commensurate with the tissue requirements of the plant as shown by quantitative analyses. The benefit is far greater than such analyses would indicate. Fred and Hart (4, p. 36) and Lipman (11, p. 179) have considered a part of the benefit to higher plants from phosphorus treatment due to cellular stimulation and to the quickening of bacterial processes in the soil. In the case of legumes, however, there appears to be some additional factor which has not hitherto been considered.

It must not be supposed that the writer means that in all cases there is a greater absolute or percentage increase in the yield of legumes than in that of other crops. But, upon the basis of the relative phosphorus contents of the plant tissues, the increase seems to be greater in the case of the leguminous plants.

An examination of some plant analyses will serve to make this relation clearer. In the following figures certain legumes and non-legumes are compared in regard to their chief constituents.

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<sup>1</sup> Paper from the laboratory of agricultural bacteriology of the University of Wisconsin.

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## PERCENTAGE COMPOSITION OF CERTAIN LEGUMES AND NON-LEGUMES

(N, P, K:20, p. 714-720) (Ca:1, p. 751) (S:6, p. 3)

	Nitrogen	Phosphorus	Potassium	Calcium	Sulfur
Alfalfa ( <i>Medicago sativa</i> ) .....	2.45	0.22	1.74	1.51	0.29
Red Clover ( <i>Trifolium pratense</i> )..	2.10	0.22	1.65	1.43	0.16
Corn ( <i>Zea mays</i> ) .....	1.75	0.24	0.75	0.37	0.12
Timothy ( <i>Phleum pratense</i> ) .....	1.25	0.24	0.83	0.23	0.19

Since the process of nitrogen assimilation may be different in the two groups, legumes and non-legumes, it will not be considered. A comparison of the mineral elements in the two groups shows that potassium, calcium, and sulfur occur in much larger amounts in the legumes, while phosphorus occurs in about equal quantities in both groups. Exception must be taken in the case of the sulfur content of red clover and that of timothy. However, red clover is much lower than other legumes in sulfur content (6, p. 3). It is to be expected, therefore, that phosphorus fertilizers should have a proportionately slighter effect than potassium, calcium or sulfur fertilizers when applied to legumes as compared with similar treatments of non-legumes. Under average conditions no such relation has been reported. On the contrary, the evidence seems to indicate a disproportionate increase for phosphorus treatment in the case of legumes.

In attempting to explain the beneficial effect of phosphorus on legumes, a factor must be found which is not common to both legumes and non-legumes. Consequently, the possibilities of antagonism, neutralization of toxins, and stimulation of the common groups of soil bacteria may be dismissed as being in no way specific to legumes.

The stimulation of plants caused by phosphorus treatment is worthy of greater consideration. This stimulation must not be confused with simple nutrition, but rather must be associated with a nuclear stimulation resulting in increased cell division. While such stimulation obtains with all plants, it is possible that, in the case of legumes such as alfalfa, there may be a greater stimulation or that the plant, by means of its larger root system, is enabled to use this stimulation to greater advantage than are other plants. The writer noted an astonishingly strong growth of alfalfa plants fertilized with phosphorus. The beneficial effect of phosphorus was seen in very early stages before nodule formation had become very prominent.

There remains the possibility of the indirect increase in nitrogen nutrition of the leguminous host as a result of the increase in number of the nodule bacteria (*Bacillus radicicola*) in the soil, and consequent increase in numbers of nodules, as a result of more numerous infections and the greater proliferation of the bacteria within the nodules. This appears to be a highly probable explanation.



In order to understand the possibilities of stimulation of the lower plants, including the root-nodule bacteria, by phosphorus treatment, it may be well to give comparative figures showing the analyses of bacteria and legumes. Unfortunately no analyses are available for the nodule bacteria but it seems probable that they would be somewhat similar to *Azotobacter chroococcum*, a non-symbiotic nitrogen-fixing organism, for which figures are given.

PERCENTAGE COMPOSITION OF CERTAIN BACTERIA  
(19, p. 495)

	Phosphorus	Potassium	Ash
<i>Azotobacter chroococcum</i> .....	2.23	2.00	8.20
<i>Bacillus mycoides</i> .....	1.77	1.88	7.50
<i>Bacillus fluorescens liquefaciens</i> .....	2.31	0.69	6.48

It will be noted from these figures that bacteria have a high ash content, a high potassium content, and an exceedingly high phosphorus content. The ratio of phosphorus to potassium, it will be noted, is very high.

PERCENTAGE COMPOSITION OF ROOTS AND NODULES FROM LUPINE  
(*Lupinus luteus*) (18: p. 843)

	Total Ash
Nodules .....	6.32
Roots .....	4.55

ASH CONSTITUENTS

	Nodules	Roots
Silicon .....	1.59	1.90
Sulfur .....	4.90	6.38
Phosphorus .....	6.51	4.28
Potassium .....	17.31	12.05
Sodium .....	16.94	19.94
Magnesium .....	7.41	7.05
Calcium .....	7.64	12.04
Iron .....	0.83	0.75

It will be seen from the preceding figures that, in the case of lupine, the nodules are richer in ash, phosphorus, and potassium, than are the roots. It may be observed in passing that sulfur, an element essential to plant growth, which occurs in about the same quantities in soils as phosphorus, and which does not stimulate plants to any unusual growth; does not occur in as large quantities in the nodules as does phosphorus. By converting the percentages given above to a dry weight basis and comparing them with *Azotobacter chroococcum*, the following figures are obtained.



PERCENTAGE COMPOSITION OF LUPINE ROOTS AND NODULES, AND BACTERIA  
(19, p. 495; 18, p. 843)

	Phosphorus	Potassium	Ash
Lupine ( <i>Lupinus luteus</i> ) roots .....	0.19	0.54	4.55
Lupine ( <i>Lupinus luteus</i> ) nodules .....	0.34	1.09	6.30
<i>Azotobacter chroococcum</i> .....	2.23	2.00	8.20

From the preceding figures, it will be noted that the nodule analyses represent an approximate mean between the roots and bacteria in all three columns. Since the nodules are composed of bacterial cells and hypertrophied host tissues, this relation is what should be expected.

PERCENTAGE COMPOSITION OF ALFALFA AND BACTERIA

	Phosphorus	Potassium
Alfalfa ( <i>Medicago sativa</i> ) (20, p. 714) .....	0.22	1.74
<i>Azotobacter chroococcum</i> (19, p. 495) .....	2.23	2.00

It will be noted that, whereas there is not a great difference in the potassium content of alfalfa and bacteria, there is more than ten times as much phosphorus in the latter as in the former.

The foregoing figures indicate that phosphorus is the dominant element of the bacterial cell, and, from comparative compositions, phosphorus should cause a greater increase in growth of bacteria than in growth of higher plants. Consequently they seem to support the theory of the increase to the leguminous host as a result of increased activity of the nodule bacteria as outlined on page 78.

#### REVIEW OF INVESTIGATIONS ON THE INFLUENCE OF PHOSPHORUS ON BACTERIAL ACTIVITY AND GROWTH

While the increase in growth of the nodule organism, due to phosphorus treatment, has not heretofore received any direct attention, there are some interesting data, relating to bacterial stimulation and nodule formation caused by such treatment, which relate more or less closely to the subject.

The results of Lipman and Owen (13, p. 302) show that 1 per cent of acid phosphate causes a marked increase in the total number of bacteria in soil. Similar results were obtained by Fred and Hart (4, p. 54) as given in the following figures.

Treatment	Av. No. Bacteria in 1 gm. of Soil
None .....	3,082,000
Dipotassium phosphate, 500 mg. ....	5,920,000
Tricalcium phosphate, 1,000 mg. ....	3,176,000
Monocalcium phosphate, 1,000 mg. ....	4,230,000



Van Suchtelen (21, p. 77) found that the addition of superphosphate to soil double the evolution of carbon dioxide. Fred and Hart (4, p. 60) verified his results as shown by the following figures.

Treatment	Av. mg. CO <sub>2</sub> Evolution per Day from 100 gm. of Soil
None .....	12.75
Monocalcium phosphate, 1,000 mg. ....	19.28

Wohltmann, Fischer, and Schneider (26, p. 114) give us comparative figures for the effect of phosphorus on nitrification as shown below.

Treatment	Nitrification
None .....	100
Superphosphate .....	160

Considerable work has been done upon the effect of phosphorus on ammonification. The data of Wohltmann, Fisher, and Schneider (26, p. 108), Lipman (11, p. 179), Fred and Hart (4, p. 52), and McLean and Wilson (15) agree in showing a marked increase in ammonification for phosphorus treatment. The results of Lipman, which follow, show slighter benefit for phosphorus treatment than those of some other investigators.

Treatment	Ammonia Nitrogen found in 100 gm. of Soil mg.
None .....	133.7
Monocalcium phosphate .....	141.2
Dicalcium phosphate .....	142.0
Tricalcium phosphate .....	133.7

There have been several investigators working on the independent type of nitrogen fixation, especially that of *Azotobacter chroococcum*. Gerlach and Vogel (5, p. 638), working with solutions, made some interesting experiments concerning the effect of phosphorus. The results of these tests are given below.

Treatment	Nitrogen in 1000 c.c. of Solution mg.
Complete nutrient solution .....	28.7
Complete nutrient solution without calcium .....	4.6
Complete nutrient solution without potassium .....	24.1
Complete nutrient solution without phosphorus .....	3.7
Complete nutrient solution without phosphorus and potassium .....	1.8

It may be noted that the absence of calcium and phosphorus caused a great decrease in fixation and the absence of potassium a slight decrease. Wilfarth and Wimmer (22) secured similar results working in sand cultures. The latter likewise noted a similar relation of phosphorus to the growth and fixation of organic matter by algae. Working



in impure cultures of *Azotobacter*, Heinze (7, p. 904), and Hoffman and Hammer (8, p. 162), found a large increase in nitrogen fixed for mono-, di-, and tricalcium phosphate treatment. Koch, Litzendorff, Krull, and Alves (9, p. 413) working in soil got large increases for superphosphate added with dextrose as shown below.

Treatment	Nitrogen in 100 gm. of Soil mg.
None .....	46.4
Dextrose, 3 gm. ....	57.6
Dextrose, 3 gm., and superphosphate, 1 gm. ....	65.6

Their results show no increase for potassium sulfate and a decrease for potassium chloride treatment. Lipman (12, p. 139) found an increase of 50 per cent in nitrogen fixation for the addition of dipotassium phosphate in pure culture of *Azotobacter chroococcum*.

Of greatest interest from the standpoint of this paper are the data of many investigators on the effect of phosphorus treatment upon root nodule formation on the commoner legumes. Marchal (16, p. 1033) and Flamand (3) working in water cultures and Laurent (10, p. 1243), Wohltmann and Bergené (25), Löhnis (14, p. 26), Dehérain and Demoussy (2, p. 78), Prucha (17, p. 28), and Wilson (23) working in soil, all report a marked beneficial effect for phosphorus treatment upon nodule formation. Löhnis upon averaging a large number of plants obtained the following results with clover (*Trifolium pratense*).

Treatment	Nodules per Plant
None .....	31
Potassium phosphate .....	45

Wohltmann and Bergené, in an exceptionally comprehensive treatment of the subject, working with alfalfa treated with tripotassium phosphate, basic slag, and ammonium nitrate on many types of soils, got the following average results.

Treatment	Character of Nodules
None .....	Poor
Ammonium nitrate .....	Lacking
Basic slag .....	Good
Tripotassium phosphate .....	Very good

It will be seen from the preceding data that all investigations show an increased bacterial activity for phosphorus treatment.

#### EXPERIMENTAL WORK

The vital question involved in this investigation is the discovery of a reason for the beneficial influence of phosphorus upon alfalfa and other legumes. As shown by chemical analyses, simple nutrition is insufficient



to explain this beneficial influence. This benefit cannot be explained by any factor which is common to both legumes and non-legumes.

The symbiotic nitrogen fixation of legumes does not occur in the common non-legumes. A likely explanation therefore, for the favorable influence of phosphorus in the former case is that it may cause greater growth and activity of the root bacteria, resulting in greater nitrogen fixation, and more rapid growth of the leguminous host. It is proposed to test the validity of this explanation experimentally.

One step favorable to the establishment of this theory has been made by the earlier investigators who observed the beneficial influence of phosphorus upon nodule formation. The effect of phosphorus upon the growth of the root bacteria and upon the nitrogen content of legumes has apparently not received careful study. The latter considerations will receive especial attention in this investigation.

The relation of phosphates to the growth of alfalfa, to the growth of alfalfa bacteria, or to the growth of a combination of these two factors furnishes the point of attack for the problem. The work naturally falls into two parts:—first, that which treats of the influence of phosphates upon the growth of the alfalfa organism (*B. radicola*) as indicated by numerical counts; and second, that which treats of the influence of phosphates upon alfalfa, as regards nodule formation, rate of growth, dry weight of plants, and percentage and absolute content of nitrogen.

#### *The Effect of Phosphorus in the Form of Various Phosphates on the Growth of Bacillus Radicola from Alfalfa*

In this laboratory work, sterilized soils, to which phosphates had been added, were inoculated with a pure culture of the alfalfa organism, allowed to incubate for one and two weeks, and the number of organisms in 1 gm. at the end of these periods determined. The results were compared with the results obtained from similar, inoculated, but untreated controls.

In selecting compounds for phosphorus treatment, it was decided to use some of the phosphates commonly found in soil and commercial fertilizers. Potassium, sodium, and calcium phosphates appeared to be the most suitable. Because of their reaction, the primary phosphates, which are acid, and the tertiary phosphates, which are alkaline, were rejected. In order that bacterial growth might be influenced as little as possible in this respect, the secondary phosphates, which are only slightly alkaline, were employed. The quantities of the phosphates expressed in the phosphorus equivalents 0.1, 0.02 and 0.002 per cent were thought to represent a suitable range of fertilizers.

The soil used in the experiment was neutral and presented a suitable medium for bacterial growth.



### Effect of Dipotassium Phosphate on the Alfalfa Organism

In the first determination, dipotassium phosphate ( $K_2HPO_4$ ) was used. This salt has the advantage, of having no harmful physical effect on soil and, on account of its solubility, of yielding its maximum benefits within a short period of time. It has the disadvantage of carrying much potassium, an important plant-food constituent, which may itself cause marked increase in growth.

Twenty-five-gram portions of Madison field soil (Miami silt loam) were placed in large test tubes. Dilutions of dipotassium phosphate were made, in such a way that 1-c.c. portions of the stock solution contained 0.125, 0.025, and 0.0025 gm. of the salt which, when added to the soil samples, was equivalent to 0.1, 0.02, and 0.002 per cent of elemental phosphorus. The salts were added as indicated in the plan below. A dilution of a pure culture of *B. radicola* from alfalfa was made by transferring a loopful of culture from an agar slope to a sterile 100-c.c. water blank containing quartz sand and transferring a 5-c.c. portion to another 100-c.c. water blank. After sterilization for 4 hours at 15 pounds pressure, the soil tubes were inoculated, 5-c.c. portions from the highest dilution of the alfalfa organism being used. The water added with the inoculant brought the moisture content of the soil samples to about 28 per cent. The amount of the inoculation was determined by plate counts. The plan of the experiment is given below.

No. and Treatment	No. of Tubes in Each Group
1. Uninoculated .....	4
2. Inoculation .....	4
3. Inoculation with 0.002% phosphorus .....	4
4. Inoculation with 0.020% phosphorus .....	4
5. Inoculation with 0.100% phosphorus .....	4

Plate counts, to ascertain the number of bacteria in each soil sample, were made at the end of 7 and 14 days. In making the counts, duplicate tubes from each group were emptied into 400-c.c. water blanks, 25-c.c. portions from the latter transferred to other water blanks, and the pro-

TABLE I  
THE EFFECT OF DIPOTASSIUM PHOSPHATE UPON THE GROWTH OF  
*B. RADICOLA* FROM ALFALFA

Treatment in per cent of Phosphorus	Bacteria in 1 gm. of Dry Soil			
	After 7 Days		After 14 Days	
	Total	Increase Due to Treatment	Total	Increase Due to Treatment
None	24,400,000	.....	16,700,000	.....
0.002	28,500,000	4,100,000	18,100,000	1,400,000
0.02	158,000,000	133,600,000	113,100,000	96,400,000
0.1	58,500,000	34,100,000	40,300,000	23,600,000



cess repeated until five dilutions had been made. From the fourth and fifth dilutions plates were poured in triplicate, mannite agar being used. After five days of incubation at 30° C., the total number of colonies per plate was determined and the number of organisms in the original soil calculated. The results of the experiment are given in Table I.

It will be noted that there is an appreciable increase for the lowest dipotassium phosphate treatment, an enormous increase for the next treatment, and a somewhat smaller increase for the highest treatment. Obviously, the lowest treatment was insufficient to cause any large gain and the highest probably tended to have inhibitory as well as nutritive action, while the middle treatment approached more nearly the optimum concentration for the development of legume bacteria in this soil.

The objection may be raised that the results do not show the benefit for phosphorus alone since the potassium contained in the salt very probably caused a large part of the increased growth of the bacteria. In order to determine what the real effect of phosphorus may be in this case, the results must be compared with those obtained from the use of other phosphorus salts which do not contain potassium.

#### The Effect of Disodium Phosphate on the Alfalfa Organism

The experiment was next run with disodium phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ ). This salt, it was thought, would not be open to the same criticism as dipotassium phosphate, inasmuch as sodium, unlike potassium is not usually considered to be essential to plant growth. On the other hand, unlike dicalcium phosphate, it is quite soluble. Its greatest defect is its tendency to deflocculate soil.

The process as outlined for dipotassium phosphate was repeated with disodium phosphate. In order to simplify the work, the highest phosphorus concentration was omitted. The stock solutions were made such that 2-c.c. portions contained 0.056 and 0.0056 gm. of the salt, and, when added to the 25-gm. samples of soil, were equivalent to 0.02 and 0.002 per cent of phosphorus. The results are shown in Table II.

TABLE II  
THE EFFECT OF DISODIUM PHOSPHATE ON THE GROWTH OF  
*B. RADICICOLA* FROM ALFALFA

Treatment in per cent of Phosphorus	Bacteria in 1 gm. of Dry Soil			
	After 7 Days		After 14 Days	
	Total	Increase Due to Treatment	Total	Increase Due to Treatment
None	222,900,000	.....	260,600,000	.....
0.002	Lost	.....	481,100,000	220,500,000
0.02	392,800,000	169,900,000	534,500,000	273,900,000

Although sodium is supposedly a non-nutrient element, it may be seen from Table II that there is a very great numerical increase for the addi-



tion of disodium phosphate. This increase should, it would appear, be considered due to the phosphorus rather than to the sodium.

It will be seen that the counts were much higher in the case of disodium phosphate, although the percentage increase for treatment was much greater in the case of dipotassium phosphate. The difference may be due to a difference in moisture content or to a difference in soil. Unfortunately, the soil used for the dipotassium phosphate experiment was inadvertently discarded during the summer, and the remaining tests had to be run on a different sample of soil, though of the same type.

However, with soils containing such an enormous number of bacteria as 500,000,000 per gram, comparison by percentage with soils containing 100,000,000 or less bacteria per gram, seems unjustifiable. In the former case, the bacteria are very likely approaching a point where they are self-inhibitory and thus cannot proliferate freely in response to an external stimulus, whereas, in the latter case, expansion is not limited.

Results similar to those shown in Table II were obtained by a subsequent determination. Flask cultures of the organism were treated with disodium phosphate and incubated as previously outlined. Counts were made in 7 days. The results which are represented by the sets of Petri dishes in Plate I, are given below.

Treatment in Per cent of Phosphorus	Bacteria in 1 gm. of Dry Soil
None .....	346,000,000
0.002 .....	350,000,000
0.02 .....	505,000,000

#### The Effect of Dicalcium Phosphate on the Alfalfa Organism

The final trial was made with dicalcium phosphate ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ). This salt is one of common occurrence in soil and a constituent of many phosphatic fertilizers. Although calcium is a recognized constituent of plant-food, it is generally believed to be present in the soil in sufficient quantities to supply the needs of plants, and it is not believed to cause any great increase in plant growth when applied in the form of its salts, unless it is applied in forms which may alter the reaction of the soil, or its physical character. It is likewise true that calcium salts are not reported to have any injurious effects upon plants or soil. However, the chief objection to the use of dicalcium phosphate was that it is very insoluble and, unless there is much carbon dioxide production, a large part of it is likely to remain unavailable within the limits of a short experiment.

The method as outlined for dipotassium phosphate was first employed. However, owing to the small increase in bacterial growth for the application of dicalcium phosphate, the relative variation was sufficient to render the results doubtful. It was, therefore, decided to repeat the experiment, using Erlenmeyer flasks. Portions of soil were weighed into each of ten 300-c.c. flasks, and treated as outlined for test tubes on page 84. For



each treatment duplicate flasks were used in place of the quadruplicate test tubes. In this case, 0.091 and 0.0091 gm. of the dicalcium phosphate, equivalent to 0.02 and 0.002 per cent of phosphorus, were weighed, and, on account of the insolubility of the salt, mixed directly with the soil in the flasks. The outline of the experiment is given below.

No. and Treatment	No. of Flasks
1. None .....	2
2. Inoculation .....	2
3. Inoculation plus 0.002% phosphorus .....	2
4. Inoculation plus 0.02% phosphorus .....	2

When counts were to be made, 25-gm. portions of soil from all of the flasks were weighed and transferred to water blanks. The flasks were then replugged, and returned to the incubator. After a week, other 25-gm. portions were weighed from the same flasks for the second count. In all other respects the experiment was similar to that in which dipotassium phosphate was used. This process called for more careful technique to prevent contamination, but gave more accurate results than did the test-tube method. The results are shown in Table III.

TABLE III  
THE EFFECT OF DICALCIUM PHOSPHATE ON THE GROWTH OF  
*B. RADICICOLA* FROM ALFALFA

Treatment in per cent of Phosphorus	Bacteria in 1 gm. of Dry Soil			
	After 7 Days		After 14 Days	
	Total	Increase Due to Treatment	Total	Increase Due to Treatment
None	293,900,000	.....	227,200,000	.....
0.002	287,000,000	.....	233,800,000	6,600,000
0.02	367,400,000	73,500,000	260,500,000	33,300,000

On comparing Table III with the two preceding tables it will be observed that dicalcium phosphate produces nowhere near the increase in growth that dipotassium and disodium phosphates produce. This condition is to be expected from the insolubility of the salt.

The extreme difficulty in determining the effect of phosphorus in its calcium salts must be borne in mind. The secondary salt is insoluble, and the tertiary salt still more insoluble. The primary salt is soluble, and, from analogous results (7, p. 889) might cause greater increase than the secondary salt. However, the former has an acid reaction, and, since it is generally believed that acidity is unfavorable to the growth of the legume organism, the counts, if this salt were used, would still not be a measure of the beneficial action of phosphorus. Furthermore, in this experiment it seemed expedient to use the salt most comparable with the secondary sodium and potassium salts previously used.



With a due consideration for the character of the basic elements involved, it seems justifiable to conclude from the three preceding tables that phosphorus produces a decided increase in the growth of *B. radiculicola* from alfalfa, analogous to that produced in the case of *Azotobacter*, and other soil forms.

*The Effect of Phosphorus in the Form of Dicalcium Phosphate upon Alfalfa*

The following experiment was carried on under greenhouse conditions using pot cultures. Alfalfa, grown on unsterilized soil, was inoculated, treated with phosphorus, and with phosphorus plus nitrogen. The results, in nodule formation, dry weight, percentage and absolute nitrogen content were compared with all possible control combinations.

The Experimental Method

Into 4-gallon earthen jars were weighed 14-kg. portions of neutral Madison field soil (Miami silt loam). The pots were treated as shown in the following outline.

Inoculated			Uninoculated		
Pot No.	Phosphorus as $\text{CaHPO}_4$ Per cent	Nitrogen as $\text{CO}(\text{NH}_2)_2$ Per cent	Pot No.	Phosphorus as $\text{CaHPO}_4$ Per cent	Nitrogen as $\text{CO}(\text{NH}_2)_2$ Per cent
1, 2	.....	.....	17, 18	.....	.....
3, 4	.....	0.014	19, 20	.....	0.014
5, 6	0.005	0.014	21, 22	0.005	0.014
7, 8	0.015	0.014	23, 24	0.015	0.014
9, 10	0.045	0.014	25, 26	0.045	0.014
11, 12	0.005	.....	27, 28	0.005	.....
13, 14	0.015	.....	29, 30	0.015	.....
15, 16	0.045	.....	31, 32	0.045	.....

The dibasic calcium phosphate was used because it is not likely to cause any great change in the reaction of soil to which it is added, and it should be expected to become soluble at a rate suitable to supply the needs of plants. The percentages of phosphorus, 0.005, 0.015, and 0.045, are equivalent to field applications of 700, 2100, and 6300 pounds of rock phosphate, the only comparable field fertilizer. As a nitrogenous fertilizer, urea [ $\text{CO}(\text{NH}_2)_2$ ] was used because it was thought that it would not, either directly or indirectly by residues of decomposition, cause any marked change in the reaction of the soil, and because it has been shown that it does not injure nodule formation (3, p. 739). A quantity of urea equivalent to 0.014 per cent of nitrogen was used in order to furnish an excess of that element.

When the soil and fertilizer had been thoroughly mixed, the pots were ready for planting. Alfalfa seed was sterilized with a solution of mercuric chloride, washed in sterile water, and a portion of it soaked in a sus-



pension of alfalfa organisms. Pots 1 to 16 were planted with uninoculated seed and pots 17 to 32 with inoculated seed. In a week the seedlings were thinned to 30 plants to each pot.

Throughout the experiment the alfalfa was watered with distilled water in sterilized watering pots. The plants were grown for 7 months, during which four cuttings were made. When the experiment was discontinued, the roots were carefully removed, washed and the root nodules noted.

When the material had become thoroughly air-dry, it was weighed. In the case of the roots and of the first and third cuttings, the samples were ground and the percentage content of nitrogen determined by the Kjeldahl-Gunning method. The results, determined in percentage dry weight, were multiplied by the dry weights to give the absolute nitrogen content.

#### Early Effects of Phosphorus upon Alfalfa Seedlings

As previously stated (p. 78), it was observed in the earlier stages that there was a marked increase in growth for the addition of phosphorus. Nitrogen fertilization appeared to exert an injurious effect while inoculation seemed to have no effect whatever. The following figures will indicate the extent of this difference three weeks after planting.

Treatment—Inoculated and Uninoculated Series	Average Height in cm.
None .....	11.0
Nitrogen .....	7.0
0.005 per cent phosphorus with nitrogen .....	12.0
0.015 per cent phosphorus with nitrogen .....	14.0
0.045 per cent phosphorus with nitrogen .....	15.5
0.005 per cent phosphorus .....	15.0
0.015 per cent phosphorus .....	16.0
0.045 per cent phosphorus .....	16.5

The beneficial effect of phosphorus on plant growth was noted almost from the first, but, when the foregoing measurements were made, there was greater uniformity, permitting of more accurate measurement, than at any earlier period. At this early stage, the nodule bacteria could hardly be expected to have furnished the plant with sufficient nitrogen to cause such a difference in growth.

The rapid growth of the phosphorus-treated plants, it appears to the writer, may be accounted for only as a result of direct nutrition and stimulation of the plant by phosphorus, and as a result of the quickening of bacterial actions other than that of nitrogen fixation.

#### The Effect of Phosphorus on Alfalfa Nodule Formation

When the roots were removed the relative occurrence of the nodules was noted. Some nodules were found on all of the uninoculated plants although they were not so numerous as those on the inoculated plants. That the soil was already naturally inoculated with the alfalfa organisms



had previously been shown from the results of a preliminary test. However, it will be seen from the tables which follow, that there were perceptible differences resulting from inoculation notwithstanding the presence of the organism in the uninoculated controls.

On the roots of the phosphorus-treated plants there were more abundant nodules than upon those of the untreated controls. This is in agreement with the results of all previous investigators. Where both phosphorus and nitrogen were used, there was much variability but, on the whole, there were fewer nodules than where phosphorus alone was employed. Where nitrogen alone was used the results were similar to the controls.

#### The Effect of Phosphorus on the Air-dry Weight of Alfalfa

Although the air-dry weights of the alfalfa given in Tables IV and V fluctuate somewhat, the average of the duplicate checks, given in Table VI, brings out a definite relation between treatment and yield. Slight differences in the diameter of pots, chance inoculations, and attacks by insects were possible causes of the occasional disparity of duplicate checks.

TABLE IV  
THE EFFECT OF PHOSPHORUS AND NITROGEN TREATMENT UPON THE  
AIR-DRY WEIGHTS OF ALFALFA: UNINOCULATED SERIES

Treatment		1st	2nd	3rd	4th		Total
P as CaHPO <sub>4</sub>	N as CO(NH <sub>2</sub> ) <sub>2</sub>	Cutting	Cutting	Cutting	Cutting	Roots	Roots and Tops
Per cent	Per cent	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
.....	.....	6.7	9.0	9.8	8.1	13.8	47.4
.....	.....	5.5	8.2	9.5	8.5	13.0	44.7
.....	0.014	5.7	10.1	12.2	10.4	18.8	57.2
.....	0.014	2.5	7.6	10.0	8.1	16.6	44.8
0.005	0.014	5.8	8.8	13.9	8.0	22.2	58.7
0.005	0.014	5.3	7.9	11.6	9.5	18.6	52.9
0.015	0.014	6.4	8.2	11.1	9.2	22.1	57.0
0.015	0.014	7.2	8.8	12.2	10.3	19.5	58.0
0.045	0.014	7.9	11.2	13.2	10.6	20.3	63.2
0.045	.....	6.0	8.5	9.7	9.8	17.0	51.0
0.005	.....	8.0	11.9	11.3	9.6	17.2	58.0
0.005	.....	6.7	9.8	10.0	9.0	17.3	52.8
0.015	.....	8.3	11.6	11.2	10.1	16.0	57.2
0.015	.....	7.9	10.5	11.7	10.9	16.9	57.9
0.045	.....	8.6	11.6	12.7	12.9	18.2	64.0
0.045	.....	9.8	10.4	10.9	10.7	16.7	58.5
Total .....	.....	108.3	154.1	181.0	155.7	284.2	883.3

On comparing the vertical columns, it will be seen that the first cutting and the roots showed the greatest variation both between checks and between pots of unlike treatment, and that the third cutting was the most uniform. Nitrogen treatment had apparently a depressing effect in the first cutting, as previously noted, but had no harmful effects in subse-



TABLE V

THE EFFECT OF PHOSPHORUS AND NITROGEN TREATMENT UPON THE AIR-DRY WEIGHTS OF ALFALFA: INOCULATED SERIES

Treatment		1st Cutting	2nd Cutting	3rd Cutting	4th Cutting	Roots	Total Roots and Tops
P as $\text{CaHPO}_4$	N as $\text{CO}(\text{NH}_2)_2$						
Per cent	Per cent	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
.....	.....	6.0	9.8	11.0	10.9	16.7	54.4
.....	.....	5.0	10.4	9.8	8.8	13.6	47.4
.....	0.014	3.6	9.0	11.0	9.1	18.3	51.6
.....	0.014	3.0	7.6	11.8	9.8	15.0	47.2
0.005	0.014	7.8	12.3	14.0	9.7	18.2	62.0
0.005	0.014	4.0	8.8	13.0	9.9	16.2	50.9
0.015	0.014	6.2	11.1	14.0	12.0	21.3	64.6
0.015	0.014	6.8	9.6	12.7	11.5	17.5	58.1
0.045	0.014	6.5	10.2	12.2	11.6	18.0	58.5
0.045	0.014	7.5	8.5	13.4	9.9	16.2	55.5
0.005	.....	7.3	10.2	10.8	10.2	15.6	54.1
0.005	.....	6.4	8.5	12.4	9.9	16.0	53.2
0.015	.....	8.0	11.3	13.7	11.7	16.9	61.6
0.015	.....	7.0	8.8	14.2	10.4	17.2	57.6
0.045	.....	6.5	9.4	13.9	9.0	18.6	57.4
0.045	.....	6.6	8.7	12.7	10.5	17.8	56.3
Total .....		98.2	154.2	199.6	164.9	273.1	890.0

TABLE VI

THE EFFECT OF PHOSPHORUS AND NITROGEN TREATMENT UPON THE AIR-DRY WEIGHTS OF ALFALFA: AVERAGE OF DUPLICATES

UNINOCULATED

Treatment		1st Cutting	2nd Cutting	3rd Cutting	4th Cutting	Total Tops	Roots	Total Roots and Tops
P as $\text{CaHPO}_4$	N as $\text{CO}(\text{NH}_2)_2$							
Per cent	Per cent	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
.....	.....	6.10	8.60	9.65	8.30	32.7	13.40	46.1
.....	0.014	4.10	8.85	11.10	9.25	33.3	17.70	51.0
0.005	0.014	5.55	8.35	12.75	8.75	35.4	20.40	55.8
0.015	0.014	6.80	8.50	11.65	9.85	36.8	20.80	57.6
0.045	0.014	6.95	9.85	11.45	10.40	38.7	18.65	57.3
0.005	.....	7.35	10.85	10.65	9.30	38.2	17.25	55.4
0.015	.....	8.10	11.05	11.45	10.50	41.1	16.45	57.6
0.045	.....	9.30	11.00	11.80	11.80	43.8	17.45	61.3

INOCULATED

.....	.....	5.50	10.10	10.50	9.85	36.0	15.10	51.1
.....	0.014	3.30	8.30	11.40	9.45	32.5	16.65	49.1
0.005	0.014	5.90	10.55	13.00	9.80	39.3	17.20	56.5
0.015	0.014	6.50	10.35	13.45	11.75	42.1	19.40	61.5
0.045	0.014	7.00	9.35	12.80	10.75	39.9	17.10	57.0
0.005	0.014	6.85	9.35	11.60	10.05	37.9	15.80	53.7
0.015	.....	7.50	10.05	13.95	11.05	42.6	17.50	60.1
0.045	.....	6.55	9.05	13.30	9.75	38.7	18.20	56.9



quent cuttings. A matter of interest, for which no explanation will be given, is that those pots to which nitrogen had been added had very large root systems.

In a very general survey of the yields for the different treatments it may be noted that, upon comparison with the appropriate controls, there is a slight increase in growth for nitrogen, in the uninoculated, but not in the inoculated, a great increase for phosphorus and an equal increase for phosphorus plus nitrogen. A comparison of the uninoculated with the inoculated series indicates a trifling advantage for the latter.

#### The Effect of Phosphorus on the Air-dry Weight and Nitrogen Content of Alfalfa Roots

Table VII gives the dry weights, percentage of nitrogen, and the total nitrogen content of the alfalfa roots. Table VIII gives the averages of the duplicate jars. It will be noted that there is considerable variation in the percentage of nitrogen as well as in dry-weight.

TABLE VII  
THE EFFECT OF PHOSPHORUS AND NITROGEN TREATMENT ON THE ROOTS OF ALFALFA

Treatment		Uninoculated			Inoculated		
P as CaHPO <sub>4</sub>	N as CO(NH <sub>2</sub> ) <sub>2</sub>	Dry Weight	Nitro- gen	Total Nitrogen	Dry Weight	Nitro- gen	Total Nitrogen
Per cent	Per cent	Gm.	Per cent	Mg.	Gm.	Per cent	Mg.
.....	.....	13.8	2.74	378.1	16.7	2.78	454.3
.....	.....	13.0	2.60	338.0	13.5	2.83	382.1
.....	0.014	13.8	2.68	503.8	18.3	2.89	528.9
.....	0.014	16.6	3.01	500.0	15.0	2.81	421.5
0.005	0.014	22.2	2.56	568.3	18.2	2.82	513.2
0.005	0.014	18.6	2.71	504.1	16.2	2.97	481.1
0.015	0.014	22.1	2.66	587.9	21.3	2.83	602.8
0.015	0.014	19.5	2.54	495.3	17.5	2.80	490.0
0.045	0.014	20.3	2.56	519.7	18.0	2.82	507.6
0.045	0.014	17.0	2.64	448.8	16.2	2.72	440.6
0.005	.....	17.2	2.43	418.0	15.6	2.70	421.2
0.005	.....	17.3	2.51	434.2	16.0	2.65	424.0
0.015	.....	16.0	2.33	372.8	16.9	2.65	447.9
0.015	.....	16.9	2.35	397.2	17.2	2.96	509.1
0.045	.....	18.2	2.46	441.2	18.6	3.21	597.1
0.045	.....	16.7	2.60	434.2	17.8	2.80	498.4

The figures for the percentage of nitrogen appear to have little significance. It is apparent, however, that there is an increase in the total nitrogen content of the roots for the addition of phosphorus to the soil, and an even greater increase for the addition of nitrogen, and nitrogen with phosphorus. The total nitrogen content varies largely with the dry weights.

#### The Effect of Phosphorus on the Air-dry Weight and Nitrogen Content of the First Cutting of Alfalfa

In Table IX are shown the air-dry weights, the percentage of nitrogen and the total nitrogen for the first cutting. The averages for the duplicate jars are given in Table X.

From a comparison of these tables it will be seen that the percentage of



nitrogen, with a single exception, varies in inverse proportion with the dry-weights. This is in agreement with frequent observations that rapidly growing plants contain a smaller percentage of nitrogen, figured on the dry-weight basis, than slower growing plants. In this case, the phosphorus-treated plants grew much more rapidly than the controls, and the percentage of nitrogen is accordingly lower. It will, however, be noted that the total nitrogen content is greatest in the case of the phosphorus-treated jars.

TABLE VIII  
THE EFFECT OF PHOSPHORUS AND NITROGEN TREATMENT ON THE ROOTS OF ALFALFA: AVERAGE OF DUPLICATES

Treatment		Uninoculated			Inoculated			Avg. Inoculated and Uninoc. Total Nitrogen
P as $\text{CaHPO}_4$	N as $\text{CO}(\text{NH}_2)_2$	Dry Weight	Nitrogen	Total Nitrog'n	Dry Weight	Nitrogen	Total Nitrog'n	
Per cent	Per cent	Gm.	Per cent	Mg.	Gm.	Per cent	Mg.	Mg.
.....	.....	13.40	2.67	358.1	15.1	2.80	423.0	390.6
.....	0.014	17.70	2.85	501.9	16.7	2.85	475.2	488.6
0.005	0.014	20.40	2.64	536.2	17.2	2.90	497.2	516.7
0.015	0.014	20.80	2.60	641.6	19.4	2.82	546.8	544.2
0.045	0.014	18.65	2.60	484.3	17.1	2.77	474.1	479.2
0.005	.....	17.25	2.47	433.4	15.8	2.68	422.6	428.0
0.015	.....	16.45	2.34	385.0	17.1	3.81	478.5	431.8
0.045	.....	17.45	2.53	440.1	18.2	3.01	547.8	494.5
Average .....		17.80	2.59	460.1	17.1	2.83	483.3	.....

#### The Effect of Phosphorus on the Air-dry Weight and Nitrogen Content of the Third Cutting of Alfalfa

Neither the analyses for the roots nor those for the first cutting appear to the writer to be entirely satisfactory. That there is an increase in total nitrogen for phosphorus treatment is certainly evident, but the relation of the treatments to the percentage of nitrogen is not clearly shown.

The difficulty in the case of the roots is due to a number of conditions; first, it is difficult to remove all of the fine roots from the soil; second, it is difficult to free the roots from the small particles of soil; third, varying numbers of nodules are likely to be torn off of the roots, and fourth, the stubble retained on the roots may be quite variable. It will be readily seen that the loss of secondary roots will reduce the weight of the tissue, and the presence of dirt will increase the dry-weight and reduce the percentage of nitrogen. Likewise the quantity of the highly nitrogenous nodules and the amount of the comparatively, highly nitrogenous stem tissues retained may cause even greater variations in the nitrogen content of the roots.

In the case of the first cutting the difficulty, as previously mentioned, is the great influence of the rate of growth upon the nitrogen content. It is obvious that small differences due to treatment would, in this case, be entirely masked.



It seemed, therefore, advisable to take a later cutting, which showed a smaller difference in dry-weight indicating a more uniform growth. It was likewise thought that, at this later period, the normal metabolism of the mature plant would be more thoroughly established than at the time of the first cutting.

TABLE IX  
THE EFFECT OF PHOSPHORUS AND NITROGEN TREATMENT ON THE FIRST CUTTING OF ALFALFA

Treatment		Uninoculated			Inoculated		
P as CaHPO <sub>4</sub>	N as CO(NH <sub>2</sub> ) <sub>2</sub>	Dry Weight	Nitro- gen	Total Nitrogen	Dry Weight	Nitro- gen	Total Nitrogen
Per cent	Per cent	Gm.	Per cent	Mg.	Gm.	Per cent	Mg.
.....	.....	6.7	3.67	244.9	6.0	3.84	230.4
.....	.....	5.5	3.79	208.5	5.0	3.74	187.0
.....	0.014	5.7	4.33	246.8	3.6	4.77	171.7
.....	0.014	2.5	4.63	115.8	3.0	4.73	141.9
0.005	0.014	5.8	4.07	236.1	7.8	4.15	323.7
0.005	0.014	5.3	4.18	221.5	4.0	4.54	181.6
0.015	0.014	6.4	4.17	266.9	6.2	4.23	262.3
0.015	0.014	7.2	3.91	281.5	6.8	4.09	278.1
0.045	0.014	7.9	3.81	301.0	6.5	3.87	259.6
0.045	0.014	6.0	3.91	234.6	7.5	3.91	293.3
0.005	.....	8.0	3.80	304.0	7.3	3.68	268.6
0.005	.....	6.7	3.77	252.9	6.4	3.60	230.4
0.015	.....	8.3	3.69	306.3	8.0	3.61	288.8
0.015	.....	7.9	3.78	298.6	7.0	3.53	247.1
0.045	.....	8.6	3.78	325.1	6.5	3.42	222.3
0.045	.....	9.8	3.65	357.7	6.6	3.57	235.6

TABLE X  
THE EFFECT OF PHOSPHORUS AND NITROGEN TREATMENT ON THE FIRST CUTTING OF ALFALFA: AVERAGE OF DUPLICATES

Treatment		Uninoculated			Inoculated			Avg. Inoculated and Uninoc. Total Nitrogen
P as CaHPO <sub>4</sub>	N as CO(NH <sub>2</sub> ) <sub>2</sub>	Dry Weight	Nitro- gen	Total Nitrog'n	Dry Weight	Nitro- gen	Total Nitrog'n	
Per cent	Per cent	Gm.	Per cent	Mg.	Gm.	Per cent	Mg.	Mg.
.....	.....	6.10	3.73	226.7	5.50	3.79	208.7	217.70
.....	0.014	4.10	4.48	180.3	3.30	4.75	156.8	168.60
0.005	0.014	5.60	4.13	228.8	5.90	4.35	252.7	240.80
0.015	0.014	6.80	4.04	274.2	6.50	4.16	270.2	272.20
0.015	0.014	6.95	3.86	367.8	7.00	3.89	276.5	272.15
0.005	.....	7.35	3.79	278.5	6.85	3.64	249.5	264.00
0.015	.....	8.10	3.74	302.5	7.50	3.57	368.0	285.25
0.045	.....	9.20	3.72	341.4	6.55	3.50	229.0	285.20
Average .....		6.77	3.94	262.5	6.15	3.96	239.0	.....

Table XI shows the results for the third cutting and Table XII gives the averages for the duplicate treatments. This cutting was grown in the best of the season and as shown by Table VI, it had the maximum dry-



weight of all the cuttings. It represents a comparatively mature stage in the growth of the plant at a time when it was fairly free from insect and fungous attacks.

TABLE XI  
THE EFFECT OF PHOSPHORUS AND NITROGEN TREATMENT ON THE THIRD CUTTING OF ALFALFA

Treatment		Uninoculated			Inoculated		
P as CaHPO <sub>4</sub>	N as CO(NH <sub>2</sub> ) <sub>2</sub>	Dry Weight	Nitro- gen	Total Nitrogen	Dry Weight	Nitro- gen	Total Nitrogen
Per cent	Per cent	Gm.	Per cent	Mg.	Gm.	Per cent	Mg.
.....	.....	9.8	3.88	380.2	11.2	3.51	393.1
.....	.....	9.5	3.86	366.7	9.8	3.65	357.7
.....	0.014	12.2	3.60	434.2	11.0	3.49	383.9
.....	0.014	10.0	3.50	350.0	11.8	3.67	433.1
0.005	0.014	13.9	3.63	504.6	14.0	3.87	541.8
0.005	0.014	11.6	3.59	416.4	12.0	3.83	459.6
0.015	0.014	11.1	3.48	386.3	14.0	3.57	499.8
0.015	0.014	12.2	3.16	385.3	12.7	3.56	452.1
0.045	0.014	13.2	3.46	456.7	12.2	3.41	416.0
0.045	.....	9.7	3.49	330.5	13.4	3.92	525.3
0.005	.....	11.3	3.77	426.0	10.8	3.85	415.8
0.005	.....	10.0	3.99	399.0	12.4	3.50	434.0
0.015	.....	11.2	4.10	459.2	13.7	3.85	527.5
0.015	.....	11.7	4.09	478.5	14.2	3.79	538.2
0.045	.....	12.7	3.84	487.7	13.9	3.73	518.5
0.045	.....	10.9	3.95	430.6	12.7	Lost	Lost

TABLE XII  
THE EFFECT OF PHOSPHORUS AND NITROGEN TREATMENT ON THE THIRD CUTTING OF ALFALFA: AVERAGE OF DUPLICATES

Treatment		Uninoculated			Inoculated			Avg. Inoculated and Uninoc. Total Nitrogen
P as CaHPO <sub>4</sub>	N as CO(NH <sub>2</sub> ) <sub>2</sub>	Dry Weight	Nitro- gen	Total Nitrog'n	Dry Weight	Nitro- gen	Total Nitrog'n	
Per cent	Per cent	Gm.	Per cent	Mg.	Gm.	Per cent	Mg.	Mg.
.....	.....	9.65	3.87	373.5	10.50	3.58	378.2	375.4
.....	0.014	11.10	3.55	394.6	11.40	3.58	408.5	401.6
0.005	0.014	12.75	3.61	460.5	13.00	3.84	500.7	480.1
0.015	0.014	11.65	3.32	385.8	13.35	3.57	476.0	430.9
0.045	0.014	11.45	3.48	393.6	12.80	3.66	470.7	432.2
0.005	.....	10.65	3.88	412.5	11.60	3.68	424.9	418.7
0.015	.....	11.45	4.09	468.9	13.95	3.82	532.9	500.9
0.045	.....	11.80	3.90	459.2	13.30	3.73	518.5	488.9
Average .....		11.30	3.71	418.6	12.50	3.68	463.8	.....

The increase in total nitrogen and in dry weight of the tops due to phosphorus treatment is in entire agreement with the results for both the roots and the first cutting. But, *in this case, there is an increase in the percentage of nitrogen in the tops for phosphorus treatment.* It will likewise be noted that an abundant nitrogen treatment did not cause either as great a quantity or percentage of nitrogen to be stored in the tops as did the phosphorus treatment.



These results are quite clear cut and the data of the inoculated and uninoculated series agree throughout. Consequently this difference in the percentage of nitrogen must unquestionably be considered as resulting from phosphorus treatment.

The evidence points to a greater efficiency in fixing and storing nitrogen as well as an increase in growth as a result of phosphorus treatment. The nodule bacteria apparently have not only supplied more nitrogen to meet the needs of the larger, phosphorus-treated plants, but they have stored a larger percentage of nitrogen in the tops of these plants than in the untreated controls and the nitrogen-treated plants.

The data for the jar cultures of alfalfa seem to indicate an increased activity of the root bacteria due to phosphorus treatment, resulting in larger quantities of nitrogen being fixed and stored in the plant. In the case of the third cutting this relation is especially evident, since in the phosphorus-treated plants there are found to be not only greater quantities, but also a greater percentage of nitrogen than that occurring in the controls.

#### SUMMARY AND CONCLUSIONS

1. The relatively large increase in the growth of alfalfa and some other legumes resulting from phosphorus treatment, as compared with some non-legumes, seems to indicate some previously unaccounted-for benefit due to phosphorus treatment.

2. The relatively low phosphorus content of higher plants in comparison with relatively high phosphorus content of bacteria indicates that phosphorus may be expected to cause greater increases in the latter than in the former case.

3. That phosphorus does cause large increases in the growth and activity of various groups of bacteria, including some nitrogen-fixing forms is shown by the work of numerous investigators.

4. The work of some investigators has likewise shown that there are more numerous and larger nodules on the roots of leguminous plants as a result of phosphorus treatment.

5. The treatment of pure cultures of *B. radicola* from alfalfa with phosphates resulted in large increases in the number of organisms varying with the character and solubility of the salt.

6. The fertilization of alfalfa plants with phosphate resulted in a much more rapid growth in the young seedling stage.

7. The fertilization of alfalfa with phosphorus resulted in increased nodule formation, dry weight, and total nitrogen content.

8. In the case of the third cutting, which was most nearly representative of normal, average conditions, not only was there an increase in the total nitrogen, but *there was an increase in the percentage of nitrogen associated with the addition of phosphorus fertilizer.*



9. The early increase noted in the growth of the young phosphorus-treated seedlings may be interpreted as a result of the nutrition of the plant and the stimulation frequently associated with cell reproduction and to the quickening of bacterial processes in the soil.

10. The ultimate increases in growth resulting from phosphorus treatment may, to a large extent, be due to the increased infection with the alfalfa organisms, increased growth and proliferation of the organism within the nodule, and consequently greater fixation of nitrogen. The increase in the percentage of nitrogen in the third cutting, resulting from phosphorus treatment, points strongly to this conclusion.

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PLATE I

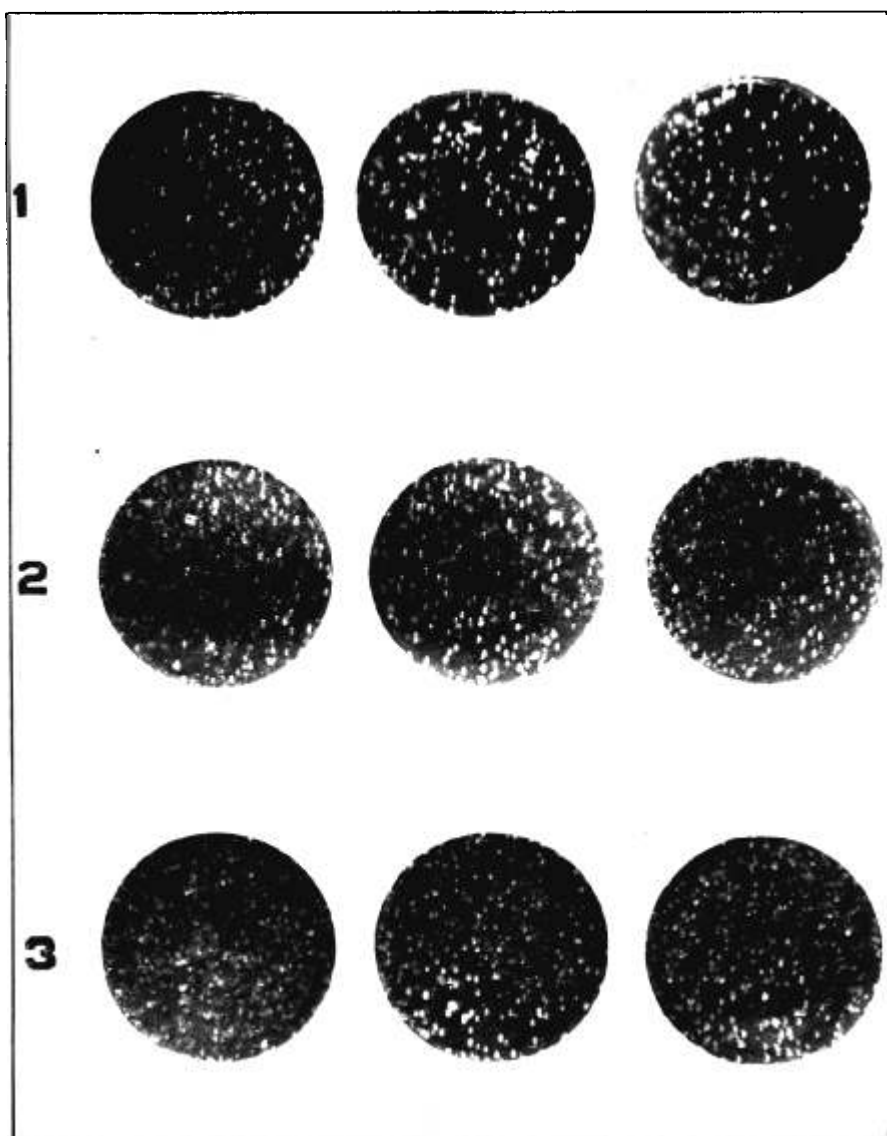
Colonies of *B. radicola* from alfalfa in plate dilutions, showing the effect of phosphorus on the numerical increase of a pure culture of this organism in sterilized soil:

Fig. 1.—Control, representing 346,000,000 bacteria in 1 gm. of the original soil.

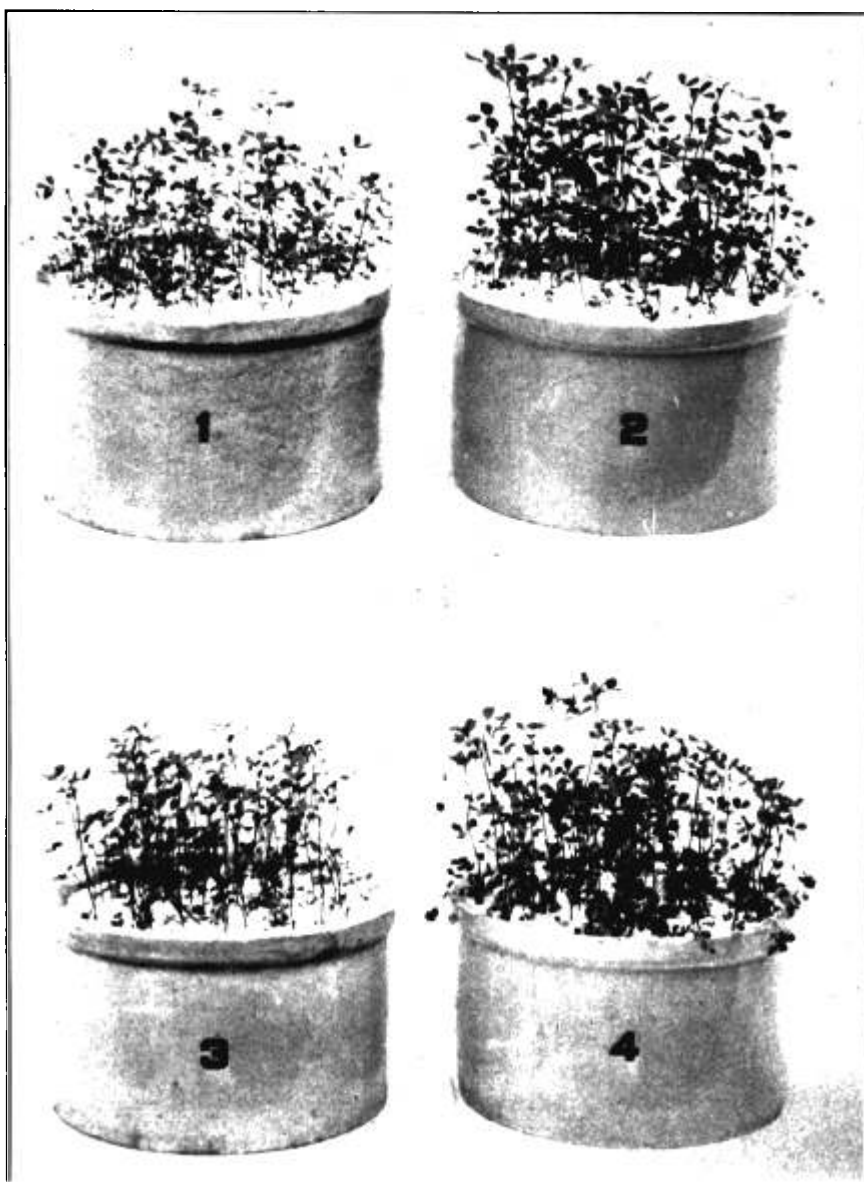
Fig. 2.—Disodium phosphate equivalent to 0.002 gm. of phosphorus added to the soil, representing 350,000,000 bacteria in 1 gm. of the original soil.

Fig. 3.—Disodium phosphate equivalent to 0.02 gm. of phosphorus added to the soil, representing 505,000,000 bacteria in 1 gm. of the original soil.











## PLATE II

**Alfalfa plants, showing the effect of phosphorus upon the rate of growth.**

**Fig. 1.—Uninoculated; no phosphorus.**

**Fig. 2.—Uninoculated; dicalcium phosphate, 5 gm.**

**Fig. 3.—Inoculated; no phosphorus.**

**Fig. 4.—Inoculated; dicalcium phosphate, 5 gm.**