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Studies on the Assimilation of Organic Phosphorus by Plants

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STUDIES ON THE ASSIMILATION OF ORGANIC PHOSPHORUS BY PLANTS

BY

ARTHUR FLOYD HECK

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THESIS

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

ARTHUR FLOYD HECK

ENTITLED Studies on the Assimilation of Organic Phosphorus by Plants

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

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STUDIES ON THE ASSIMILATION OF ORGANIC PHOSPHORUS BY PLANTS

Introduction

One of the great problems confronting the farmer of today is that of supplying the element phosphorus in amounts sufficient for maximum yields. In amount phosphorus ranks with sulphur, about 0.11 percent of the earth's crust[1], but in physiological significance it stands with nitrogen, of which there is an inexhaustible supply in the air. Unlike nitrogen, however, the only source of phosphorus is, directly or indirectly, from the earth's crust. Phosphorus is a necessary element in the existance of every living cell whether animal, vegetable or bacterial. The vital substances which go to make up the living protoplasm must contain the element phosphorus. It is then a constant need for the normal development of plants and the production of normal yields.

The largest source of phosphorus and that of greatest agricultural significance is the great supply found in the soil; while the mineral phosphates stand second and of somewhat lesser importance. The supply in the soil exists in two forms, first, inorganic or natural mineral phosphates and, second, organic phosphorus compounds. The first form is thought to be fairly constant throughout the entire depth of the soil and also tends to be more or less unchanged throughout the types, while the

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1. Hopkins, "Soil Fertility and Permanent Agriculture".

latter form exists for the most part in the surface soil, decreasing with the nitrogen and organic matter through the subsurface and subsoil. This latter source of the element phosphorus not only varies with the depth but is also present in vastly different amounts in the different types of soils, in some types being reduced to such an extent as to be of little value.

The question as to which form of soil phosphorus is the more valuable to the plants as a source of available phosphorus and from which crops obtain their greatest supply, is one which yet remains for the most part unanswered. It is generally thought that the sooner the insoluble mineral elements are incorperated into organic compounds or in combination with organic matter, the sooner do they become readily available for plant growth, and that a rather small amount of organic phosphorus is much more valuable to the plant than a very much larger amount of phosphorus in the insoluble mineral form. In order to bring more light to bear upon the assimilation of soil phosphorus and that supplied in the way of fertilizers, this present work on the assimilation of organic phosphorus was undertaken.

LITERATURE STUDIES

It is generally conceded that phosphorus in different combinations has a widely different availibility for plant growth. Müntz and Gaudechon[1] report a comparative test with mono-, di- and tricalcium phosphates in soil cultures in which lime and all other elements of plant food were supplied. The first year all three forms produced a marked increase over the check: the second year the increase was much less, while the third year there was no increase at all. The following table gives the total crop yields for the three years.

Table 1.- Yields from Mono-, Di- and Tricalcium Phosphate (grams)

Monocalcium phosphate	5867.2
Dicalcium phosphate	5356.1
Tricalcium phosphate	5509.0
Check	4429.6

A great number of culture experiments has been carried on using inorganic phosphorus compounds, but very few using organic phosphates. Nagaoka[2] investigated the relative values of organic phosphates from animal and vegetable origin and found those from animal origin to be more valuable for plant growth. Later Aso and Yoshida[3] studied the comparative values of different forms of vegetable substances containing phosphorus and showed that phytin was more valuable than

1. Ann. Sci. Agron. (1912), II, p 200.
2. Bul. Col. Agr. Tokio, II, No.3.
3. Jour. Col. Agr. Tokio, VI, No.3.

protein phosphate (nucleo-protein) but less valuable than lecithin. The experiment was carried out in pots holding 2½ kilograms of soil and into each was placed 0.395 grams of phosphorus in the various forms with the following results:

Table 2.- Comparative Yields from Organic and Inorganic Phosphorus.

Substance	Height cm	Dry Matter gr	Grain gr
Lecithin	20.4	11.7	4.5
Phytin	11.1	2.5	0.5
Nucleo-protein	9.3	2.3	0.0
Na_2HPO_4	15.9	6.0	1.8
$FePO_4$	9.6	3.5	0.8
$AlPO_4$	10.2	3.0	0.5
$Ca_3(PO_4)_2$	21.0	9.9	3.2
Check	7.8	1.4	0.0

Rose[1] grew lupine seedlings for a term of 6 to 9 days in different solutions and carefully measured the amount of root elongation every 3 days. He used such solutions as calcium nitrate, calcium sulphate, dipotassium phosphate, calcium phytate, magnesium phytate, potassium nitrate, etc. He found that the addition of phytin did not cause any stimulation of growth due to the acid radicle.

In this present work phytin was used as a source of organic phosphorus, for the reason that it was the most easily obtained in pure form and more was known of its chemical constitution. It is perhaps the first organic compound of phosphorus to be isolated and also has the advantage of being rather abundant and easily isolated and purified. In a resume on inosite-

1. Biochem. Bul., (1911-12),I, p 428.

hexaphosphate, Rose[1] reports no definite formula, but says that the ratio of C : P is 6 : 6, showing six phosphoric acid molecules united with one of inosite. It has a probable molecular weight of 714 or differing from this by three molecules of water. He thinks that phyto-phosphoric acids whether they be inosite esters or other compounds play a very significant role in higher plants, being chiefly concerned in the process of photosynthesis or in the changes of photosynthetic products. It is more than a reserve food material and in all probability is an intermediate step in the synthesis of phospho-proteins and lipoids.

Anderson[2] isolated the phytin from oats, corn and cotton seed meal and compared it with commercial phytin and found the barium salts to be identical. For phytic acid or inosite hexaphosphate he says the formula must be either $C_6H_{18}O_{24}P_6$ or an isomer. In a previous work[3] he was unable to isolate phytin or phytic acid from wheat bran. Later[4] he came to the conclusion that the amorphous salts isolated from the wheat bran were not homogeneous but mixtures of various salts of organic phosphoric acids. He was able however, to isolate a new compound, inositemonophosphate, $C_6H_{13}O_9P$. Later[5] in working with the organic phosphoric acid in wheat bran he was able to isolate inosite-triphosphoric acid, $C_6H_{15}O_{15}P_3$. He found

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1. Biochem. Bul., (1912-13), 2, p 21.
2. N.Y. Agr. Exp. Sta. Tech. Bul. 32.
3. Ibid. 22.
4. Ibid, 36.
5. Ibid. 40.

also that phytin was hydrolyzed by the enzyme phytase and that the resulting products were intermediate, consisting of inosite tri-, di- and monophosphoric acids. When he isolated the organic phosphoric acid from wheat bran in the ordinary way he found that he did not have phytin or any one acid but a mixture of intermediate products due to hydrolysis by the phytase in the wheat bran. When the bran was digested in 1% hydrochloric acid, which is sufficiently strong to destroy the enzyme phytase, the the barium salts of phytic acid may be isolated, $C_6H_{12}O_{24}P_6Ba_3$, and hence innositehexaphosphate or phytic acid, $C_6H_{18}O_{24}P_6$.

Thompson[1] isolated phytin from rice bran and unpolished rice but was unable to obtain more than a trace from the polished grain, from which she concludes that the phytin is in the outer layer and is removed by the polishing. She also found phytin hard to oxidize by aqua regia and was best determined by the Neumann method. Her analyses approach closely those of Anderson's for inosite hexaphosphate.

The knowledge concerning the organic phosphorus of the soil is somewhat meagre on account of inadequate methods of separation. Hopkins and Pettit[2] found that in certain soils the mineral composition had a tendancy to be constant in the surface, subsurface and subsoil. This was shown by the uniform potassium content and by the fact that the different samples of the same type showed a wide variation in the phosphorus content of the surface soil which largely disappeared in the subsoil.

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1. Jour. Agr. Res. III, No.5, p 425.
2. Ill. Agr. Exp. Sta. Bul., 123, p 204.

The potassium exists for the most part in the inorganic form, the nitrogen in the organic form, while the phosphorus exists in both forms. These facts suggest, therefore, a method for calculating the organic phosphorus in the soil. The difference in the amount of nitrogen in the surface and subsoil and the difference in the amount of phosphorus in the surface and subsoil give apparently the amount of nitrogen and phosphorus associated in organic combination. By means of this ratio and the total amount of nitrogen in the surface soil, the total amount of organic phosphorus in the surface soil can be calculated.

Stewart[1] thinks that the phosphorus associated with the matiere noire is not a quantitative method for the determination of organic phosphorus in soils but only a good qualitative method. He thinks the calculation method conservative and can be relied upon in drawing broad general conclusions. The following table gives the results by four different methods:

Table 3.- Total Phosphorus and Organic Phosphorus by Different Methods.

Method	Lbs per 2 Million	% of Total
Total phosphorus	919	
Organic, by calculation	423	46
" , by solution in dil. ammonia	504	55
" , by ignition	543	60
" , by evaporation (Schmoeger)	607	66

Fraps[2] claims that part if not all of the phosphorus associated with the matiere noire is inorganic and derived from the iron or aluminum phosphates. He also[3] thinks that the

1. Ill. Agr. Exp. Sta. Bul., 145.
2. Jour. Indus. and Engin. Chem., (1911),3,No.5, p 355.
3. Am. Chem. Journal, (1908),39, p 579.

ignition of phosphates renders them more soluble and that the ignition of a soil will render inorganic phosphorus soluble and interfere with the determination of organic phosphorus.

Stewart[1] contends that the phosphorus associated with the matiere noire is organic phosphorus and not simply adsorbed inorganic phosphorus. Partial and complete removal of the inorganic phosphorus by acid gave the same phosphorus content in the ammonia extract. He found the organic phosphorus content of soil to be approximately 0.0103%.

Rogers[2] in the U.S. Geological Survey gives the organic matter content of South Carolina river rock as ranging from 3.42% to 4.81%, and the land rock from 5.37% to 6.58%.

Collison[3] has worked out a method for the separation of organic from inorganic phosphorus by calling that organic which is insoluble in a 0.2% acid alcohol solution. He used this method on plant substances.

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1. Abs. in Science, (1912), 35, No.897, p 379.
2. U.S. Geol. Survey Bul., 530-J, p 96.
3. Jour. Biol. Chem., (1912), 12, p 65.

EXPERIMENTAL

MATERIALS EMPLOYED

Pots.- The culture work was all carried on in one-gallon stone jars. Each pot had a drain in the bottom covered with glass wool. Each pot had a diameter of 6 11-16 inches which makes an approximate area of 1-178000 of an acre. If computed on the surface area, 5.1 grams per pot is equivalent to one ton per acre, while if computed on the percent basis, 7 grams per pot is equivalent to one ton per acre.

Sand.- The medium for growth was pure white quartz sand. It was thoroughly leached with dilute hydrochloric acid and then washed free from the acid with distilled water. This removed practically all of the plant food, there being left not more than a trace of phosphorus. It was then air dried and weighed out and the dry fertilizers thoroughly mixed with it before being placed in the pots.

The Lime.- In series 29-56 dolomite was used to supply the lime and also magnesium. It was of good quality, finely ground and gave no test for phosphorus. In all other series pure precipitated calcium carbonate was used to supply the lime and the magnesium in these series was supplied in the soluble form as magnesium sulphate.

Soluble Plant Foods.- Kainite free from phosphorus was used to supply the potassium in oats series 29-56, but in all other series potassium sulphate was used and applied in

solution. The magnesium when supplied in the soluble form was applied in the form of magnesium sulphate. The nitrogen was added in the form of ammonium nitrate, excepting one or two applications to the clover series where potassium nitrate was used to supply both potassium and nitrogen. The application of these soluble plant foods was made as directed by Hopkins and Pettit[1] and in amounts sufficiently large for maximum yields. The same amount of soluble plant food was added to each pot.

Phosphorus.- The phosphorus was supplied in five different forms, namely, Tennessee rock phosphate, pure phytin[2], commercial or crude phytin[3], tricalcium phosphate, alfalfa and leached alfalfa. The rock was a random sample obtained at the University farm. It was ground to pass a 100-mesh sieve and contained 12.12% of phosphorus in the air dry condition. The pure phytin, calcium phytate, had a phosphorus content of 19.2% in the air dry condition. The crude phytin was obtained from corn was probably mixed salts of phytic acid. The crude phytin contained 12.96% phosphorus. The alfalfa used as a source of phosphorus was from the third cutting and ground to a fine powder before being applied. It had a phosphorus content of 0.168% air dry. Part of the alfalfa was leached with 0.2% hydrochloric acid and then washed free from acid with distilled water. It was dried and after being ground analyzed 0.067%

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1. "Soil Fertility Labratory Manual".
2. From the Schaefer Alkalcid Works, Maywood, N.J., and kindly furnished by Dr. Grindly of the Animal Nutrition Dept.
3. Donated by the Minor Labratories, Chicago.

phosphorus. Tricalcium phosphate containing 14.6% phosphorus was used to supply phosphorus in the molecular state.

Water.- All water was ammonia-free distilled water.

METHODS OF ATTACK

The primary object of this work was to determine the relative availibility of organic and inorganic phosphates, and secondarily to throw some light on the role of organic phosphorus in plant growth. In the first place similar amounts of organic phosphorus in the form of pure and crude phytin and inorganic phosphorus in the form of rock phosphate were used and oats was grown (Series 29-56). Following this preliminary series all of the forms of organic and inorganic phosphorus mentioned above, excepting pure phytin, were applied in different amounts both singly and in different combinations in order to get the maximum yield and a better comparison(Series 101-184).

Probably the most interesting fact of the entire work came out in the clover series, half of which was planted in the oats on series 29-56 just before the harvest; the other half being a new series, 1-28. All together this series brought out strongly the durability of available phosphorus, in the presence of lime. Following the culture work sufficient analytical work was done to connect the isolated points and bind them together into something of a convincing proof.

All weights are reported on the air dry basis and in grams per pot.

OATS SERIES 29-56

The object of this preliminary series was to compare the yields of oats grown on organic and inorganic phosphates. Three different forms of phosphorus were used, namely, rock phosphate, pure phytin and crude phytin. It was the intention to add equal amounts of phosphorus in each of the different forms, but through an error in the analysis, different amounts were added. Although the treatments are not identical they will give an idea as to the relative availibility of the different forms of phosphorus. All pots excepting 29 and 30 received 28 grams of dolomite and 1.75 grams of kainite and soluble nitrogen in the form of ammonium nitrate. Seven kilograms of clean quartz sand was weighed out and the dolomite, kainite and the phosphorus-bearing substances added and thoroughly mixed before being placed in the pots. After being wet with distilled water a sufficient number of oats were planted to insure a stand and when the seedlings were a few days old they were thinned to ten plants per pot. According to analysis of of kernels of oats not more than 0.9 milligram of phosphorus was added to each pot in the seed. In the following table which which shows the treatment and yields, the treatments are expressed in milligrams of phosphorus per pot and the yields in grams per pot. All yields of the two duplicate pots, unless otherwise indicated, are averaged in the table.

Table 4.- Treatments and Yields of Oats Series 29-56.

Pot No.	Treatment mg P per pot	Wt Crop grams	Wt Grain grams
Checks			
29-30	None	0.8	----
31-32	All but P	0.8	----
Rock Phosphate Series			
33-34	212	2.7	.015
35-36	424	3.9	.133
37-38	848	9.8	.118
39-40	2545	18.9	.207
Pure Phytin Series			
41-42	221	22.9	6.743
43-44	442	18.7	4.377
45-46	883	17.8	2.375
47-48	2650	16.7	1.408
Crude Phytin Series			
49-50	175	24.2	4.598
51-52	351	25.3	5.116
53-54	702	20.4	3.197
55-56	2106	6.9	.263

It will be observed that the rock phosphate even in large amounts produced scarcely any grain. This may be largely due however, to the fact that the oats matured in January. With the pure phytin the minimum treatment produced the maximum yield. With the crude phytin the first two treatments produced the maximum yields. The experiment tends to show two points; first, that organic phosphorus as phytin is much more readily assimilated by plants than the phosphorus from rock phosphate, and second, that phytin supplied in large amounts is deleterious. By considering the amount of phosphorus supplied in the two forms of phytin it will be seen that the curves of production are very close together and that an amount of phosphorus as

phytin of about 220 milligrams per pot seems to produce about the maximum yield. Larger amounts than this seem to be deleterious. The toxic effect from the crude phytin seems to be more noticeable during the growth, due perhaps to the presence of the putrifaction products of some nitrogenous substance present.

The following table shows the analytical results of this series in tabular form:

Table 5.- Percentage and Total Milligrams of Phosphorus in Grain and Straw (Oats series 29-56)

Pot No	Treatment mg P per pt	Grain % P	Straw % P	Grain mgP	Straw mgP	Crop Total P
Checks						
29-30	None	---	.065	---	.55	.55
31-32	All but P	---	.060	---	.46	.46
Rock Phosphate Series						
33-34	212	.65	.063	.08	1.69	1.77
35-36	424	.47	.065	.62	2.41	3.03
37-38	848	.63	.082	.75	7.94	8.69
39-40	2545	.60	.125	1.25	23.3	24.55
Pure Phytin Series						
41-42	221	.62	.295	41.8	47.6	89.4
43-44	442	.64	.815	28.0	116.7	144.7
45-46	883	.74	1.39	17.6	215.1	232.7
47-48	2650	.85	1.94	11.9	246.7	308.6
Crude Phytin Series						
49-50	175	.53	.164	24.3	32.0	56.3
51-52	351	.59	.404	30.2	81.8	112.0
53-54	702	.64	.764	20.4	165,8	186.2
55-56	2106	.73	.992	1.9	64.8	66.7

From table 5 it will be noticed that there is a constant increase in the percentage of phosphorus in both grain and straw, which holds for both forms of phytin and also for the rock phosphate. The grain shows only a small amount of

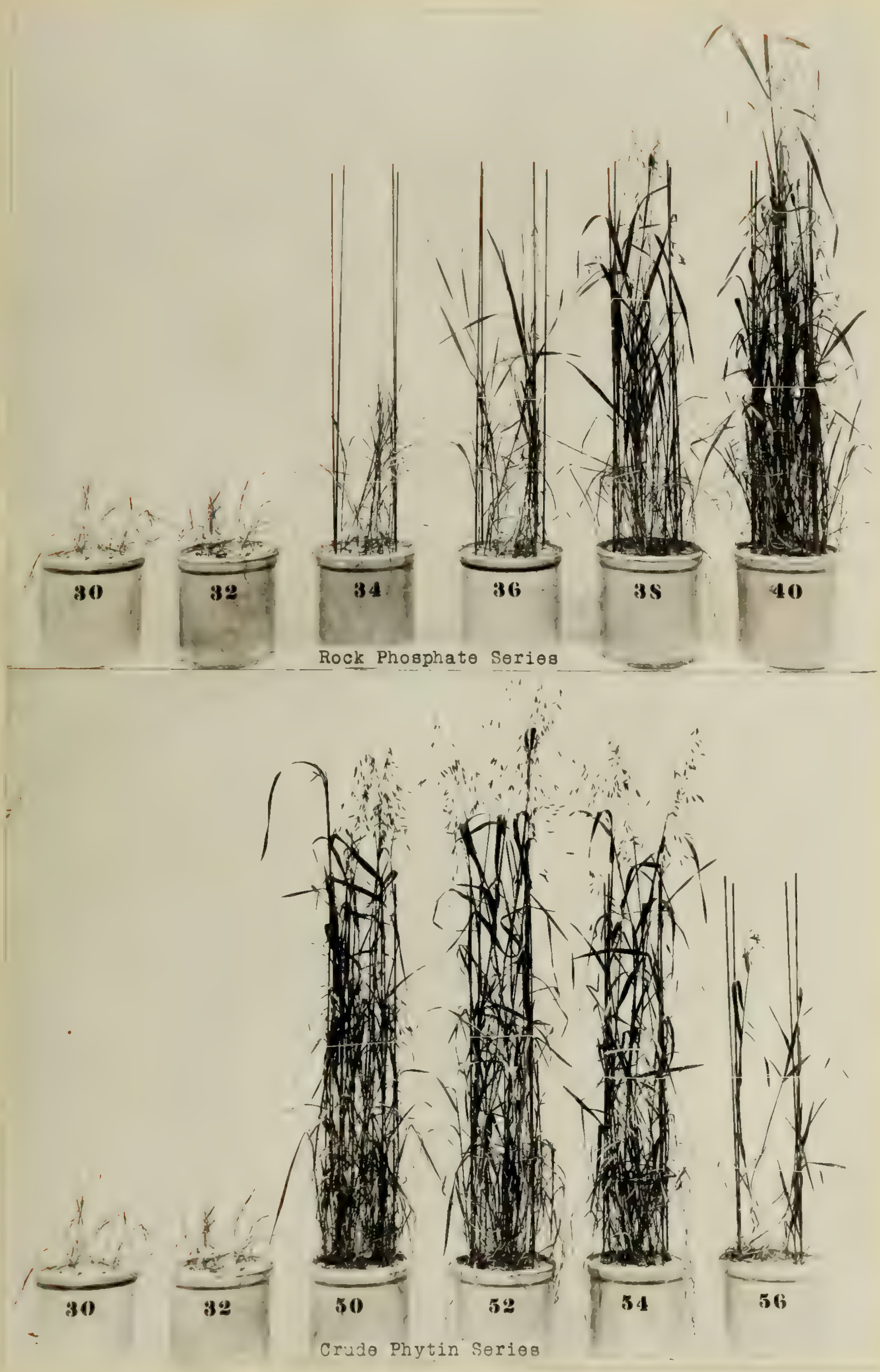


Plate I. Showing Relative Growth of Oats on Rock Phosphate and Crude Phytin.

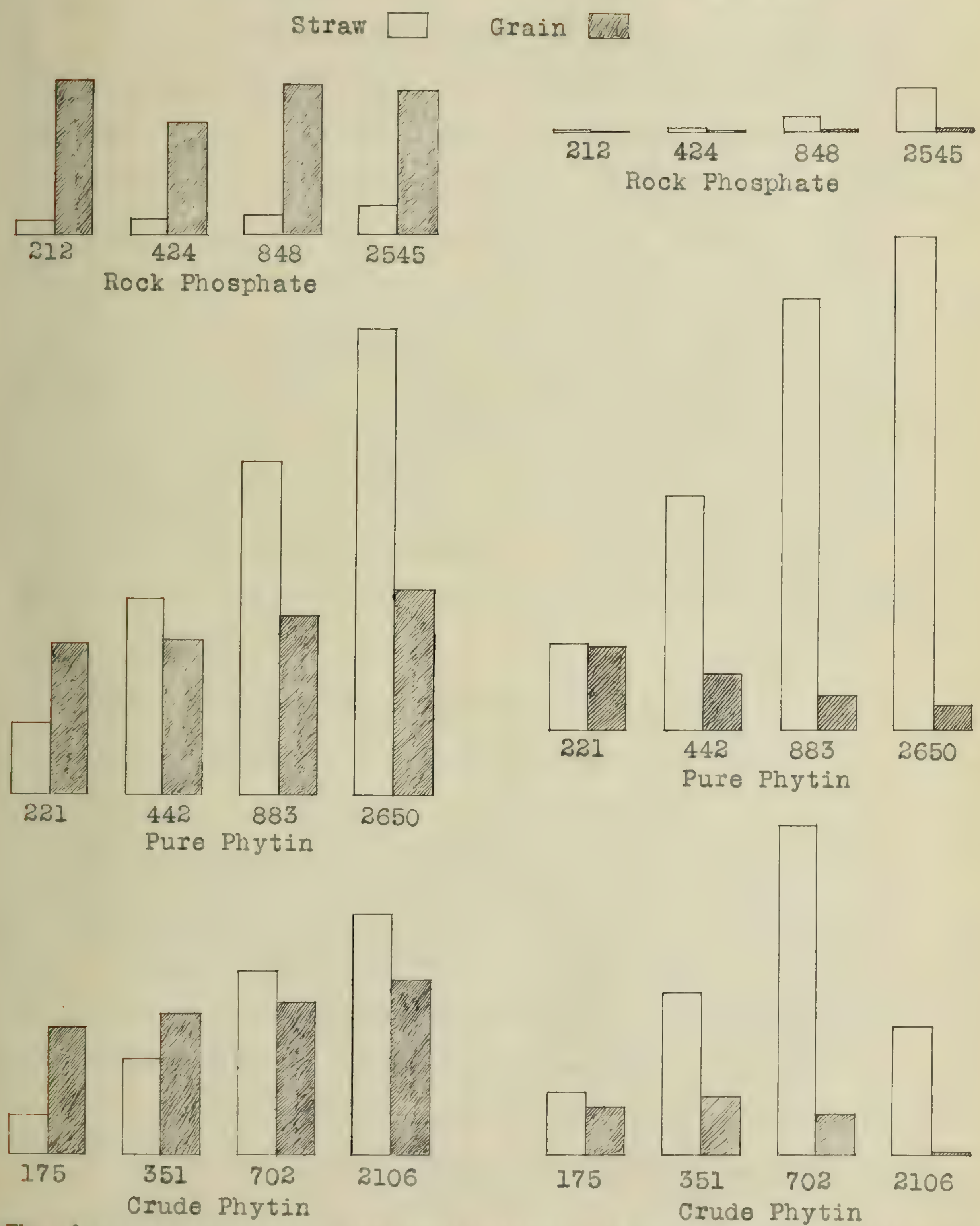
tolerance while in the straw a wide tolerance for phosphorus is to be seen.

Plate I shows the growth of oats on the rock phosphate and crude phytin series.

On the following page is shown graphically the percentages and total phosphorus content of the grain and straw.

Percentage Phosphorus in Straw and Grain of Oats

Total Phosphorus in Straw and Grain of Oats



The figures represent the application of Phosphorus in miligrams per pot

OATS SERIES 101-184

This second oats series was much larger than the first, comprising 84 pots. In this series the phosphorus was supplied in five different forms, each being added in four different amounts. In the first five series the phosphorus was added singly or only one form to a series, while in the last five series, two kinds were combined in each series, one organic and the other inorganic. The object of the first five series was mainly as a check on the combination series. An attempt was made to confine the applications to such amounts that pot limitation would not be a factor but in some cases in the higher treatments, however it did enter in. The applications of phosphorus on the single series were made in the following forms and amounts per pot;

<u>Tricalcium phosphate</u>.- 28, 55, 110 and 330 mg.

<u>Rock phosphate</u>.- 220, 440, 880 and 1320 mg.

<u>Alfalfa</u>.- 11.9, 23.8, 35.7 and 47.6 mg.

<u>Leached alfalfa</u>.- 4.9, 9.8, 14.7 and 19.6 mg.

<u>Crude phytin</u>.- 11.9, 23.8, 35.7 and 47.6 mg.

The alfalfa and leached alfalfa were applied at the rate of 7, 14, 21 and 28 grams per pot respectively, thus adding to each pot a comparative amount of organic matter.

The combination series contained in each pot one organic and one inorganic phosphatic substance and were made up as follows;

<u>Alfalfa plus rock phosphate</u>.- To each pot of an alfalfa series was added 880 milligrams phosphorus as rock phosphate.

<u>Leached alfalfa plus rock phosphate</u>.- To each pot of a leached alfalfa series was added 880 milligrams of phosphorus as rock phosphate.

<u>Phytin plus rock phosphate</u>.- To each pot of a phytin series was added 880 milligrams of phosphorus as rock phosphate.

<u>Rock phosphate plus alfalfa</u>.- To each pot of a rock phosphate series was added 21 grams of alfalfa containing 35.7 milligrams of phosphorus.

<u>Rock phosphate plus phytin</u>.- To each pot of a rock phosphate series was added 35.7 milligrams phosphorus as phytin.

In this way by combining inorganic phosphorus, first, with organic phosphorus combined with large amounts of readily decomposing organic matter and, second, with organic phosphorus combined with small amounts of organic matter, a comparison with a theoretical yield obtained by taking the sum of the yields on the singly treated pots can be made.

In order to allow ample time for the decomposition of the alfalfa and the establishment of an equilibrium in the sand, the fertilizers were mixed with the sand, potted and wet down on December 28th with a liberal amount of soil infusion. On January 20th, 16 kernels of oats were planted in each pot and in a week there was a fine stand. In a few days most of the plants in the pots containing alfalfa or leached alfalfa had died or were suffering severely. On February 4th these affected

pots were replanted and came on without any further noticeable difficulty.

The following table gives the treatments and yields on the oats on the singly treated series:

Table 6.- Treatment and Weights of Grain and Total Crop on Oats Series 101-144

Pot No	Treatment mg P per pt	Wt Crop grams	Wt Grain grams
Checks			
101- 2	None	0.9	----
103- 4	All but P	1.6	----
Tricalcium Phosphate Series			
105- 6	28	2.0	.09
107- 8	55	2.0	.12
109-10	110	3.5	.69
111-12	330	4.6	1.17
Rock Phosphate Series			
113-14	220	7.0	1.08
115-16	440	10.3	2.15
117-18	880	15.5	3.59
119-20	1320	16.2	4.84
Alfalfa Series			
121-22	11.9	6.6	.96
123-24	23.8	7.0	1.73
125-26	35.7	10.5	2.26
127-28	47.6	8.5	2.48
Leached Alfalfa Series			
129-30	4.9	1.4	----
131-32	9.8	1.2	----
133-34	14.7	1.1	----
135-36	19.6	1.1	----
Crude Phytin Series			
137-38	11.9	3.7	.48
139-40	23.8	6.3	1.09
141-42	35.7	9.5	2.04
143-44	47.6	15.7	4.07

It will be seen that for each increment of phosphorus there is an increase in the yield of grain until the maximum is

reached. The alfalfa series produced better than the phytin in the lower treatments but drops off in the higher treatments.

Table 7.- Treatment and Yields on Combination
Series 145-184

Pot No	Treatment mg P per pt	Wt Crop grams	Wt Grain grams	Theor. Yield
Alfalfa plus Rock Phosphate				
145-46	Alfalfa 11.9, R.P. 880	13.6	3.72	4.55
147-48	" 23.8, " "	15.7	4.10	5.32
149-50	" 35.7, " "	12.8	3.56	5.85
151-52	" 47.6, " "	11.0	2.85	6.08
Leached Alfalfa plus Rock Phosphate				
153-54	Lea.Alf. 4.9, R.P. 880	10.6	2.21	3.59
155-56	" 9.8, " "	7.0	1.79	3.59
157-58	" 14.7, " "	7.6	2.06	3.59
159-60	" 19.6, " "	7.0	1.76	3.59
Phytin plus Rock Phosphate				
161-62	Phytin 11.9, R.P. 880	18.6	4.91	4.07
163-64	" 23.8, " "	17.0	4.81	4.68
165-66	" 35.7, " "	21.2	6.07	5.63
167-68	" 47.6, " "	19.1	5.45	7.66
Rock Phosphate plus Alfalfa				
169-70	R.P. 220, Alfalfa 35.7	12.6	3.74	3.34
171-72	" 440, " "	15.4	3.48	4.41
173-74	" 880, " "	12.8	3.56	5.85
175-76	" 1320, " "	15.5	4.48	7.10
Rock Phosphate plus Phytin				
177-78	R.P. 220, Phytin 35.7	15.7	3.80	3.12
179-80	" 440, " "	16.0	3.88	4.19
181-82	" 880, " "	21.2	6.07	5.63
183-84	" 1320, " "	21.6	6.22	6.90

As has been pointed out in table 6 the alfalfa in the three lower treatments produced a better yield than phytin, the treatments being the same as regards phosphorus. When 880 milligrams of phosphorus as rock phosphate is added to each pot of the alfalfa and phytin series, the alfalfa combination in

no case equals the theoretical sum, while the phytin combination produces in three cases a larger yield than the theoretical sum. This same trend is noticed also when the organic phosphates are added to the rock phosphate as in series 169-76 and 177-84.

Plates II, III, IV, V, VI and VII show at the time of harvest the different series both singly and in combination.

Plate II. Tricalcium Phosphate, Leached Alfalfa and Leached Alfalfa plus Rock Phosphate

Plate III. Alfalfa and Alfalfa plus Rock Phosphate.

Plate IV. Rock Phosphate and Rock Phosphate plus Alfalfa

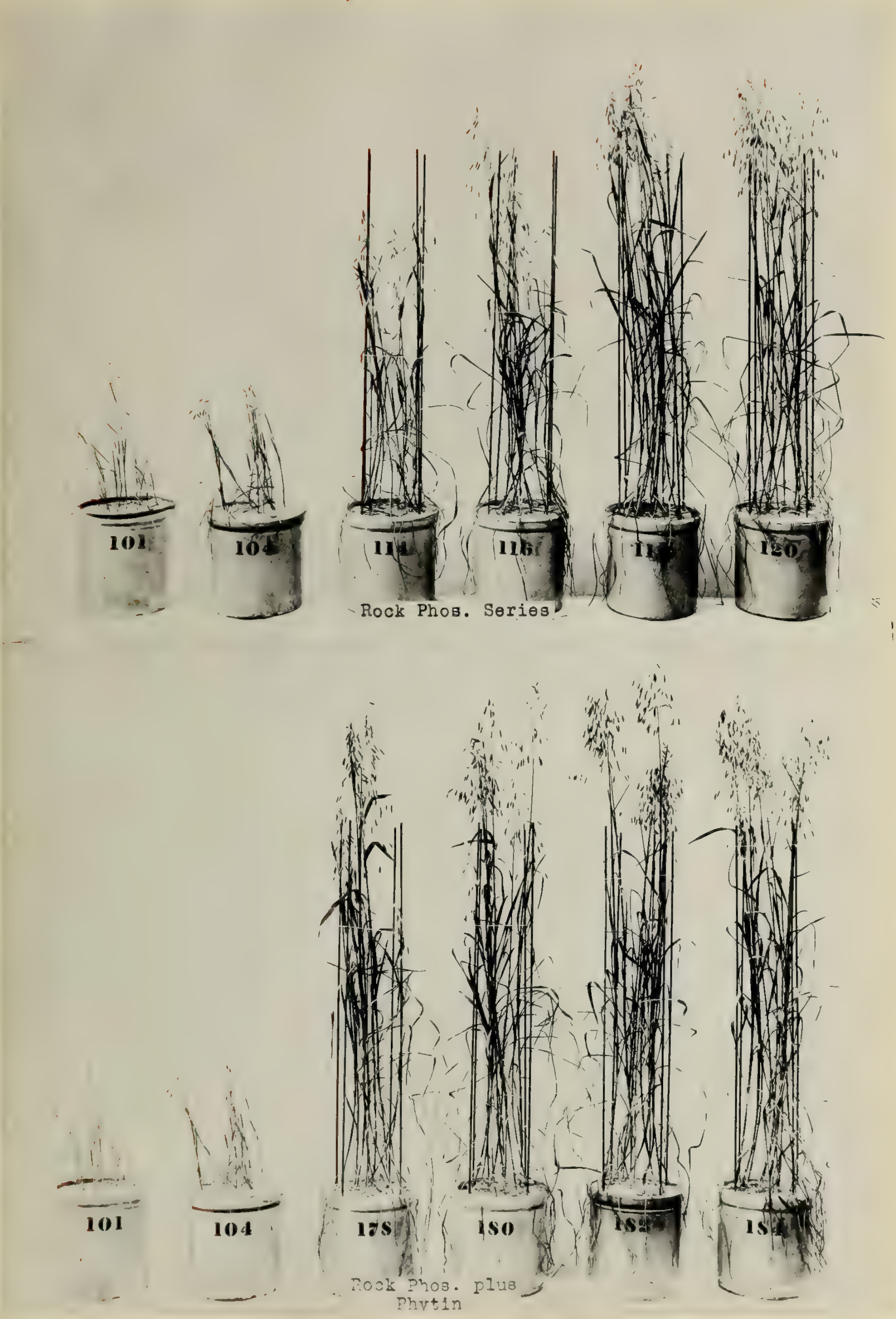


Plate V. Rock Phosphate and Rock Phosphate plus Phytin

Plate VI. Crude Phytin and Crude Phytin plus Rock Phosphate

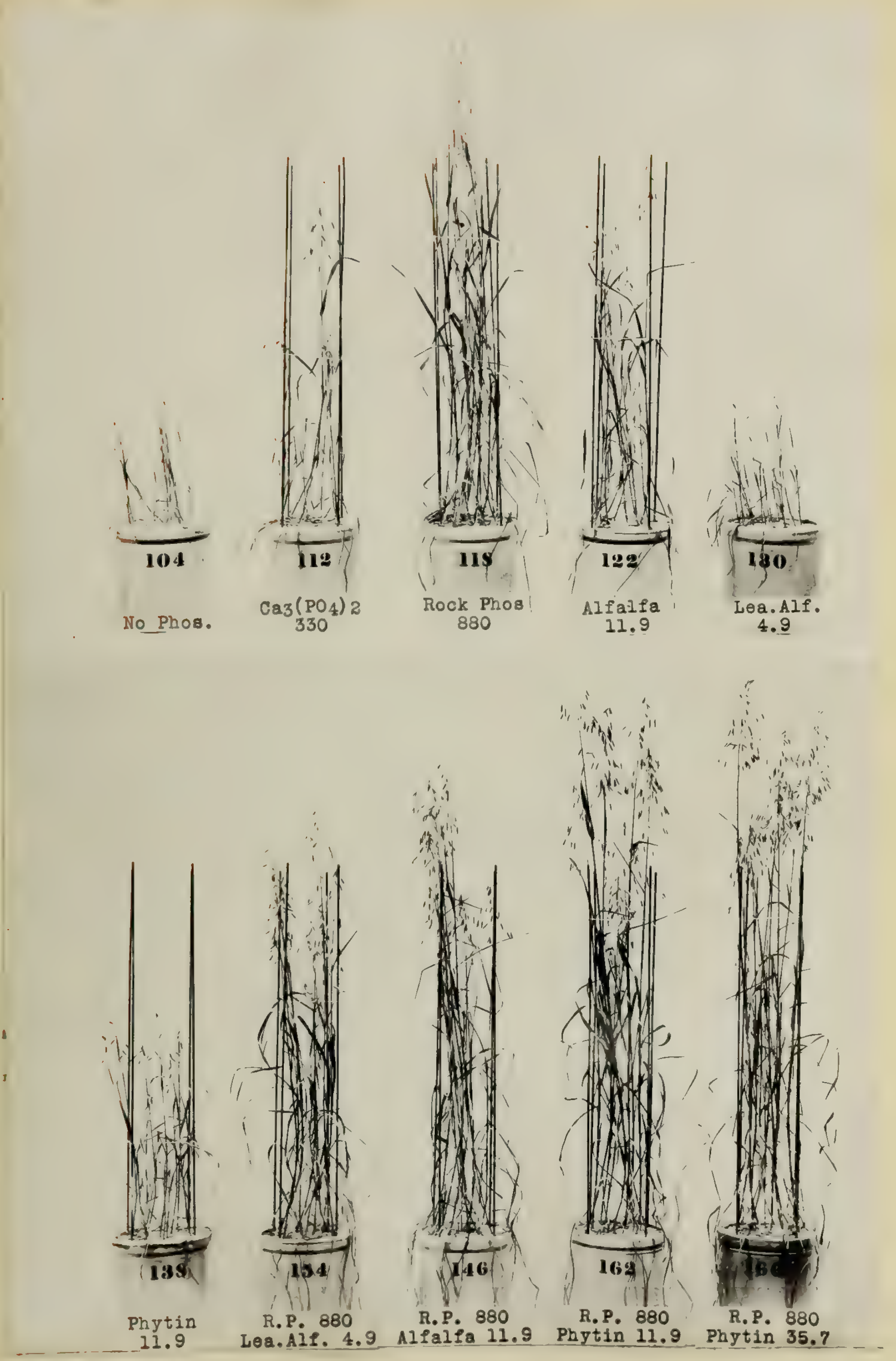


Plate VII. The Figures Show the Number of Milligrams of Phosphorus Added in the Forms Indicated

CLOVER SERIES 1-28 and 29-56

At the same time that the first clover series (29-56) was started, a clover series (1-28) was also started with the same treatments as the oats. On account of injuries by red spider and soap spray the clover was damaged to such an extent that it was discarded.. Later the same series was reconstructed according to the following plan;

1 - 2.- Sand alone, no treatment.
3 - 4.- These and all succeeding pots received the following treatment;
28 grams precipitated calcium carbonate.
Soluble potassium, magnesium and nitrogen.
Liberal application of soil infusion.

Rock Phosphate Series				
5 - 6.-	220	milligrams	of	phosphorus
7 - 8.-	440	"	"	"
9 -10.-	880	"	"	"
11-12.-	2640	"	"	"
Tricalcium Phosphate Series				
13-14.-	36	milligrams	of	phosphorus
15-16.-	73	"	"	"
17-18.-	146	"	"	"
19-20.-	440	"	"	"
Crude Phytin Series				
21-22.-	18	milligrams	of	phosphorus
23-24.-	36	"	"	"
25-26.-	73	"	"	"
27-28.-	220	"	"	"

This series was planted to red clover on Jan. 26th. At the same time the oats series 29-56, which was harvested Feb. 10th, was also planted to red clover. In the seed planted about 0.2 milligram of phosphorus was added to each pot. The clover was allowed to reach a stage where it was evident from

the growth that it would live when it was thinned to 12 plants per pot. The red spider and white fly were held in check by daily spraying with cold distilled water. Some injuries which were thought to be due to the ammonium nitrate were noticed, but were not permanent and were not seen on series 29-56.

On April 26th the first cutting was made. It will be noted, however, that some of the pots were not harvested because the plants were too small. Those pots not harvested are as follows;

Checks, 1-4.

Tricalcium phosphate, 13-20.

Checks, 29-32.

Rock phosphate, 33-38 and 40.

The treatments on series 29-56 are obtained by subtracting from the total number of milligrams of phosphorus originally placed in each pot, the number of milligrams of phosphorus removed in the grain and straw of the oats crop. Aside from leaching there is no possible way of loss and it is perhaps safe to say that the leaching can be disregarded.

The following table gives yields of the first cutting together with the percent of phosphorus and the total number of milligrams of phosphorus in the crop. The figures are averages of the two duplicate pots.

Plates VIII and IX show the clover at the time of the first cutting.

Table 8.- Yields and Analyses of the First Cutting of Clover

Pot No	Treatment mg P per pt	Yield grams	Per Ct P	Mg P in Crop
Rock Phosphate Series				
5 - 6.	220	.48	.154	.74
7 - 8.	440	.82	.179	1.47
9 -10.	880	1.57	.175	2.78
11-12.	2640	3.91	.212	8.28
Crude Phytin Series				
13-14.	18	.74	.16	1.18
15-16.	36	1.65	.253	3.57
17-18.	73	3.26	.33	10.70
19-20.	220	6.40	.335	21.47
Rock Phosphate Series (after oats)				
39	2520	.79	.266	2.10
Pure Phytin Series (after oats)				
41-42.	132	.65	.522	3.40
43-44.	297	1.38	.555	7.69
45-46.	550	2.18	.691	15.10
47-48.	2341	2.08	.853	17.56
Crude Phytin Series (after oats)				
49-50.	119	.38	.615	2.43
51-52.	239	.44	.895	3.92
53-54.	516	1.76	.768	12.87
55-56.	2040	5.00	.678	33.89

A nice comparison exists between pots 7-8 and 21-22, 9-10 and 23-24, and 11-12 and 25-26 (See plate VIII, middle figure), both in yields and total number of milligrams assimilated by the plant. From the data on these pots a ratio of availibility could be calculated for the phosphorus in phytin and rock phosphate.

On May 15th a second cutting was made and the roots removed and washed as free from sand as possible. It was impossible to remove all of the sand but what remained did not

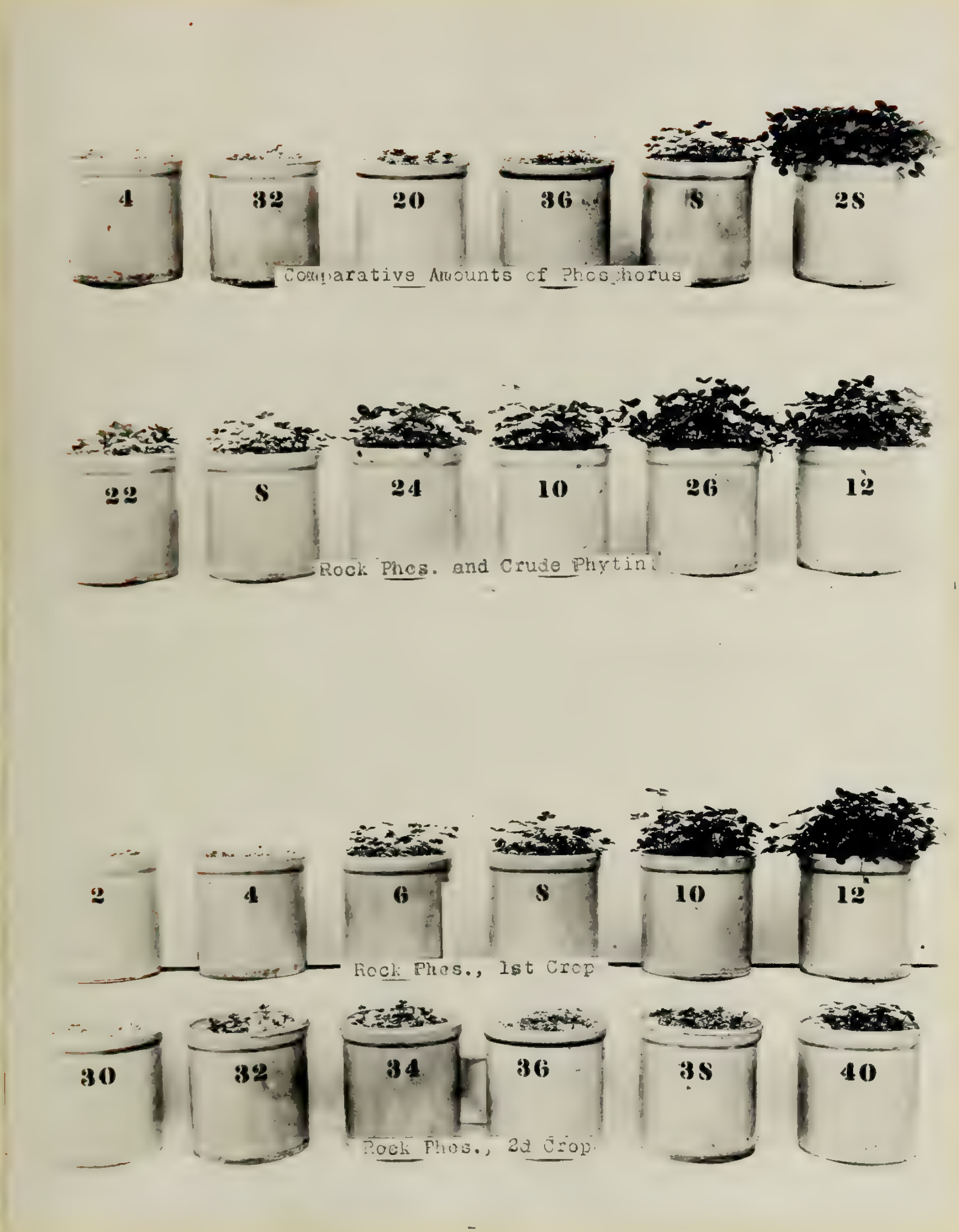


Plate VIII.- Clover at the Time of First Cutting.

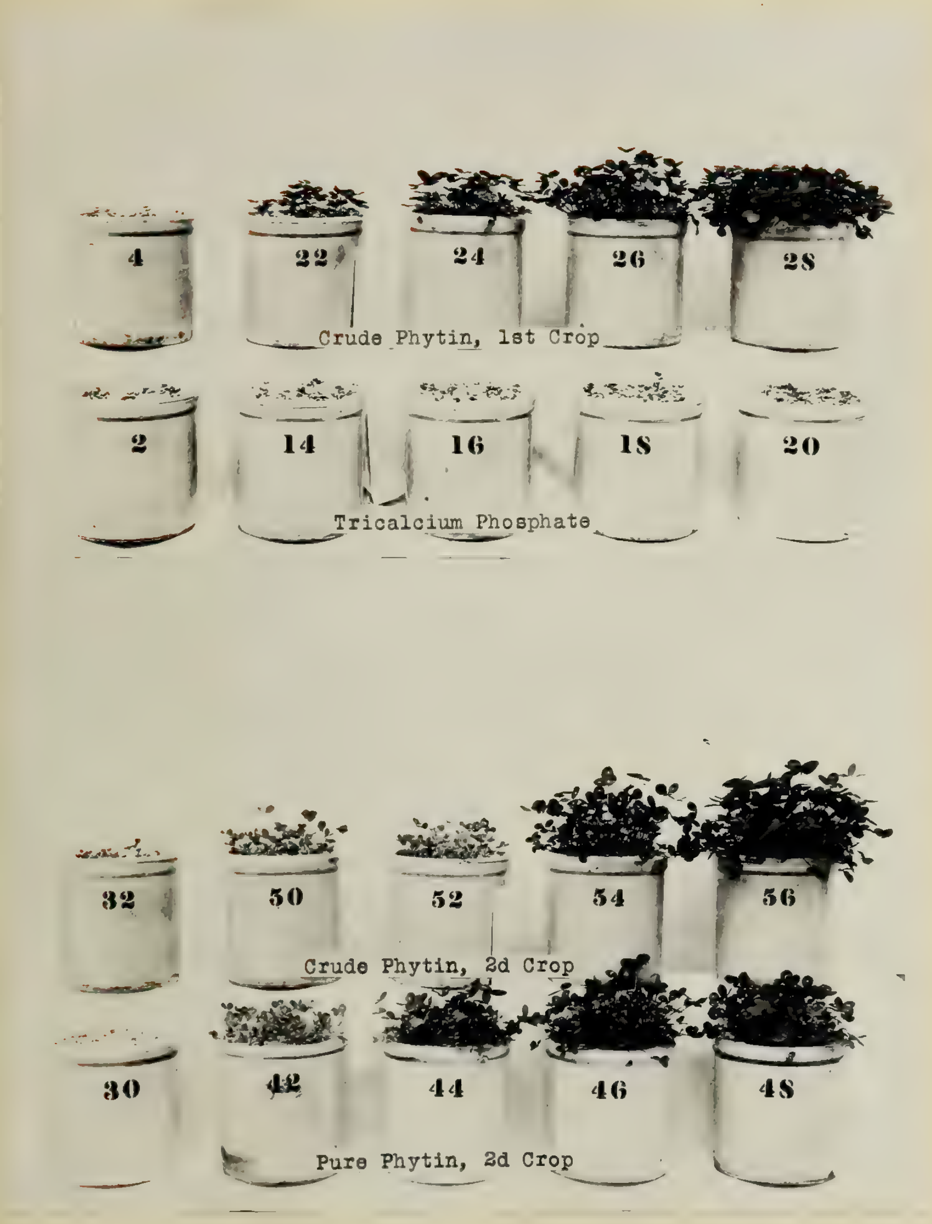


Plate IX. Clover at the Time of First Cutting

interfere seriously with the reliability of the weights. In the following tables where total crop weights are given it means that the plants never attained a size large enough to harvest and the total weight is of the entire plant. The following table gives the yields on clover series 1-28.

Table 9.- Yields of Clover on Series 1-28.

Pot No	Treatment mg P per pt	Tops(grams) 1st c't'g	Tops(grams) 2d c't'g	Roots grams	Total grams
Checks					
1 - 2.	None	----	----	----	.39
3 - 4.	All but P	----	----	----	.28
Rock Phosphate Series					
5 - 6.	220	.48	.57	1.17	
7 - 8.	440	.82	1.02	1.60	
9 -10.	880	1.57	1.86	3.00	
11-12.	2640	3.91	3.70	5.68	
Tricalcium Phosphate Series					
13-14.	36	----	----	----	.50
15-16.	73	----	----	----	.40
17-18.	146	----	----	----	.56
19-20.	440	----	----	----	.90
Crude Phytin Series					
21-22.	18	.74	.78	1.63	
23-24.	36	1.65	1.95	2.79	
25-26.	73	3.26	3.20	3.96	
27-28.	220	6.40	2.96	5.70	

It will be noted that although the tricalcium phosphate was not applied in sufficient amounts, its availibility is insufficient for the needs of the plant. A very small amount of phosphorus in phytin is equivalent in yield to a large amount of either tricalcium phosphate or rock phosphate, while the tricalcium phosphate stands much below the rock phosphate in productivity. The following table gives the yields on the

series where the clover followed oats;

Table 10.- Yields of Clover on Series 29-56.

Pot No	Treatment mg P per pt	Tops(grams) 1st c'tg	Tops(grams) 2d c'tg	Roots grams	Total grams
Checks					
29-30.	None	----	----	----	---
31-32.	All but P	----	----	----	.51
Rock Phosphate Series					
33-34.	210	----	----	----	.52
35-36.	421	----	----	----	.75
37-38.	840	----	----	----	1.06
39	2341	.79	2.68	4.34	
40	2341	----	.92	2.75	
Pure Phytin Series					
41-42.	132	.65	1.92	2.75	
43-44.	297	1.38	2.51	3.45	
45-46.	550	2.18	3.85	4.36	
47-48.	2341	2.08	4.07	5.78	
Crude Phytin Series					
49-50.	119	.38	1.14	1.92	
51-52.	239	.44	Removed for analysis		
53-54.	516	1.76	3.09	4.73	
55-56.	2040	5.00	4.22	5.89	

Plates VIII and IX show the clover at the time of the first cutting and plate X shows it at the time of the final harvest.

In series 33-40, the second crop on the rock phosphate or where the clover followed oats, it will be seen that the low yields produced are in a way identical with those on the tricalcium phosphate series 13-20. An exception exists however, on pots 39 and 40, but this may be explained by the fact that the roots of the oats were not removed. In these two pots, judging from the amount of oats straw produced, con-

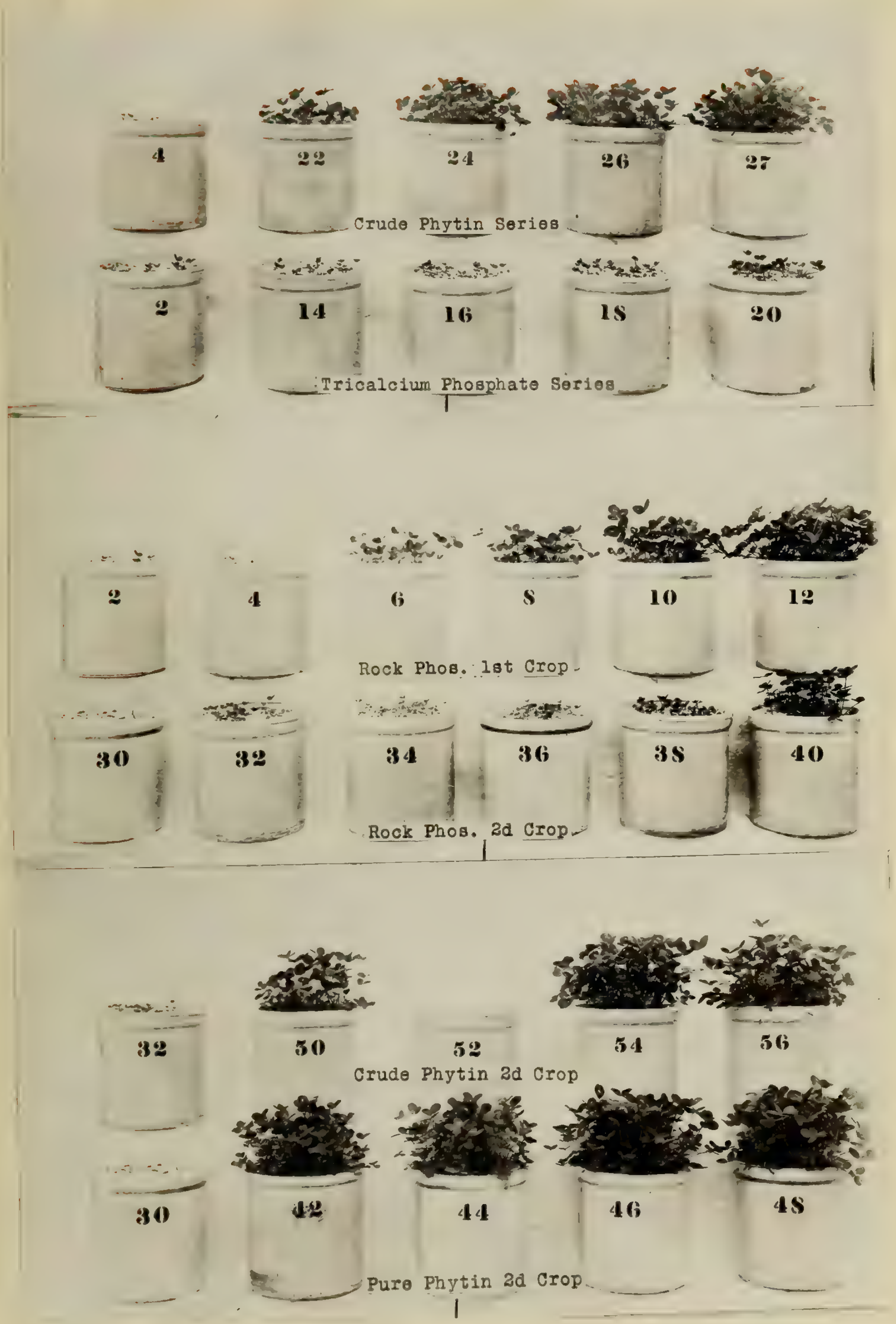


Plate X. Clover at the Time of Second Cutting

siderable quantities of roots must have been left which on decomposition liberated organic phosphorus which was utilized by the clover. The percentage phosphorus content of the clover on these pots would also indicate organic origin. It will be noted that most of the growth was during the last month, which indicates that growth was delayed until the phosphorus was liberated by decomposition.

ANALYTICAL

In any kind of experimental work such as this it is always necessary to do enough analytical work to either confirm or disprove the points that may arise, and this plan was carried out in connection with the present work.

In the crude phytin series on which clover followed oats, it was noticed that the clover crop was poorest on the lower treatments where the best crop of oats was produced, although a sufficient amount of phosphorus was present to grow a maximum crop. Originally it was thought that all of the phosphorus in crude phytin was organic but on observing this phenomenon the question arose as to whether the phosphorus in crude phytin might not be in part inorganic. Collison[1] had separated organic and inorganic phosphates by the use of acid alcohol and his method was tried in this case. As a solvent he used 94-96% alcohol containing 0.2% hydrochloric acid. The finely ground substance is shaken up with this acid alcohol solution for several hours or until all of the inorganic phosphorus goes into solution. The difference between the total phosphorus and that in solution being organic.

In cases where mineral phosphates were to be separated it was found that 0.2% hydrochloric acid was not sufficiently strong to dissolve all of the inorganic phosphorus. The acid

1. Jour. Biol. Chem., (1912), 12, p 65.

was then increased to one per cent, thus making a final solution of about 94% alcohol containing 1% hydrochloric acid.

A weighed amount of the substance was placed in a shaker bottle with 200cc of the 1% acid alcohol, shaken at intervals and allowed to stand until the reaction had reached an equilibrium, which on the mineral phosphates was as long as ten days or two weeks. Trial separations were made on phytin and pure tricalcium phosphate and the method checked up in this way.

Table 11.- Trial Separation of Organic and Inorganic Phosphorus.

Substance	Wt Sample grams	Inorg. P. mg	Org. P. mg
Separately			
$Ca_3(PO_4)_2$	0.05	7.31	----
Phytin	0.05	.84	5.64
Together			
$Ca_3(PO_4)_2$	0.05	8.20	5.59
Phytin	0.05		

These results which are averages of four determinations check within the limit of experimental error and indicate a complete separation in 1% acid alcohol.

In view of the fact that the trial separation was complete and accurate, a separation was tried on pots 51 and 52, on which only a small amount of clover was produced, but on which had grown the maximum oats crop of this series. In the beginning 351 milligrams of phosphorus as crude phytin was added to each pot. According to the above analysis this con-

tained only 305 milligrams of organic phosphorus. The total oats crop removed in the grain and straw 112 milligrams of phosphorus. The clover crop removed in all 11 milligrams of phosphorus, making a total 123 milligrams of phosphorus removed by the crops, and leaving 182 milligrams of presumably organic phosphorus together with the 46 milligrams of inorganic phosphorus applied in the phytin at the beginning. As an average of four determinations on the sand from pots 51 and 52, only 31 milligrams of organic phosphorus per pot could be found. This shows that at least 151 milligrams of organic phosphorus has gone over to the molecular inorganic state and in the presence of lime to tricalcium phosphate. The total milligrams of phosphorus assimilated in the first cutting from pots 51 and 52 check very closely with that assimilated in pots 23 and 24 into which was placed 36 milligrams of phosphorus each in the form of phytin and carrying 31.3 milligrams of organic phosphorus.

It was also noticed early that the clover grown on the rock phosphate series which had previously grown oats, did not make the growth of the clover grown on rock phosphate which had not previously grown oats(See tables 9 and 10). The difficulty seemed to be similar to that affecting the crude phytin clover. Since the rock phosphate and phytin had been treated exactly alike and had both shown the same phenomenon namely that of reduced yields, it was considered worth while to attempt to determine whether or not the rock phosphate contained any organic phosphorus. Samples of rock phosphate were run for

organic phosphorus with the following results;

Table 12.- Organic Phosphorus in Rock Phosphate.

	Wt Sample grams	Milligrams Phos.	Per cent Phos.
Total Phosphorus	0.05	6.06	12.12
Acid Alcohol Soluble	0.05	5.75	11.50

This shows that 5.1% of the phosphorus in rock phosphate is organic or 0.62% of the mineral is organic phosphorus. There is the possibility however, that this figure is too high but coupled with the fact that rock phosphate may contain over 6% of organic matter would indicate perhaps that it does contain some organic phosphorus. In this connection it might be well to note that rock phosphate contains about 0.5 milligram of organic nitrogen per gram. These points together with the fact that the clover following oats on the rock phosphate was inferior, all tend to indicate some form of readily available phosphorus in the rock, which tends in the presence of lime to go to the tricalcium state or some form which is not available. The nitrogen in rock phosphate is low in comparison with the organic matter and organic phosphorus and this would tend to indicate non-nitrogenous phosphates such as phytin.

By taking the sum of the first and second cuttings of clover on similarly producing pots in the rock phosphate and phytin series, and computing from the treatment the amount of organic phosphorus present it will be seen that there is sufficient organic phosphorus in the rock to produce these yields.

A marked similarity exists in the amounts of organic phosphorus present in the two treatments. The following table shows the number of milligrams of organic phosphorus (by calculation) present in each pot, the total yield from the two cuttings and the number of milligrams assimilated in the first cutting.

Table 13.- Comparative Yields and Treatments on Rock Phosphate and Crude Phytin.

Pot No	Treatment	Org Phos mg	Yield grams	mg P* 1st ct'g
7 - 8.	Rock Phos.	26.4	1.84	1.47
21-22.	Phytin	15.6	1.52	1.18
9 -10.	Rock Phos.	52.8	3.43	2.78
23-24.	Phytin	31.3	3.60	3.57
11-12.	Rock Phos.	158.4	7.60	8.28
25-26.	Phytin	63.5	6.48	10.70

*From table 8

All of these data tend to show that the rock phosphate used in this work contained enough organic phosphorus to grow the crops produced without using any of the inorganic form.

SUMMARY

1. Phosphorus in organic combination as phytin is more readily assimilated by growing plants than that in inorganic form as insoluble phosphates.

2. Large amounts of phosphorus as phytin are found deleterious to the growing plant.

3. An increase in application tends to increase the percentage phosphorus content of the clover and of both the grain and straw of the oats, but to a greater extent in the straw. This is found true with both organic and inorganic phosphates.

4. There seems to be a rather marked toleration for phosphorus.

5. When applied with limestone, organic phosphorus as phytin tends to go over to the insoluble inorganic form.

6. Organic phosphorus applied in the form of alfalfa in combination with rock phosphate produces no increase over the theoretical sum, while organic phosphorus supplied in the form of phytin combined with rock phosphste gives better yields than the sum of the two.

7. The rock phosphate employed in this work contains some readily available form of phosphorus, which in the presence of limestone tends to go over into the unavailable state.

8. Analyses tend to show that rock phosphate contains organic phosphorus.

In connection with this work, I wish to acknowledge the grateful assistance of Prof. C. G. Hopkins and Dr. A. L. Whiting under whom the work has been planned and carried out, and for the many valuable suggestions offered.