JULY, 1938 RESEARCH BULLETIN 288

UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

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MAGNESIUM AS A FACTOR IN NITROGEN FIXATION BY SOYBEANS

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(Publication Authorized July 1, 1938)

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ABSTRACT

A study of the influence of magnesium on the nitrogen fixing power and composition of soybeans by using colloidal clay as a carrier of this cation, in combination with calcium, and with calcium and potassium, revealed the fact that nitrogen fixation increased with higher magnesium levels and higher degrees of saturation of the clay by this nutrient when accompanied by a constant calcium level and degree of clay saturation. Nitrogen fixation did not occur at a low calcium level in the absence of magnesium, but occurred when a small amount of magnesium was added to the low calcium level. Though increased nitrogen fixation was always accompanied by increased growth, the converse did not necessarily hold true. Plants grown on colloidal clay cultures treated with the cations calcium, magnesium, and potassium grew well without fixing nitrogen in commensurate amounts. The ability of soybean plants to fix nitrogen decreased rapidly with consecutive croppings, suggesting that the supply of exchangeable bases on the colloidal clay may be reduced by one crop to the point where they become the limiting element in legume growth and their nitrogen fixation.

ACKNOWLEDGMENT

The author expresses his sincere appreciation to Dr. Wm. A. Albrecht for his advice and kindly criticisms during the course of this investigation and the preparation of this manuscript.

Magnesium as a Factor in Nitrogen Fixation by Soybeans

Ellis R. Graham

Introduction

Nitrogen fixation by nodule bacteria of legumes has long been of agricultural significance. Farmers planted such legumes as lupines to make their fields more fertile for succeeding crops even before the time of Caesar. Though they did not know of nitrogen fixation, they were dependent upon it. The explanation of this process has been such a tempting problem that its bacteria have received extensive study particularly under conditions outside of the soil. Further studies under carefully controlled soil conditions are needed if this process is to be fully understood as it functions under field conditions.

Not all farmers have been able to grow legumes successfully. It is a well established fact that lime must be added to many soils to grow these crops and that this treatment increases the nitrogen fixing power of the plants. Since calcium is important for nodulation and the growth of legumes, and since magnesium resembles it so closely in chemical properties, a study was undertaken to determine the influence of magnesium on nitrogen fixation by the soybean plants.

Historical

Studies on the importance of the element magnesium by means of nutrient solutions show that in the absence of magnesium a rather characteristic chlorosis develops in green plants. Its function in the plant is important since it is a constituent of chlorophyll. It has been suggested that it is involved in some way in the formation of oils and fats and that it acts as a carrier of phosphorus from one part of the plant to another. Whenever it has been absent from solution, poor growth and low vigor of the plant have been noted.

McMurtrey's (19)* experiments on the effects of deficiencies of certain essential elements showed that a deficiency of magnesium had a decided effect on the growth of tobacco plants. The leaves curled upwards and a loss of green color was noted on the lower

*See "References", page 30.

leaves. Ginsburg (11) reported that soybeans growing in a culture solution lacking magnesium had 18.41 percent less ash content than the soybeans grown in full nutrient solution.

As early as 1892 Loew (17) proposed a hypothesis regarding a calcium-magnesium ratio in relation to crop growth. Loew and May (18) also showed that deleterious effects occurred from an excess of magnesia, while an excess of lime produced symptoms of starvation within the plant.

Bernardi and Siniscalchi (7) concluded that the calcium-magnesium ratio is not responsible for the alteration in growth but may influence the solubility of the phosphate.

Moser (20) working with calcium-magnesium ratios in soils concluded that no significant relation could be found between the calcium-magnesium ratio and the crop yield; also that the beneficial effect of adding lime to the soil was not due to an alteration of the calcium-magnesium ratio, but to an increase of the replaceable calcium content of the soil. Lipman (15) after making a critical review of numerous papers on the question of lime-magnesium ratios, concluded that there was little or no evidence to support belief in the necessity of proper calcium-magnesium ratio for plant growth.

The importance of calcium as a factor in plant growth and nitrogen fixation has been widely appreciated. Lipman and Blair (16), Albrecht and Davis (4), and many others have reported the beneficial effects of lime on the growth and inoculation of legumes on acid soils. Albrecht et al. (1), (2), (3), (4), (5), have shown that lime is essential for successful legume crops, not merely as a neutralizing agent but because its calcium serves as a nutrient; also that the requirement of the plant for calcium must be met and then as additional amounts give increased plant growth there is improved nodulation and nitrogen fixation.

Horner (12) and Hutchings (13) grew soybeans on cultures of sand mixed with colloidal clay on which there were adsorbed varying amounts of exchangeable magnesium and calcium. They reported extreme variations in composition and growth of the soybeans. Hutchings (13) noted that a high degree of magnesium and a low degree of calcium saturation produced a plant with high protein content, while the reverse produced a plant with a low protein content.

These observations on the importance of magnesium as an essential element; its close resemblance and relation to calcium; and the effect of the latter on growth, composition, and nodulation suggest that magnesium may affect the nitrogen fixing power of soybeans.

Since water culture studies do not duplicate soil conditions and since the influence of calcium on soybean growth and nodulation has been successfully attacked in the work of Albrecht et al. (1), (2), (3), (4), (5), (14), by means of sand-colloidal clay mixture, it was deemed desirable to approach the problem of magnesium as related to nitrogen fixation by employing these same methods. The colloidal fraction of Putnam silt loam as used by the workers mentioned above affords a medium which can be standardized at the outset of the growth period and again at the close. With the knowledge of the chemical composition of the clay soil and seed at the outset, and of the plant and the clay soil at the close, the movement of the plant nutrients during the growth process from the soil to the plant or vice versa may be measured. The amount of nitrogen fixed can be considered as equal to the increase of nitrogen in the system for a given growth period. The nitrogen fixation can then be related to the behavior of the other nutrient elements as magnesium, calcium, phosphorus, potassium and others whose behavior in the clay is chemically ascertainable and whose significance and function in the plant can be more carefully observed and interpreted.

Experimental

PLAN AND METHOD OF THE EXPERIMENT

The study was designed to test the effects of various amounts of magnesium on the nitrogen fixing power of soybeans when the level of the calcium is constant. The amount of calcium considered necessary under the experimental conditions used, as shown in previous work by Albrecht (3) was 0.2 milligram equivalents* per plant. As a means of controlling the amounts of calcium and magnesium, a medium was used which contained quartz sand mixed with varying amounts of specially prepared colloidal clay which had been electrodialyzed and then given different amounts of calcium and magnesium to be held in the sorbed† form. This made possible a medium that could be standardized and the amounts of nutrients controlled while at the same time it had excellent physical properties.

*Milligram Equivalents will be abbreviated as M. E. in the following pages.

†Sorbed is a term used to include both surface accumulation and penetration of cations into the crystal lattice of the colloidal clay, in avoidance of the confusion attendant upon the use of the terms adsorbed and absorbed.

The Virginia variety of soybeans was chosen for this investigation because of the previous extensive work with them. Information is available regarding their response to the seasons and to greenhouse culture so that comparisons can be made with previous work.

Colloidal Clay Preparation

The colloidal clay was extracted from the heavy subsoil layer of the Putnam silt loam and purified by electrodialysis according to the methods of Bradfield (8). A conductometric determination of the exchange capacity according to the carbonation method of Bradfield and Allison (9) showed this to be 68 M. E. per 100 grams of clay as given in Figure 1.

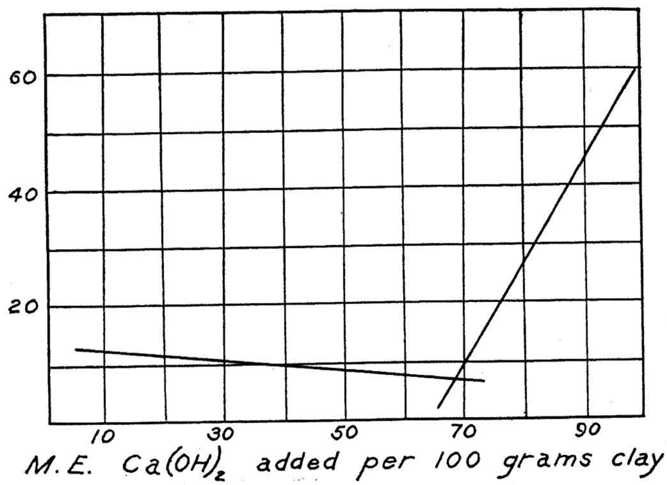


Fig. 1.—Conductometric graph of hydrogen clay and calcium hydroxide.

The media were then prepared for the cultures by taking sufficient quantities of the H-clay* to sorbe the desired bases, and then adding calcium and magnesium to it. In some cases barium was added as a supplement to completely saturate the clay. Calcium, magnesium and barium were added as hydroxides so that after their reaction with the H-clay the resulting suspension would not be disturbed in subsequent reactions by the presence of some nonessential ions. The hydroxides were titrated into the desired quantity of H-clay, stirred vigorously, and allowed to stand for 3 weeks. The pH of the mixture was determined to assure complete saturation, which was calculated from the determination of the exchange

*H-clay is an abbreviation of Hydrogen clay and will be used in the following pages.

capacity and a standardization of the concentration of the colloidal suspension. Then 2000 grams of quartz sand that had been previously leached with 1:1 HCl for 48 hours and washed free of chlorides was added to each culture. The sand-colloidal clay mixture was then allowed to evaporate to optimum moisture content.

Selected soybean seeds were germinated. After they had produced a radicle about 2 cm. long they were inoculated and planted in the sand-colloidal clay cultures. Fifty seedlings were used in each and allowed to grow for a period of 5 weeks. In order to eliminate undesirable effects of short day growing seasons the plants were grown during the months from May to October. Artificial light was used during October. After 5 weeks, the tops and roots were harvested, placed in paper bags, and dried in the oven at 60°C. for 48 hours.

The plants were grown in two different groups designated group A and group B. Group A which included combinations of calcium and magnesium; of calcium, magnesium, and barium; and of calcium and barium as the exchangeable cations, was planted in May. Group B which included combinations of calcium and magnesium; of calcium, magnesium, and hydrogen; and of calcium, magnesium, and potassium, was planted in September. The cultures as prepared may be schematically represented in the following arrangement in which the figures are given as M. E. of element per 50 plants.

Group A

Used for Three Successive Crops

No. 1	Calcium	10 M. E.	No. 4	Calcium	20 M. E.
	Barium	10 M. E.		Barium	20 M. E.
No. 2	Calcium	10 M. E.	No. 5	Calcium	20 M. E.
	Barium	5 M. E.		Barium	10 M. E.
	Magnesium	5 M. E.		Magnesium	10 M. E.
No. 3	Calcium	10 M. E.	No. 6	Calcium	20 M. E.
	Magnesium	10 M. E.		Magnesium	20 M. E.

Group B

Used for a Single Crop

No. 1	Calcium	10 M. E.	No. 3	Calcium	10 M. E.
	Magnesium	5 M. E.		Magnesium	5 M. E.
				Potassium	5 M. E.
No. 2	Calcium	10 M. E.	No. 4	Calcium	10 M. E.
	Magnesium	5 M. E.		Magnesium	5 M. E.
	Hydrogen	15 M. E.		Potassium	10 M. E.

All cultures were grown in duplicate. Three successive crops were grown on the sand-colloidal cultures of group A without any further treatments. Group B included but a single crop. The plants were harvested and the pans reseeded with inoculated seedlings.

Analytical Methods

The analytical data represent the analyses of both the tops and roots from cultures of fifty plants each grown in duplicate. The nitrogen analyses were made according to the standard Kjeldahl (13) procedure used for total nitrogen. The analyses for calcium, magnesium, potassium, phosphorus, and barium were made on aliquots of a nitric and perchloric acid digestion of the pulverized plants. This digestion converted the elements into chlorides, for which standard analytic methods were used (13).

RESULTS

Nitrogen Fixation—Group A

Relation of Magnesium to Nitrogen Fixation.—The amount of nitrogen fixed by the soybean plants was related directly to the amount and availability of the magnesium present, as is evident from the data in Table 1, and shown graphically by Figure 2. Plate I shows the plants at the ages of three and five weeks. These results show that a culture of fifty plants treated with 10 M. E. of calcium and 10 M. E. of barium, or without magnesium, did not fix nitrogen, while a culture treated with 5 M. E. of magnesium, 10 M. E. of calcium, and 5 M. E. of barium fixed 71 mgms. of nitrogen. The culture treated with 10 M. E. of magnesium and 10 M. E. of calcium, fixed 164 mgms. of nitrogen. The latter case was an increase of 41% of nitrogen over the amount contained in the seeds. The crop growths of these three cultures, 1, 2, and 3, as determined

TABLE 1.—NITROGEN FIXATION BY SOYBEANS AS INFLUENCED BY MAGNESIUM. (DATA FOR 50 PLANTS, GROUP A, CROP 1.)

Culture Number	Treatment: Exchangeable cations M. E. Mg	Ca	Ba	Crop Weight gms.	Nodules	Nitrogen %	total mgms.	Fixed
1	0	10	10	14.802	297	2.63	391	—3
2	5	10	5	18.198	456	2.56	465	71
3	10	10	0	21.460	490	2.59	558	164
4	0	20	20	15.316	210	2.86	432	38
5	10	20	10	18.288	322	2.49	455	61
6	20	20	0	21.866	365	2.32	541	147

Fifty Soybean seeds contained 394 mgms. of nitrogen.

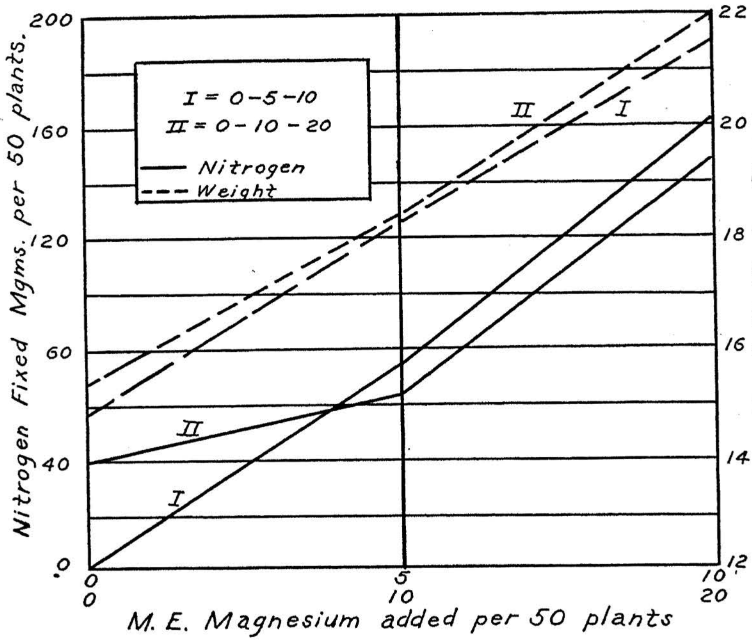


Fig. 2.—Nitrogen fixation and growth of soybeans as influenced by magnesium (Group A, Crop 1).

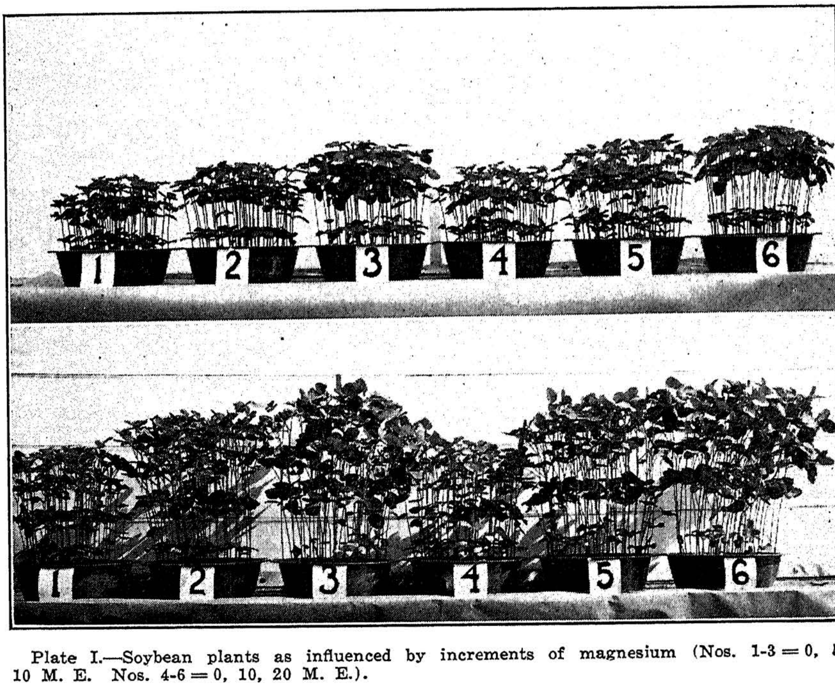


Plate I.—Soybean plants as influenced by increments of magnesium (Nos. 1-3 = 0, 5, 10 M. E. Nos. 4-6 = 0, 10, 20 M. E.).

by dry weight of 50 plants were 14.802, 18.198, and 21.460 grams respectively.

Cultures 4, 5, and 6 were treated with twice the amount of the bases contained in cultures 1, 2, and 3 but in the same ratio. With these larger amounts of bases all three cultures fixed nitrogen. The non-magnesium culture fixed 38 mgms. That with 10 M. E. magnesium fixed 61 mgms. and the culture with 20 M. E. fixed 147 mgms. of nitrogen. The growths in relation to the increased magnesium additions were 15.316, 18.288, and 21.866 gms. respectively.

Magnesium Content of Plants (Group A, Crop 1).—The amount of magnesium in the plant tissue when expressed either as percentage or as total was directly proportional to the amount of magnesium added to the clay. Table 2, shows that cultures treated with increments of 0.0, 5.0, and 10.0 M. E. of magnesium had a percentage content of .13, .27, and .47 and a total content of 20,2, 48.3, and 100.1 mgms. respectively per 50 plants. The cultures treated with magnesium increments of 0.0, 10.0, and 20.0 M. E. had a percentage content of .12, .55, and .86 and a total content of 19.5, 92.2, and 178.1 mgms. respectively per 50 plants. When plotted as functions of the magnesium increments, as given in Figure 3, the total magnesium approaches a straight line.

TABLE 2.—COMPOSITION OF SOYBEAN PLANTS AS INFLUENCED BY MAGNESIUM. (DATA FOR 50 PLANTS,* GROUP A, CROP 1.)

Culture Number*	Magnesium (Mg) %	Magnesium (Mg) total mgms.	Calcium (Ca) %	Calcium (Ca) total mgms.	Phosphorus (P) %	Phosphorus (P) total mgms.	Potassium (K) %	Potassium (K) total mgms.	Barium (Ba) %	Barium (Ba) total mgms.
1	.13	20.2	.45	66.2	.24	35.1	1.10	172	.17	25.9
2	.27	48.3	.44	79.5	.19	36.2	1.03	185	.07	13.2
3	.47	100.1	.42	89.8	.15	33.1	.76	162	.00	0.0
4	.12	19.5	.50	76.8	.25	39.2	.97	147	.38	52.0
5	.55	92.2	.52	87.6	.22	41.9	1.09	203	.03	5.4
6	.86	178.1	.51	114.1	.15	35.5	.85	185	.00	0.0

*Exchangeable cations same as Table 1.
Fifty Soybean seeds contained 16.7 mgms. of magnesium, 12.2 mgms. of calcium, 39.4 mgms. of phosphorus, 171 mgms. of potassium, and no barium.

Influence by Magnesium on the Plants' Use of the Calcium Offered by the Clay.—The presence of magnesium in the culture had a pronounced influence on the amount of calcium utilized by the plants. The data of Table 3, show that cultures numbers 1, 2, and 3 each of which offered 212.2 mgms. of calcium in the seed and clay along with increments of magnesium as 0-5-10 M. E. per 50 plants contained 66.2, 79.5, and 89.8 mgms. of calcium respectively at the end

TABLE 3.—COMPOSITION OF SOYBEANS AS INFLUENCED BY MAGNESIUM CONTENT OF THE CLAY MEDIUM. (GROUP A, CROP 1.)

	Calcium (Ca)			Magnesium (Mg)		
Culture Number	Amount offered in mgms.	taken mgms.	%	Amount offered mgms.	taken mgms.	%
1	212.2	66.2	31.2	20.2	20.2	00.0
2	212.2	79.5	37.5	80.2	48.3	60.2
3	212.2	89.8	42.3	140.2	100.1	71.2
4	412.2	76.8	18.7	19.5	19.5	00.0
5	412.2	87.6	21.0	139.5	92.2	66.1
6	412.2	114.1	27.0	259.5	178.1	68.5

Fifty seeds contained 12.2 mgms. of calcium and 16.7 mgms. of magnesium.

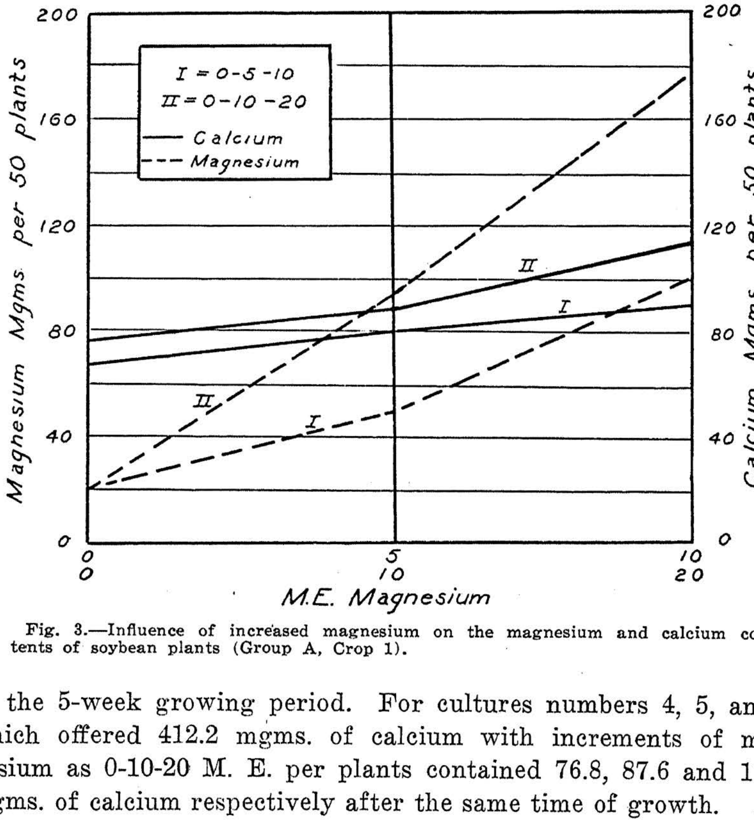


Fig. 3.—Influence of increased magnesium on the magnesium and calcium contents of soybean plants (Group A, Crop 1).

of the 5-week growing period. For cultures numbers 4, 5, and 6 which offered 412.2 mgms. of calcium with increments of magnesium as 0-10-20 M. E. per plants contained 76.8, 87.6 and 114.1 mgms. of calcium respectively after the same time of growth.

Influence of Magnesium on Some Other Elements in the Composition of the Soybeans.—Increasing the magnesium in the cultures was without noticeable effect on the amounts of phosphorus and potassium, as determined by analyses of the seed at the outset and of the plants after the growing period. The data for these analyses, as given in Table 2 and shown graphically in Figure 4, point out that the total phosphorus found in the fifty plants agreed closely with the amount found in the seeds. Likewise, potassium showed no consistent difference. The inactive element barium, which was added to cultures 1, 2, 4 and 5, was taken in quantities of 25.9 and 13.2 mgms. for cultures 1 and 2 respectively, while cultures 4 and

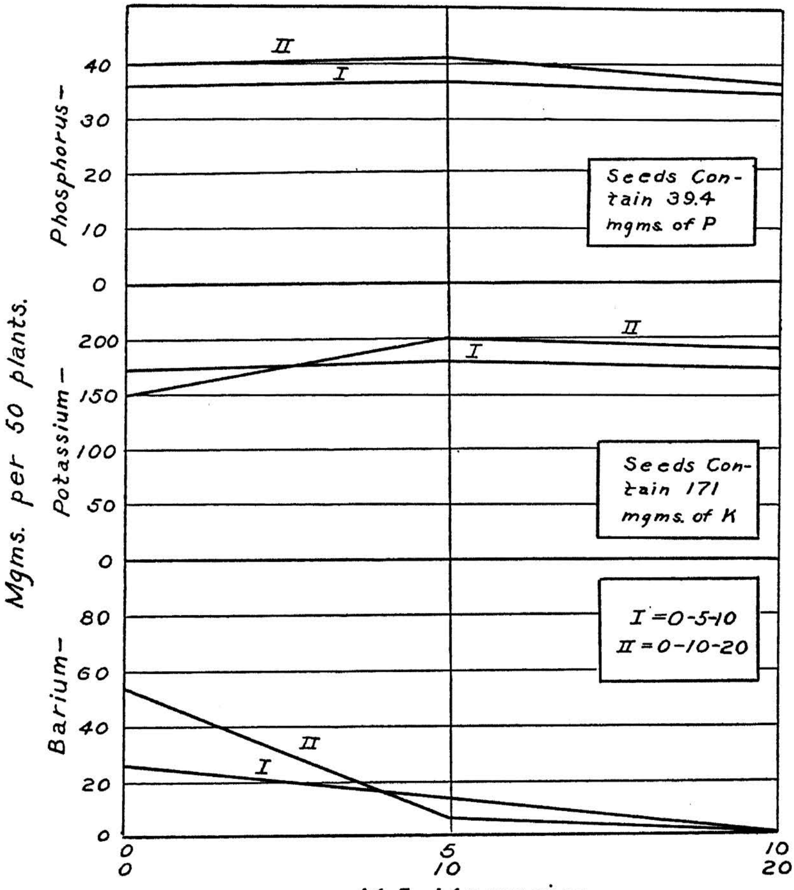
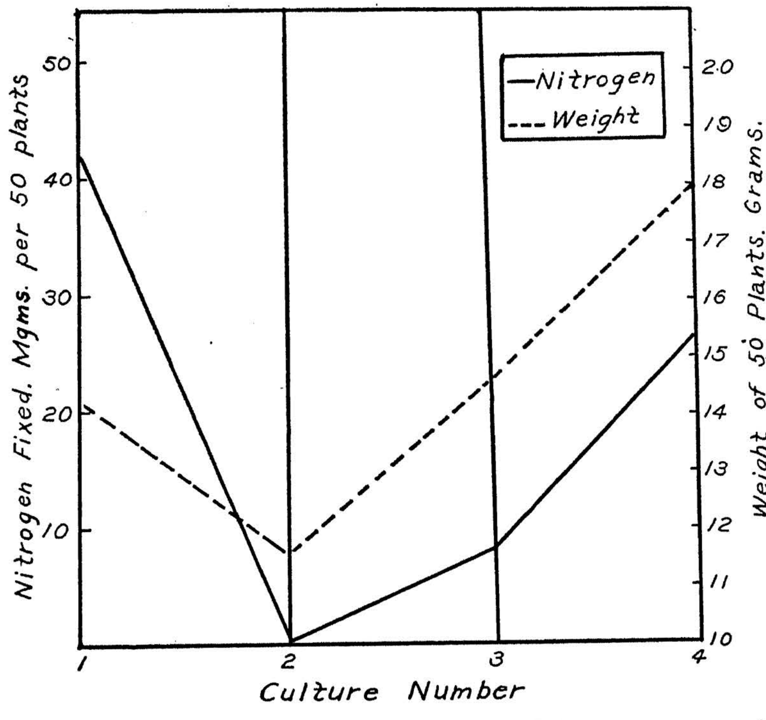


Fig. 4.—Phosphorus, potassium and barium contents of soybean plants as influenced by increased magnesium (Group A, Crop 1).

5 contained 52.0 and 5.4 mgms. of this element. It is of interest to note that cultures 1, 2, and 4 contained amounts of barium directly proportional to the amounts added to the clay, while the plants of culture 5 which had 10 M. E. of barium added to it contained only a very small amount of barium. Perhaps this is due to the fact that the plants of culture 5 contained large amounts of magnesium and calcium.

Nitrogen Fixation—Group B, Crop 1

Nitrogen Fixation by Magnesium-Calcium Cultures as Influenced by the Presence of Hydrogen and of Potassium.—Both hydrogen and potassium had a very marked effect on the nitrogen fixation by soybeans grown in cultures of sand-colloidal clay treated with magnesium and calcium, as shown by the data assembled in Table 4 and



EXCHANGEABLE CATIONS (M. E.)

	Culture 1	Culture 2	Culture 3	Culture 4
Mg	5	5	5	5
Ca	10	10	10	10
K	0	0	5	10
H	0	15	0	0

Fig. 5.—Nitrogen fixation by soybean plants as influenced by potassium and hydrogen as supplements to magnesium and calcium (Group B, Crop 1).

shown graphically in Figure 5. Plate II gives a photographic presentation of the difference. These results show that the culture which had been treated with 10 M. E. of calcium and 5 M. E. of magnesium fixed 43 mgms. of nitrogen per 50 plants during the five weeks growth period. Culture 2 which had been treated with 10 M. E. of calcium, 5 M. E. of magnesium but had 15 M. E. of hydrogen lost 5 mgms. of nitrogen for the same growth period. The third culture treated with 10 M. E. of calcium, 5 M. E. of magnesium, supplemented by 5 M. E. of potassium fixed 8 mgms. of nitrogen.

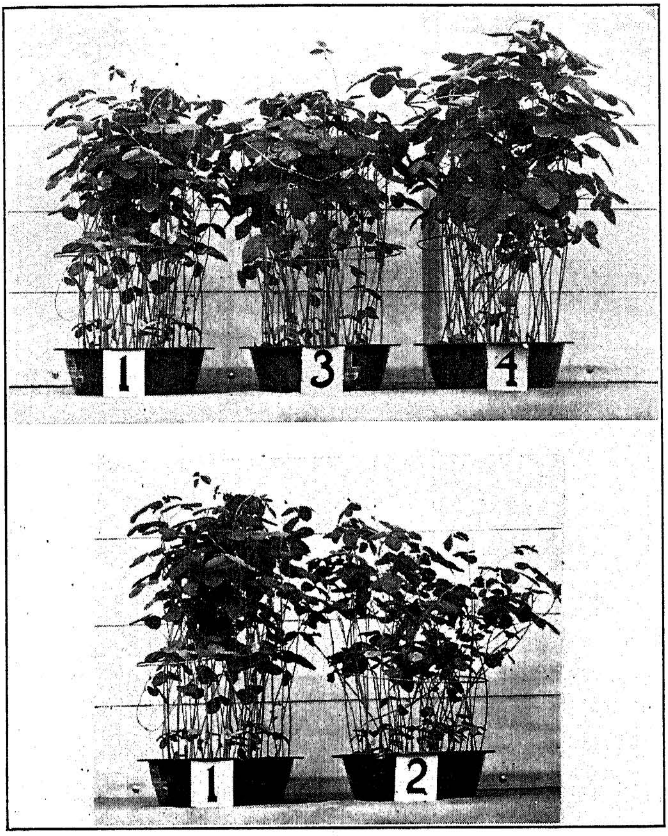


Plate II.—Soybean plants as influenced by potassium and hydrogen as supplements to calcium and magnesium. (No. 3 = 5 M. E., No. 4 = 10 M. E. of potassium, No. 2 = 15 M. E. of hydrogen as supplements.).

The fourth culture with 10 M. E. of calcium and 5 M. E. of magnesium coupled with 10 M. E. of potassium, fixed the second largest amount in this series which was 26 mgms. per 50 plants. The growth weights as determined by oven dry weight of the 50 plants of cultures 1, 2, 3, and 4 were 14.207, 11.492, 14.592 and 17.807 grams respectively.

TABLE 4.—NITROGEN FIXATION AS INFLUENCED BY POTASSIUM AND HYDROGEN AS SUPPLEMENTS TO MAGNESIUM AND CALCIUM. (DATA FOR 50 PLANTS, GROUP B, CROP 1.)

Culture Number	Treatment: Exchangeable cations M. E. Mg	Ca	K	H	Crop Weight gms.	Nodules	Nitrogen %	total mgms.	Fixed
1	5	10	0	0	14.207	101	2.86	407	43
2	5	10	0	15	11.492	13	3.24	359	—5
3	5	10	5	0	14.592	64	2.55	372	8
4	5	10	10	0	17.807	62	2.19	390	26

Fifty Soybean seeds contained 364 mgms. of nitrogen

Calcium and Magnesium Contents of Soybeans as Influenced by the Presence of Potassium and Hydrogen (Group B, Crop 1).—The addition of hydrogen and potassium to the ionic atmosphere of the colloidal clay had little if any effect on the amount of magnesium taken by the fifty soybean plants. The data assembled in Table 5 and given graphically in Figure 6 show that cultures 1, 2, 3, and 4 took 52, 48, 54, and 55 mgms. of magnesium respectively from the constant supply of 80 mgms. This was not found to be the case for the constant supply of 212.2 mgms. of calcium. The calcium intake was greatly affected by both hydrogen and potassium, as is shown by the data of Table 5, and by the curves for them in Figure 6. Culture 2, the clay of which contained 15 M. E. of hydrogen, took up only 46 mgms. of calcium from the 212.2 mgms. available, while

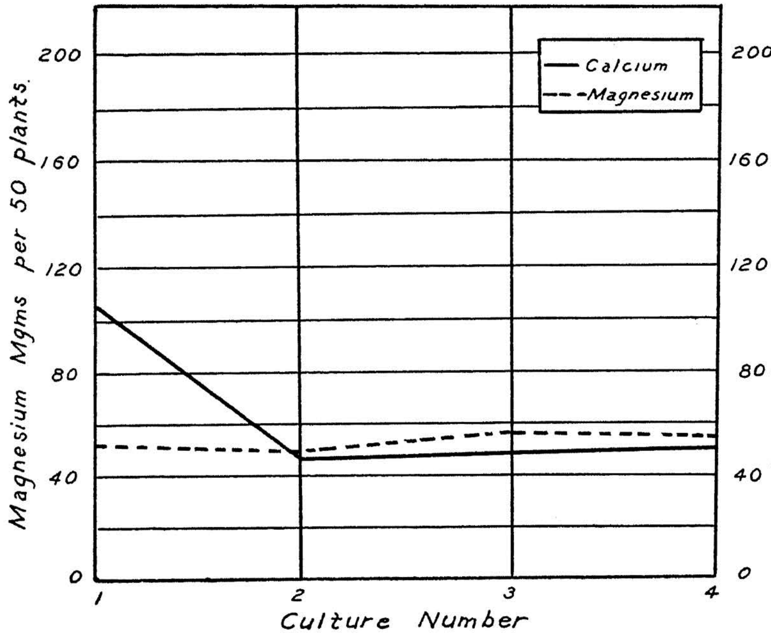
TABLE 5.—COMPOSITION OF SOYBEAN PLANTS AS INFLUENCED BY POTASSIUM AND HYDROGEN AS SUPPLEMENTS TO MAGNESIUM AND CALCIUM. (DATA FOR 50 PLANTS,* GROUP B, CROP 1.)

Culture Number	Magnesium (Mg) %	Magnesium (Mg) total mgms.	Calcium (Ca) %	Calcium (Ca) total mgms.	Phosphorus (P) %	Phosphorus (P) total mgms.	Potassium (K) %	Potassium (K) total mgms.
1	.36	52	.74	105	.25	39	1.01	150
2	.33	48	.40	46	.27	30	1.15	134
3	.36	54	.32	46	.18	26	1.90	285
4	.30	55	.27	48	.14	25	2.15	384

*Exchange cations same as Table 4.

culture 1 containing the same amount of calcium and magnesium but without hydrogen took 105 mgms. of the 212.2 mgms. of calcium available in the culture. The third culture similar to culture one but containing 5 M. E. of potassium on the clay took up only 46 mgms. of calcium, while the fourth culture differing from it only by its content of 10 M. E. of potassium on the clay took from this source but 48 mgms., or almost the same amount as when the potassium offered was but half as much.

If the calcium in the plants is calculated on the basis of percentage taken from the amount offered, we find the cultures in consecutive order took 49.5, 21.7, 21.7, and 22.6 percent.



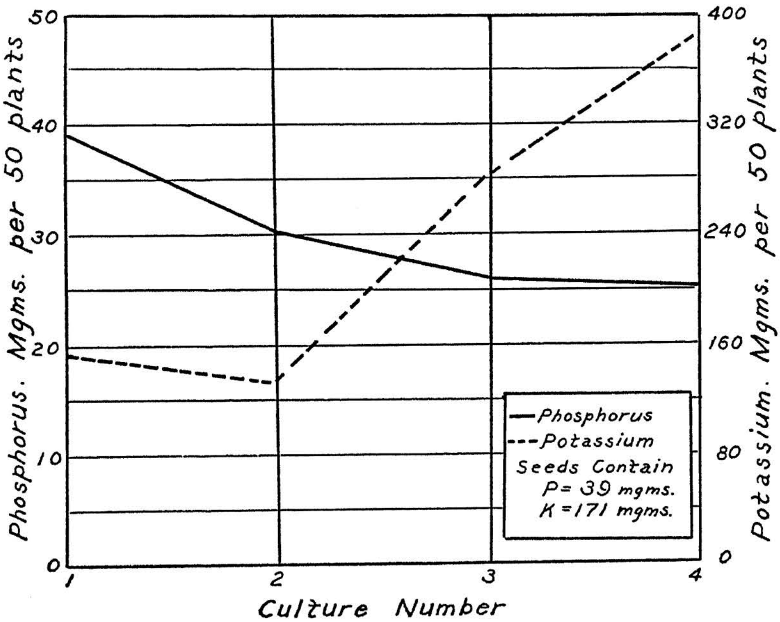
EXCHANGEABLE CATIONS (M. E.)

	Culture 1	Culture 2	Culture 3	Culture 4
Mg	5	5	5	5
Ca	10	10	10	10
K	0	0	5	10
H	0	15	0	0

Fig. 6.—Magnesium and calcium contents of soybean plants as influenced by the presence of hydrogen and of potassium (Group B, Crop 1).

Phosphorus and Potassium Contents of Soybean Plants as Influenced by Hydrogen and Potassium on the Clay (Group B, Crop 1). —The phosphorus and potassium contents of the soybean plants varied widely with the addition of hydrogen and potassium to the colloidal clay cultures carrying also sorbed calcium and magnesium. An examination of the data of Table 4 and the graphs in Figure 7 shows that cultures 1, 2, 3, and 4 contained 39, 30, 26, and 25 mgms.

of phosphorus per 50 plants respectively. The first culture contained phosphorus in the exact quantity contained in the original seeds. However, the remainder of the cultures contained less phosphorus in the 50 plants than was in the seeds at the outset. The fifty plants of each of the four cultures in sequence contained 150, 134, 285, and 384 mgms. of potassium per 50 plants respectively.



EXCHANGEABLE CATIONS (M. E.)

	Culture 1	Culture 2	Culture 3	Culture 4
Mg	5	5	5	5
Ca	10	10	10	10
K	0	0	5	10
H	0	15	0	0

Fig. 7.—Phosphorus and potassium contents of soybean plants as influenced by the presence of hydrogen and of potassium (Group B, Crop 1).

The extremely large amount of potassium in cultures 3 and 4 may be due to the fact that culture number 3 had 5 M. E. of potassium and culture 4 had 10 M. E. added to the clay. In these two the amounts of potassium taken from the clay, or seed and clay, were proportional to the amount added to the clay. Cultures 1 and 2 contained less potassium than the quantity contained in the original seeds.

Nitrogen Fixation—Group A, Second and Third Crops

Nitrogen Fixation by Successive Soybean Crops as Related to Initial Magnesium Applications (Group A, Crops 2 and 3.— Cropping the original cultures of Group A to the second and third crops had a very marked effect on the nitrogen fixation by the soybeans. The second crop fixed very little nitrogen and none was fixed by the third crop. The data of Tables 6 and 8, and the curves of Figure 9, show that of cultures 1, 2, and 3 which originally had increments of 0-5-10 M. E. of magnesium, only culture 3 fixed nitrogen in the second crop. The remainder of the cultures of this crop,

Plate III.—Successive soybean crops as influenced by initial treatments of magnesium. (Nos. 1-3 = 0-5-10 M. E., Nos. 4-6 = 0-10-20 M. E.).

and all of those in the third crop failed to fix nitrogen. The total crop weights decreased with successive croppings even though the growth period was the same duration for all. These physical and chemical differences of the harvested crops were not evident from the general appearances of the crops during growth as is shown in Plate III.

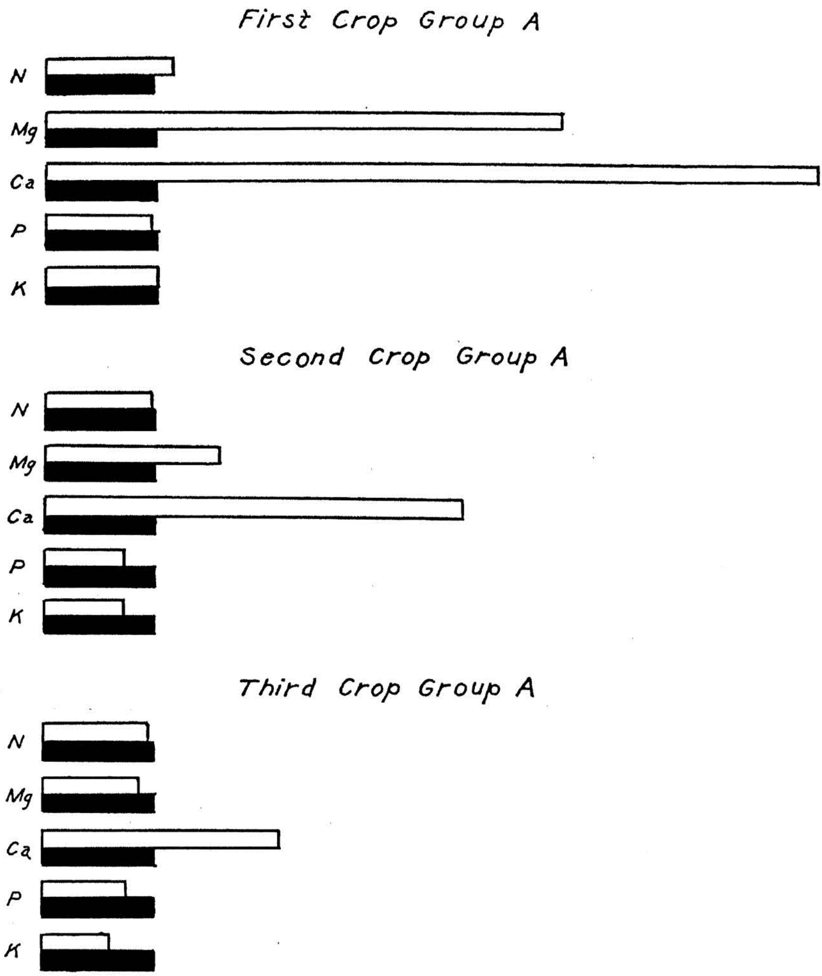


Fig. 8.—Composition of successive crops of soybeans. (Total nutrients in 6 cultures, 50 plants each. Seed content represented by solid columns, crop content by open columns.).

TABLE 6.—NITROGEN FIXATION BY THE SECOND CROP OF SOYBEANS AS INFLUENCED BY MAGNESIUM. (DATA FOR 50 PLANTS, GROUP A, CROP 2.)

Culture Number	Treatment: Exchangeable cations M. E. Mg	Ca	Ba	Crop Weight gms.	Nodules	Nitrogen %	total mgms.	Fixed
1	0	10	10	12.838	82	2.85	366	—28
2	5	10	5	13.706	81	2.73	374	—20
3	10	10	0	17.260	82	2.51	434	40
4	0	20	20	13.397	81	2.66	358	—36
5	10	20	10	14.587	90	2.62	382	—12
6	20	20	0	14.151	107	2.49	353	—41

Fiity Soybean seeds contained 394 mgms. of nitrogen.

TABLE 7.—COMPOSITION OF THE SECOND CROP OF SOYBEANS AS INFLUENCED BY MAGNESIUM. (DATA FOR PLANTS,* GROUP A, CROP 2.)

Culture Number*	Magnesium (Mg) %	total mgms.	Calcium (Ca) %	total mgms.	Phosphorus (P) %	total mgms.	Potassium (K) %	total mgms.
1	.08	11.0	.33	42.3	.22	32.3	1.04	133
2	.10	14.3	.28	39.0	.19	27.4	.93	127
3	.12	21.5	.24	41.3	.18	28.4	.82	136
4	.09	12.0	.34	46.0	.21	28.6	.81	109
5	.23	34.3	.26	39.2	.20	28.5	.82	120
6	.39	54.9	.24	69.2	.18	26.0	.84	119

*Exchangeable cations same as Table 6.
Fifty Soybean seeds contained 16.7 mgms. of magnesium, 12.2 mgms. of calcium, 39.4 mgms. of phosphorus, 171 mgms. of potassium.

TABLE 8.—NITROGEN FIXATION BY THE THIRD CROP OF SOYBEANS AS INFLUENCED BY MAGNESIUM. (DATA FOR 50 PLANTS, GROUP A, CROP 3.)

Culture Number	Treatment: Exchangeable cations M. E. Mg	Ca	Ba	Crop Weight gms.	Nodules	Nitrogen %	total mgms.	Fixed
1	0	10	10	8.570	38	4.23	361	—33
2	5	10	5	10.575	9	3.68	388	— 6
3	10	10	0	9.440	27	4.02	378	—16
4	0	20	20	7.780	42	3.73	288	—106
5	10	20	10	11.580	24	3.45	396	2
6	20	20	0	9.740	19	3.91	380	—14

Fifty Soybean seeds contained 394 mgms. of nitrogen.

TABLE 9.—COMPOSITION OF THE THIRD CROP OF SOYBEANS AS INFLUENCED BY MAGNESIUM. (DATA FOR 50 PLANTS,* GROUP A, CROP 3.)

Culture Number*	Magnesium (Mg) %	total mgms.	Calcium (Ca) %	total mgms.	Phosphorus (P) %	total mgms.	Potassium (K) %	total mgms.
1	.12	11.0	.33	28.2	.30	25.7	1.18	101
2	.12	13.6	.29	30.4	.29	30.6	1.10	116
3	.08	7.1	.25	23.6	.31	28.9	1.18	111
4	.09	6.9	.29	22.6	.34	26.4	1.36	104
5	.13	16.0	.25	28.9	.27	31.7	1.12	129
6	.27	26.9	.24	23.4	.29	28.2	1.08	105

*Exchangeable cations same as Table 8.
Fifty Soybean seeds contained 16.7 mgms. of magnesium, 12.2 mgms. of calcium, 39.4 mgms. of phosphorus, 171 mgms. of potassium.

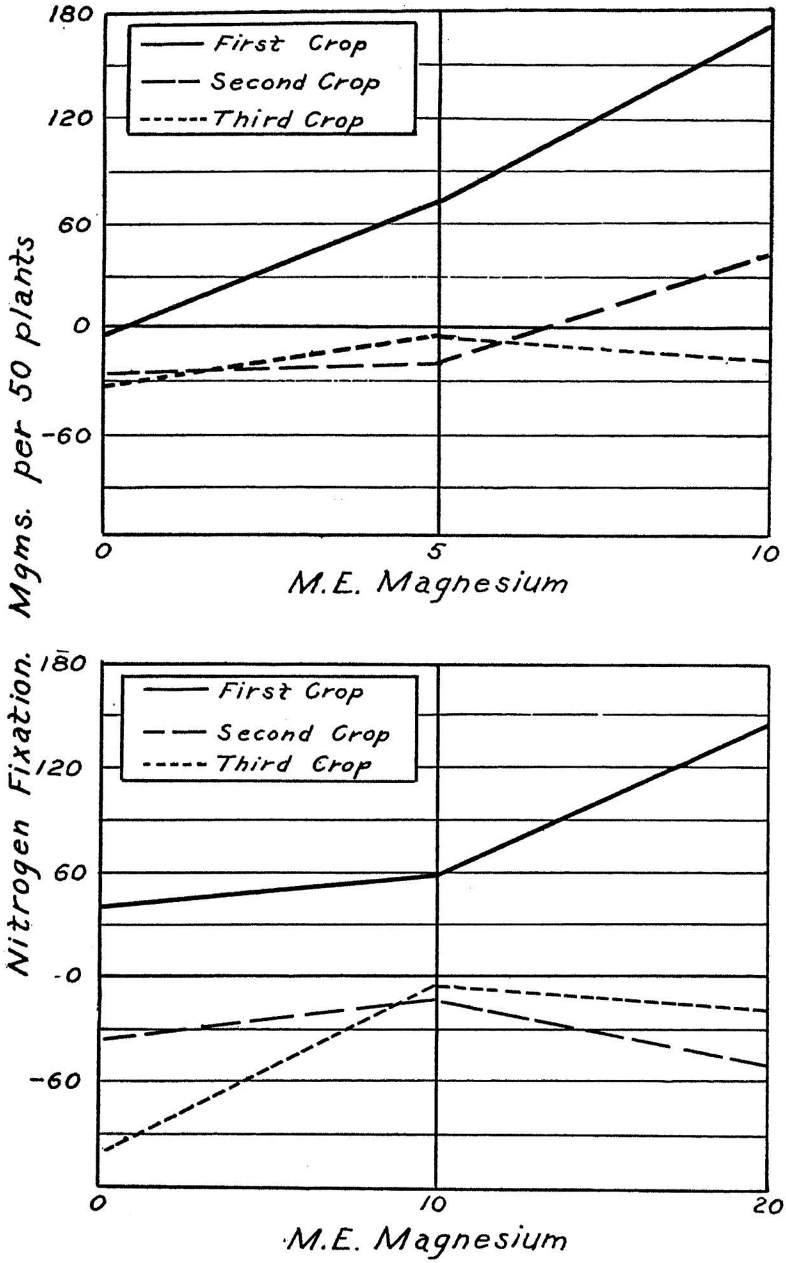


Fig. 9.—Nitrogen fixation by successive soybean crops as related to initial magnesium application.

Chemical Composition of Successive Soybean Crops in Relation to Initial Magnesium Application in Cultures of Group A.—The amounts of magnesium, calcium, phosphorus and potassium in the second crop were less than those in the first. Those in the third crop were still less than those in the second as is shown by the data of Tables 7 and 9. The magnesium contents for example in culture 3, were 100.1, 21.5 and 7.1 mgms. per 50 plants for the first and second and third crops respectively. The corresponding amounts of phosphorus in the successive crops were less than the average content of the seeds. The potassium contents of the successive crops on this third culture were increasingly less than was originally in the seed. The calcium contents for the same conditions were 89.8, 41.3, and 23.6 mgms. The other cultures of the series showed similar decreases in their calcium, phosphorus, and potassium contents. The non-magnesium treated cultures 1 and 4 lost magnesium to the sand-colloidal mixture from the seed, as was true for phosphorus and potassium. For the crops as a whole the data are given graphically in Figure 8.

Discussion

The results obtained in this study show a definite influence by magnesium on the fixation of nitrogen by soybean plants. There was a close correlation between the amount of magnesium found in the plants at the end of a five week growing period and the amount of nitrogen fixed by the plants. However, not all plants which have a high magnesium content fix significant amounts of nitrogen. The effectiveness of the magnesium is premised upon a liberal supply of calcium. Nitrogen fixation in soybeans is very probably a secondary function of magnesium. The fixation increases as magnesium improves the growth of the plants thru its influence on their more efficient use of the calcium offered.

It has repeatedly been pointed out that calcium is a very important factor in nitrogen fixation. Albrecht and Davis (4) concluded that the beneficial effect of calcium was a result of its presence within the plant tissue itself. This conclusion is verified by the facts obtained in these studies, that in every instance where nitrogen was fixed in significant amounts it was found to be accompanied by an efficient use by the plant of the calcium offered. The amount of calcium found in the plants could be correlated with nitrogen, magnesium, and potassium. Nitrogen fixation occurred when large amounts of calcium were taken by the plant. Magnesium increased the plant's consumption of calcium and its efficient use when potassium was absent. In the potassium treated cultures, calcium was not found in the plant in significant amounts even though magnesium was present. Phosphorus, also functions with calcium. Where significant amounts of calcium were taken up by the plants the amount of phosphorus also increased. The total of the latter found in the seed could be recovered in the plants in the absence of phosphorus treatments, except when the amount of calcium in the plants became low. Under such conditions not even all the seed phosphorus could be recovered in the plants, which fact suggests that with deficient calcium levels even some of the seed phosphorus may be lost from the plant to the soil.

The data and curves indicate a close relation of calcium in the plant tissue and the soybean plant's capacity to metabolize its own phosphorus. When plants had a calcium content greater than .45% under conditions of this study, the amount of phosphorus in the plants at the end of the growing period agreed with the amount originally present in the seeds. When the calcium percentage fell below .45 in the dried plants their phosphorus content was less than originally in the seeds. In no case was nitrogen fixed in significant amount where plants were found to contain less phosphorus than was in the seeds at the outset.

Data given as to the amount of growth and nitrogen fixed show that in the case of the magnesium-calcium treated cultures, the fixation was directly proportional to the amount of growth. Under these conditions any factor which would produce excellent growth would increase the nitrogen fixing capacity of the soybeans. Variation in the growing season influenced the amounts of nitrogen fixed. A set of cultures treated with 10 M. E. of calcium and 5 M. E. of magnesium growing in May fixed 71 mgms. of nitrogen, while similar cultures fixed only 43 mgms. when grown in October. This difference may be attributed to the photoperiodic effect of the different lengths of day. Though increased nitrogen fixation was always accompanied by increased growth the converse did not necessarily hold true. Growth and nitrogen fixation cannot always be correlated if calcium is low or absent. The magnesium-calcium-potassium cultures, did not fix nitrogen in significant quantities, even though the plants grew luxuriantly. The plants were low in percentage of nitrogen, and likewise low in calcium and in phosphorus. Their inability to fix nitrogen may have been occasioned by the deficiency in the last two nutrients.

On potassium treated clay mixtures, the ratio of the carbohydrate to the nitrogen found in the plants suggests that the metabolism of carbohydrate in these plants is a more dominant function than the synthesis of protein. Table 10 shows that the plants of cultures 3 and 4, Group B, produced plants rich in carbohydrate and low in nitrogen while culture 3, Group A, produced plants richer in nitrogen. The chemical composition of these cultures shows number 3, Group A, to be rich in calcium and number 3 and 4, Group B, to be rich in potassium and very low in calcium. These facts are in agreement with the suggestion that plants rich in potassium are correspondingly rich in carbohydrate, while plants rich in calcium are rich in nitrogen.

TABLE 10.—INFLUENCE OF POTASSIUM ON THE AMOUNTS OF CALCIUM AND MAGNESIUM TAKEN BY THE SOYBEAN PLANTS, AND THE CARBOHYDRATE NITROGEN PERCENT RATIO.

Culture Number 3, Group A

Weight total gms.	Nitrogen %		Nutritive cations		
			Offered mgms.	Taken mgms.	%
21.460	2.59	Magnesium	140.2	100.1	71.2
		Potassium	0.0	0.0	0.0
		Calcium	212.2	89.8	42.3

Culture Number 3, Group B

Weight total gms.	Nitrogen %		Nutritive cations		
			Offered mgms.	Taken mgms.	%
14.592	2.55	Magnesium	80.2	54	67.5
		Potassium	366	285	77.9
		Calcium	212.2	46	21.7

Culture Number 4, Group B

Weight total gms.	Nitrogen %		Nutritive cations		
			Offered mgms.	Taken mgms.	%
17.807	2.19	Magnesium	80.2	55	68.6
		Potassium	562	384	68.5
		Calcium	212.2	48	22.6

In considering the role which the plant might play in selecting nutritive cations from those available on the clay substrate the following data seem to be of interest. Plants growing on a medium with 50% saturation of calcium and the rest of the exchange cations as magnesium, took up nearly twice as much calcium as those plants growing on a culture with the same calcium saturation and level but with part of the magnesium substituted by potassium. The small amount of potassium added in the latter was taken readily by the plant. A large percentage of the magnesium offered was taken by the plants in both cases. The plant tissue analyses showed the former culture without potassium to be high in calcium and magnesium, while the latter with potassium was high in potassium and magnesium but low in calcium. Since it so happens that in this case the physico-chemical arrangement of the sorbed ions was such that potassium and magnesium were readily available for plant use and calcium not so available, one might believe the plant composition controlled by the particular ionic arrangement of the nutrients on the clay complex.

Measurements by Baver (6) on the cataphoretic velocities of colloidal clay particles saturated with different cations show the distance from a monovalent cation in the ionic atmosphere to the colloidal micelle to be greater than the distance of a divalent cation under similar conditions. This would then place potassium in the outer layer of the ionic atmosphere. By taking valence into consideration we could conclude that the cations arrange themselves in the ionic atmosphere of the colloidal particle with potassium in the outer layer, and magnesium and calcium in the inner layer. The work of Geiseking and Jenny (10) with magnesium and calcium added to hydrogen clay showed that calcium was held much more firmly by the clay than was magnesium. The high hydration of the magnesium ion is probably the factor responsible for its ease of replacement. These data indicate that the physico-chemical arrangement of the ions on the clay at the beginning of the experiment would be such that potassium and magnesium should be readily available and calcium should not be available for the plant. However, the movement of cations into the plant cannot be related wholly to the physico-chemical equilibrium viewpoint, since after the exchangeable cations pass through the cell membrane, the equilibrium may be established or disturbed as the respective ions are not or are extensively synthesized into other compounds by the plant metabolism.

The plants contained considerable quantities of iron, alumina and silica, beyond those found in the seed. Also, the plants grown on non-magnesium treated cultures showed larger amounts of magnesium than were contained in the seeds, which fact suggests that there might have been a partial breakdown of the colloidal clay. Iron, aluminum and magnesium are found in the crystal lattice of the electro-dialyzed Putnam clay in non-exchangeable form. If the clay broke down, one might expect that these would become exchangeable or available for the plant use. The fact that the amounts of iron and aluminum found in the plant were about constant for all treatments indicates that the breakdown in the colloidal clay might be related to the time period of growth of the soybeans rather than to a replacement by the added cations. If iron and aluminum in the clay complex are made more accessible or broken out of the lattice during the plant growth period, it is possible that other positive ions may behave similarly. If these are the facts, it will be essential to give some consideration to the extent to which such change in the supposedly constant clay complex occurs. Further studies only can give accurate measurements of these changes in the mineral structure or its weathering rates in the absence or presence of plants.

Summary and Conclusions

A study was made of the influence of magnesium on the nitrogen fixing power of soybeans by using colloidal clay as a carrier of this cation, along with calcium, and with calcium and potassium as the other plant nutrients. This work revealed the following items of decided importance.

1. Nitrogen fixation increased with higher magnesium levels and higher degrees of saturation of the clay by this nutrient when accompanied by a constant calcium level and degree of clay saturation.
2. Nitrogen fixation did not occur at a low calcium level in the absence of magnesium, but occurred when a small amount of magnesium was added to the low calcium level.
3. There was a close correlation between the amount of nitrogen fixed and the growth attained by the plants on colloidal clay cultures which contained constant levels of calcium and increments of magnesium. The growth increased with increments of magnesium and the nitrogen fixation ran parallel with it.
4. Though increased nitrogen fixation was always accompanied by increased growth the converse did not necessarily hold true. Plants grown on colloidal clay cultures treated with the cations calcium, magnesium, and potassium grew well without fixing nitrogen in commensurate amounts.
5. When calcium and magnesium were the only nutritive elements sorbed by the colloidal clay in the exchangeable form, the increments of magnesium made it possible for the plant to make a more efficient use of the calcium offered at a given level.
6. The addition of hydrogen and of potassium to the calcium-magnesium colloidal clay cultures reversed the previous results. Hydrogen decreased both the growth and the nitrogen fixing power of the soybeans, while potassium increased the growth but reduced the nitrogen fixing power of the plants, possibly through a disturbed carbohydrate-nitrogen ratio in these early plant growth stages.
7. The ability of the soybean plants to fix nitrogen decreased rapidly with consecutive croppings. The second crop fixed very little nitrogen and the third crop fixed no nitrogen. This suggests that the supply of exchangeable bases on the colloidal clay may be reduced by one crop to the point where they become the limiting element in legume growth and their nitrogen fixation.

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