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THE EFFECT OF LIMING AN ACID SOIL ON THE GROWTH OF CERTAIN LEGUMES.

by

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HISTORICAL*

It has long been known that the growth of legumes enriched the soil and enabled the growing of better succeeding cereal crops. The nature of this beneficial effect, however, has come to be known only in fairly recent times.

The earliest theories regarding the nutrition of plants were that all derived their nitrogen from the humus of the soil. The beneficial effect of legumes was supposed to arise quite largely from their deep-rooting habit and consequent bringing up of plant food from under layers to be mixed with the surface soil upon decay of the plants.

It was during this time that chemistry began to be an exact science. The composition of plants was studied and investigations were started as to the origin of plant food. Reasoning from the known facts of the large amount of nitrogen in the air and the small amount in the soil, many of the early chemists inclined to the belief that all plants took their nitrogen directly from the air.

This view was dispelled by the appearance, in 1840, of Liebig's book, "Chemistry in its Application to Agriculture and Physiology". In this book Liebig set forth his so called "mineral theory", namely that plants derive only certain essential mineral elements from the soil and that nitrogen, along with carbon, is taken from the air. Liebig, however, maintained that the nitrogen used was not the free nitrogen of the air, but was taken from the small amounts of ammonia present in the atmosphere.

*Some pains will be taken to review rather briefly most of the important work that has been done along the line of symbiotic nitrogen fixation as well as to pay some attention to the present status of the problem, partly for the direct bearing this will have on the experimental data to follow, and partly for interest in the problem alone.

Although widely accepted at the time, this view of Liebig's was destined to a short life for it was soon ascertained that the amount of ammonia nitrogen present in the atmosphere was entirely too small to support plant growth. Shortly afterwards, laborious investigations were made by Boussingault in France, and Lawes and Gilbert in England as to the source of plant nitrogen. As a result of these investigations, it was definitely settled that plants have no power of using atmospheric nitrogen and hence must secure their nitrogen from combined forms in the soil.

This made the strange behavior of leguminous plants all the more inexplainable.

In the meantime something of the role played by bacteria in soil processes was beginning to be known. It was discovered that through the action of bacteria nitrogenous compounds in the soil were broken down into nitrates which under normal conditions formed the entire nitrogenous food of plants.

All this paved the way for the discovery of Hellriegel and Wilfaith in 1886, which finally cleared up the mystery. In a series of sand culture experiments they found that the growth of non-leguminous plants was directly proportional to the amount of nitrates applied, but that such a relation did not always obtain for the legume plants. The legumes supplied with nitrates grew in the normal way. Of those not supplied with nitrates, most perished after the supply of plant food in the seed had been exhausted, but occasionally a plant recovered, started to grow, and made practically the same growth as those supplied with nitrates. Hellriegel and Wilfaith drew two conclusions, first that legumes could use atmospheric nitrogen, and second that this power of

using atmospheric nitrogen was not directly a power of the plant but was brought about by means of bacteria existing in the nodules on the roots of the plant.

Other investigators, notably Nobbe in Germany, Atwatér in America, Lawes and Gilbert in England, and Boussingault in France, immediately verified their results so that now the power of legumes to fix atmospheric nitrogen through the agency of the nodule bacteria, Bacillus radicicola, is scarcely questioned.

Aside from scientific interest in the matter, the immense practical ends that may be gained make the study of the Bacillus radicicola one of much interest and of great importance.

From the very first it was known that bacteria from one legume might not inoculate another. Later it was found that in some cases the bacteria would finally acquire the ability to exist on a different host plant from the first. It then became quite a question whether there were one or more species of the bacteria. Early investigators believed that the organisms were all of one species, - the different varieties being the natural result of different environmental conditions. Latterly Hiltner and Störmer have decided that there are two species, one Rhizobuim radicicola and the other Rhizobuim beyerinckii. The former includes the organisms of soy beans, lupines, and seradella, while the latter includes all other. The point cannot be said to bedefinitely settled as yet.

At first it was thought that the relation existing between the host plant and the bacteria was one of true symbiosis, the plant furnishing the bacteria carbohydrates and the bacteria furnishing the plant nitrates. In the main this is correct and the relation is, broadly speaking, a symbiotic one.

However, as pointed out by Hiltner and other recent workers, there are objections to the unqualified acceptance of this view. In the first place either the plant or the organism may exist independently of the other. The bacteria exist thus independently between crops of the legume, obtaining the necessary carbohydrates from decaying organic matter in the soil. Also conditions may arise when the host plant is injured rather than benefited by the bacteria; such a state is indicated by the weakening of a plant after the entrance of the bacteria. Similarly the plant may oppress the bacteria. The changes undergone by the bacteria in the bacteroid tissue are supposed by some to be efforts on the part of the bacteria to adjust themselves to this oppression. Furthermore, the plant seems to resist the entrance of the bacteria. It is known that on soils rich in available nitrogen legumes inoculate but slightly, resisting efforts of the bacteria to secure entrance It is only when the plant is weakened by nitrogen starvation that the bacteria are able to overcome its resistance and produce inoculation. In accord with this, Lipman[1] makes the statement that "After some tubercles are formed on legume plants, the formation of additional tubercles becomes more difficult." And he continues "Now, since plants already possessing a few nodules are better supplied with nitrogen, their vigor is increased and they can better resist the attacks of the bacteria." Hiltner claims that there exist strains of bacteria differing in their power to break down the resistance of the plant and secure entrance, and further that when a plant has been invaded by a strain of these more virulent bacteria that then it is immune to attacks of all weaker strains.

Note - Numbers refer to references listed at the close of the thesis, page 52.

The question may be raised whether the present belief is not swinging too far from the earlier one of true symbiosis. It is not as yet definitely proved that the bacteriod forms represent a stage of degeneration or transformation due to oppression by the plant. B. Heinze[2], as a result of investigations, comes to the conclusion that the utilization of the nitrogen of the air and soil goes on simultaneously and there does nothave to be a complete or even large depletion of soil nitrogen before the nodule forming process begins.

The method of entrance of the bacillus into the plant root and of subsequent development has been studied by several workers in some detail. The following statements, summarized from a recent publication by E. B. Fred of the Virginia Station,[3] express very well the results of these studies. In artificial cultures B. radicicola is for a short time actively motile but it is difficult to say how the organism will act in the soil. Infection probably depends upon the accidental nearness of roots and bacteria in the soil. The first infection is through the root hairs. The bacteria on attacking a root hair secrete a substance which causes a bending at that point. After having once gained entrance into the root hair, the bacteria multiply rapidly, forming a thread-like growth from the point of infection along the hair into the inner cells. Soon the young tubercle arises and growing outward forms a swelling on the side of the root. Growth is at the outward periphery of the tubercle and not at the base. It is due to this manner of growth that the tubercles appear as if developed exogenously to the roots instead of endogenously as they are.

The activities of the tubercle bacteria are readily influenced by most soil conditions but of particular importance are the

following three: the aeration of the soil, the presence of organic matter, and the supply of lime or other basic substances.

The B. radicicola is aerobic and hence thrives best in well aerated soils. This perhaps partly explains the location of most of the nodules in the first few inches of surface soil. However, in a paper[4] published in 1900 Hiltner makes the statement that the location of nodules is independent of their oxygen requirements. The freedom from infection of the lower roots is due, he claims, to the immunity brought about by earlier infection of the upper ones. He further states that it is not difficult to secure inoculation on the lower roots if only inoculation is prevented by some means on the upper.

In this connection some observations by Alway and Bishop[5] of the Nebraska Station are interesting. They removed sections of soil from an eight year old alfalfa field. Only a few nodules were found on the roots exposed on the pit walls within the first few inches; but at greater depths they were numerous, many large clusters being found in the second and third feet and one very large cluster at a depth of four feet. The authors state that the deeply located clusters had apparently formed in old vertical fissures.

The effect of organic matter on inoculation is two fold. The nitrates resulting from its decomposition are injurious but the increased aeration due to its presence is beneficial. The decay of organic matter furnishes the carbohydrates necessary for the bacteria when existing in an unsymbiotic state.

The third important soil condition influencing the activities of the tubercle bacteria and hence the growth of legume plants, is the presence of lime or other basic substances in the soil.

The following statements from Hopkins' "Soil Fertility and Permanent Agriculture"[6] express the generally accepted belief as to the detrimental effect of soil acidity on the growth of legumes. "Clover, alfalfa, alsike, cowpeas, soy beans, and most other valuable legumes will not thrive on soils that are strongly acid. To be sure such crops can be made to grow by the aliberal application of farm manure or other fertilizers but the nitrogen-gathering bacteria of such legume plants do not properly develop and multiply in acid soils, and consequently the legumes do not have the power which they should have to accumulate large quantities of atmospheric nitrogen by means of the root tubercle bacteria."

Lupines and seradella are known exceptions to these statements; the nodules develop better and the plants make a better growth in acid soils. It is doubtful if cowpeas are very sensitive to soil acidity. Kellerman[7] of the U. S. Bureau of Plant Industry, states that the cowpea does not respond to lime in soils where clover refuses to grow without lime.

"The favorable effect of lime on leguminous plants is due to the direct action on the plants as well as on the bacteria in the soil."[8] Aside ffom physical amelioration of the soil, limestome may directly affect the plants in the following ways: increased production of available nitrogen and potassium, and increased or decreased production of available phosphorus.

Several investigators have endeavored to ascertain the effect of calcium carbonate on the availability of phosphorus in soils. In some instances the availability was increased while in others it was diminished. The nature of the phosphorus compounds in the soil, the form in which the phosphorus fertilizer is applied if one is used, and probably other more deeply located soil condi-

tions greatly influence the results. If a conclusion can be drawn from experimental work, it is that the first effect of lime may be to decrease the amount of available phosphorous but that the final result is generally an increased supply.

Lime is believed to increase very markedly the amount of available potassium. The magnitude of this action depends largely upon the character of the potassium compounds in the soil but doubtless under most conditions it is appreciable, and in some instances may be considerable.

A considerable amount of recent work has been done as to the effect of calcium carbonate in stimulating nitrification. The results of two investigations of this nature will be noted.

Lipman, Brown and others[9] of the New Jersey Station tested the ammonifying power of varying amounts of $CaCo_3$ added to mixtures of 80 gms. silt loam, 20 gms. quartz sand, and 2 gms. dried blood. After incubating for seven days at a temperature of 27° C the ammonia found was as follows:

Mgs. $CaCO_3$ added	Mgs. NH_3 found
0	106.86
50	120.56
100	121.88
300	130.63
500	123.78

Ammonification increased up to the addition of 300 mgs. $CaCO_3$ after which a decrease set in. Three hundred mgs. $CaCO_3$ to 100 gms. soil is at the rate of three tons per 2,000,000 lbs. soil.

Brown[10], of the Iowa Station, has recently published the results of some work along this line. Part of his work had to do with the effect of ground limestone on nitrification. The soil used was of the glacial Wisconsin drift formation. Pots were filled with 30 lbs. of the soil to which varying amounts of lime-

stone had been added. One-half of the pots was left bare, to be sampled from time to time, 4 in all, as the experiment progressed for bacteriological work. In the nitrification work 100 gm. samples of the soil were taken. Two hundred mgs. dried blood were added to each. The samples were then incubated for six weeks. Optimum moisture conditions were maintained throughout. The results as given in the following table are the average of the four samplings.

Pot No.	Treatment	Av. Mgs. N.
1 & 2	No lime	15.41
5 & 6	1000 lbs. lime per acre.	17.81
9 & 10	2000 lbs. lime per acre.	19.78
13 & 14	4000 lbs. lime per acre	23.14
17 & 18	6000 lbs. lime per acre.	26.08

Brown's conclusion is as follows: "Applications of lime cause increasing nitrate production, depending on the amount of lime applied, the gains being almost proportional to the amount of lime."

Evidently in analyzing the effect of limestone on the growth of legumes considerable importance should be attached to the direct effect of the material on the plant growth.

The question arises here as to the effect on legume inoculation of the increase in nitrates due to liming. Hiltner [4] observed that pea plants grown in a liter of nutrient solution containing 5 mgs. of saltpetre remained free from nodules, whereas in a similar solution except for the absence of nitrates, abundant

nodules were formed. Hiltner's comment is that the effect here could not have been the stimulating effect on the plant of the small amount of nitrogen, but rather that of an injurious effect on the bacteria. In sand cultures Hiltner also found the nitrate to have a strong depressing effect on nodule formation. But on plants grown in rich humus soils the formation of nodules was but little depressed showing that the effect of the nitrate is not due to its food value to the plant. As further proof of this, other nitrogenous compounds such as ammonium sulphate were less harmful, and extract of horse manure was entirely without effect on nodule formation.

These results of Hiltner are interesting but are of little assistance in answering the present question. It seems probable that the only effect of the increased nitrates from liming would be that arising directly from their food value to the plant.

The statements previously quoted from Hopkins' "Soil Fertility and Permanent Agriculture" express the belief in the immense benefits accruing to legumes from the presence of lime. In accord with this, the particular richness of limestone soils in legume vegetation, and the proverbial richness of limestone countries are common facts. And yet lime in field tests on acid soils frequently does not produce as great an increase in legume growth as in the growth of cereals.* Pot culture experiments in several instances have shown depressed growths where lime was employed.

*An average of the results of 6 to 10 years cropping on five experiment fields on the acid soils of southern Illinois shows that limestone increased the yearly yield of clover one-half a ton, and of cow peas and soy beans each a tenth of a ton, whereas corn was increased 8 bushels, wheat 5½ bushels, and oats 9 bushels. The clover yield is an average of the years when some sort of a catch was secured. If the computation had been based on the number of seedings the increase would not have been a fourth of a ton.

In connection with the ammoniafication experiment already noted from New Jersey (p. 8) a green house pot experiment with barley and winter vetch was performed. Pots were filled with 20 lbs. of a soil mixture consisting of six pounds of quartz sand and 14 lbs. of silt loam soil. Four grams of acid phosphate and two grams of potassium sulphate were added to each pot.

Pot No.	Special Treatment	Crop	Av. yield of dry mat.
1 & 2	None	Barley	18.75
3 & 4	12 gms. ground oyster shell.	Barley	19.25
5 & 6	30 gms. ground Oyster shell.	Barley	16.75
11 & 12	None	Winter vetch	8.0
13 & 14	12 gms. ground oyster shell.	Winter vetch	5.85
15 & 16	30 gms. ground oyster shell.	Winter vetch.	5.5

The decrease of the vetch yield is greater than that of the barley. The author's comment as to the depressed yields when 30 gms. of oyster shell were added is, "there was a depressed growth that is sufficiently marked even when due allowance is made for experimental error."

An earlier experiment at New Jersey, by Voorhees, Lipman and Brown[11] may be mentioned in this connection. Oats and crimson clover were grown in boxes containing 50 lbs. of soil. Two soils were used. One was a sandy soil used for market gardening; the other was a red shale poor in available plant food. Different amounts of limestone were applied with and without other fertilizers. Where no other fertilizers were applied lime increased the yield of oats in the sandy soil and decreased it in the shale

soil. The explanation given was that the sandy soil was low in available N. and the lime, by stimulating increased production of nitrates, made possible increased yields. The shale soil, on the other hand, was low in available phosphorous and the lime, by fixing part of this small supply, depressed the crop yields. This explanation is borne out by the fact that when dried blood, acid phosphate, and potassium sulphate were applied in connection with the lime, that then lime neither increased the yield in the sandy soil nor decreased it in the shale soil.

On the whole, lime decreased the yields of clover in both soils whether applied with or without the other fertilizers.

Commenting on the entire results the authors say that applications of lime reduced in many instances the yields of dry matter. Bacteriological studies made at the time showed that 'lime increased nitrification in all instances, therefore the depression in yields of dry matter were not due to lack of nitrogen but to some other cause'. "This cause must be sought either in direct physiological interference with the assimilation processes in the plants, in the decreased supply of available phosphoric acid, in the accumulation of injurious substances on account of the greater bacterial development, or in the using up of a portion of the available plant food by the increased hosts of soil organisms."

As an instance of pot culture work showing increased growth of legumes due to the application of limestone, the recent work at the Penn. Station, by Gardner & Brown[12] may be mentioned. They carried on extension pot culture work to determine the effect of lime on the growth of clover. The soil used was obtained from the station fertility plots. The varying amounts of acidity were consequently due to the different fertilizer and crop treatments.

No. Plots Averaged	Av. lime required per acre as determined by the Veitch Method.	Relative Green Wt. of Clover tops		
		No Lime	Sufficient lime to Neutralize Acidity	Limestone Veitch+one ton.
24	None	100	--	103
26	190 lbs.	100	102	110
12	843 "	100	115	131
10	1395 "	100	197	222

Here lime produced largely increased growths, in the very acid plots practically doubling the yield.

In this connection the question may well be raised whether acidity due to different sources, has the same quantitative effect on plants. For instance, compare the case of an upland soil low in organic matter where the acidity is presumably largely due to the decomposition of acid silicates, with that of a marsh soil where the acidity is largely due to the incomplete oxidation of organic material, Assuming equal amounts of acidity as measured by the lime water test it would not seem to necessarily follow that equal applications of limestone would produce equal increases in crop growth.

In the experiments noted no attempt was made to separate the effect of limestone on the growth of legumes into its two factors, viz. its direct effect upon the plant and its indirect effect upon the plant through its influence on the legume bacteria. It has already been stated that the direct effect upon the plant, through the increased amounts of available plant food, must be considerable. This is verified by the fact that the growth of cereals is increased appreciably by the direct use of lime. Nevertheless, it is generally believed that the indirect effect, through providing better conditions for the bacteria, is of much more importance.

Very little definite experimental data can be found bearing on this point. In fact very little data of any sort could be found by the writer dealing with the effect of lime on legume inoculation. Indefinite statements based on rather casual observations seem to include the whole of the experimental information on this important point.

As one of the more definite of these statements that of A. V. Donnan[13] may be given. "Lime applied to (lucerne) plots caused an enormous increase in the number of plants with nodules, and also increased the number of nodules per plant."

In view of the fact that legumes are grown quite largely on account of their power to add atmospheric nitrogen to the soil, it becomes important to determine somewhat definitely the amount of nitrogen they can thus fix. Experimental work of this nature is rendered rather difficult in that relatively large amounts of nitrogen must be added to a soil before the change can be detected by present analytical methods. However, some fairly definite work of this sort has been done.

In 1902 on the Canada Experimental Farms, an experiment[14] was begun to determine to what extent the nitrogen content of a soil could be increased through turning under successive growths of a clover crop. The plan of the experiment is briefly as follows:

A plot 16 by 4 feet was laid off and boards sunk to the depth of 8 inches. The surface soil was then removed to this depth and a poor sandy soil substituted in its place. The plot was seeded to red clover and by digging and reseeding every second year has been kept in clover continuously since. At tne beginning of the experiment superphosphate and muriate of potash were applied at the rate of 400 and 200 lbs. per acre respectively. In 1909 lime

was added at the rate of one ton per acre. At intervals the plot has been sampled and determinations made for total nitrogen.

Time of Sampling	Nitrogen found. Percentage in water-free soil.	Nitrogen found. Lbs. per acre to a depth of 4 inches.
Before experiment	.0437	533
After 2 years	.0580	708
" 4 "	.0608	742
" 5 "	.0689	841
" 6 "	.0744	908
" 7 "	.0750	915
" 9 "	.0824	1005
Increase of N. in 9 years.	.0387	472

In interpreting these results it must be considered that the plants may have received some of their nitrogen from under soil layers; also that an appreciable amount of nitrogen may have been fixed by Azotobacter bacteria. On the other hand the plants growing year after year on the same plot would in all probability secure much less of their nitrogen from the air than under average farm conditions. So that on the whole the results likely do not equal the fixing power of the same number of crops grown under average farm conditions.

Hartwell and Pember[15] of the Rhode Island Experiment Station have lately published the results of a 5 year pot experiment to determine the gain in nitrogen through the growth of legumes. Two crops were grown each year, making a total of ten crops. As a winter crop, vetch was grown in all the pots and mixed with the soil. The summer crops were removed and analyzed for nitrogen.

Optimum amounts of all nutrients were added except nitrogen. Ground limestone was applied at the rate of about 2600 lbs. per acre 7 inches. The soil was a sandy loam, containing some fine gravel. The results obtained from the soy bean and cow pea series are given in the following table:

Crop	Pot No.	N. in initial soil - amount added in seeds, water & manure.	N. in aerial portion of summer legume removed.	N. in soil at end of exp.	N. in final soil & in crops removed	Net gain in N.	Av.lbs. per acre
Soy Beans	Av.of4 pots	35.25	10.95	39.38	50.32	15.07	1844
Cow Peas	Av.of4 pots	31.12	12.01	36.70	48.71	17.59	2153

The data show that in round numbers the legumes added one ton of nitrogen per acre in 5 years. If we assume that the fixing power of the vetch was equal to the summer legumes, this is at the rate of 200 lbs. per crop. The average weight of the moisture free crops was approximately 5 tons per acre. Forty pounds, there fore, was the amount of nitrogen added per ton of dry weight of crops. It should be borne in mind that this experiment presented optimum conditions for the fixation of atmospheric nitrogen.

In this connection reference may be made to the well known Rothamstead work on Geescroft Field where in three years land cropped to barley and clover gained 175 lbs. nitrogen per acre and in addition 319 lbs. were removed in the crops, making a total of 494 lbs. secured from the air per acre in three years time[16].

EXPERIMENTAL

PURPOSE

The experimental work for this thesis was planned to secure information as to the effect of limestone applied to an acid soil (1) on the growth of legumes, (2) on the activities of tubercle bacteria, and (3) on the amount of nitrogen secured from the air by symbiotic fixation. A minor experiment was added to determine the effect of quantities of organic matter on the location of nodules.

In working on these problems it was found possible to obtain information on other points of interest. These will be presented as they more or less naturally introduce themselves in connection with the other work.

PLAN

The experiment was a greenhouse pot experiment with cowpeas, soy beans, and mammoth red clover. A strongly acid soil was used. The main experiment consisted of four series with the following special soil treatments:

Series 1 - No limestone.

Series 2 - Medium limestone (at the rate of one ton per 2,000,000 lbs. of soil over the amount required to neutralize the acidity.)

Series 3 - Heavy limestone (five tons over amount to neutralize acidity.)

Series 4 - Heavy limestone (five tons excess) and dried blood at the rate of 1100 lbs. per 2,000,000 lbs. of soil.

Each series included three pots of each legume and also two fallowed pots. This series (1) included three pots of clover, three of cowpeas, three of soy beans, and the two fallowed pots.

All pots were inoculated for their respective legumes and all received treatments of bone meal and potassium sulphate that optimum growing conditions might be secured with respect to phosphorous and potassium.

In addition there was a minor experiment including three series of two pots each in which mammoth clover was grown and in which the special soil treatments were as follows:

Series 1 - Fifty grams limestone (at the rate of 3 tons over the amount required to neutralize the acidity.)

Series 2 - Fifty grams limestone and 150 grams manure (at the rate of 15 tons per acre.)

Series 3 - 150 grams of manure.

The manure, well rotted yard manure, was not mixed throughout the soil but was added in lumps to various parts of the pots while filling with soil. It was hoped in this way to secure information as to the effect of organic matter on the location of nodules. The pots were inoculated and received treatments of bone meal and potassium sulphate.

THE SOIL.

The soil was secured from just outside the Bowling Green outlying Experiment Field in N. E. Missouri. In type it is a gray silt loam on tight clay. The tight layer begins at 12 to 15 inches and extends on to the point of a forty inch soil auger.

A chemical analysis of the Bowling Green soil previously made by the Experiment Station chemists gave the following results:

Soil layer	Organic matter	Total N.	Ac. Sol. P_2O_5	Acid Soluble K_2O
0-7 inches	5.77%	.175%	.236%	.292%

An acidity determination by the Veitch lime water method of a composite sample from the soil secured, showed that one gram of soil required 0.0012885 gms. $CaCO_3$ to neutralize the acidity. This is at the rate of 2577 pounds per 2,000,000 pounds of soil (an acre 7 inches.).

Only the surface seven to eight inches was used. When secured the soil was in grass sod. The soil was secured directly after a heavy rain and while it was still almost muddy.

Preparatory to putting in the jars, the soil was sieved and thoroughly mixed.

TECHNIQUE.

Three gallon glazed earthenware jars were used. No drainage was provided. To secure if possible a more uniform distribution of roots than is generally secured in pot culture work, to prevent excessive evaporation, and to prevent baking and packing of the surface soil, the following scheme was tried out for the addition of the water needed by the plants. Three inch porous flower pots were inverted on the bottoms of the jars from each of which a glass tube extended up above the surface of the soil. This arrangement served its purpose very well indeed. The soil remained in fine physical condition throughout the experiment, and when harvested the roots were found well distributed throughout the soil. Had it not been necessary towards the close of the experiment to add water to the surface of the soil in applying spray mixtures to keep in check the red green house spider, fairly accurate data would have been secured as to transpiration of water.

The amount of moisture in the pots was kept at one-half the saturation amount as nearly as could be done by adding distilled water once a week during the early part of the experiment and twice a week during the remainder of the time. This amount was found to be twenty percent of the air dry weight of the soil.

The limestone used was sifted through a 100 mesh sieve, the dried blood through a 40 mesh, and the bonemeal through a 60 mesh. As analyzed by the station chemists, the dried blood contained 12.84% nitrogen; the steamed bone meal, 1.17% nitrogen and 30.66% P_2O_5.

In the main experiment 9247 grams of the air dry soil were used per pot. The complete fertilizer treatment in this part of

the work is shown in the following outline.*

Series 1-	....	1 gm. bone meal, 1 gm. K_2SO_4
"	2-30 gms. limestone...............	" " " " " " "
"	3-75 " "	" " " " " " "
"	4-75 " " 5 gms. dried blood	" " " " " " "

The inoculating material and fertilizers, excepting the potassium sulphate, were added and well mixed with the soil before putting it in the jars. The potassium sulphate was added in solution afterwards.

In the minor experiment the amounts of soil and the fertilizers used in each series were as follows:

Series 1 - 10,000 gms. soil, 50 gms. limestone, 2½ gms. bone meal, 2 gms. K_2SO_4.

Series 2 - 9850 gms. soil, 150 gms. manure, 50 gms. limestone, 2½ gms. bone meal, 2 gms. K_2SO_4.

Series 3 - 9850 gms. soil, 150 gms. manure, 2½ gms. bone meal, 2 gms. K_2SO_4.

With the exception of the manure, all the fertilizers were mixed with the soil before putting it in the jars. The 150 gms. of manure were roughly divided into 10 lumps and added to various parts of the jar while putting in the soil. Four of the lumps were placed near the surface of the soil, four near the center, and two well towards the bottom.

In both experiments inoculation was secured by adding soil from the station farm on which the various legumes had grown the previous season and also by adding liquid cultures kindly furnished by the Bureau of Plant Industry of the U.S. Dep't of Agriculture.

*Two crops of soy beans were grown in the same pots. The first crop made a very small growth and no weights were taken. Before planting the second time an additional 1½ grams of bone meal and likewise of K_2SO_4 were added to each pot.

All the clover pots and half the fallowed pots were kept in the horticultural greenhouse at a temperature of 70^{o} - 75^{o} C. during the day, and 60^{o} - 70^{o} C. during the night. The cow pea and soy bean pots were kept in the agronomy greenhouse at a temperature of 85^{o} - 95^{o} C. during the day and 70^{o} - 75^{o} during the night.

The time of planting and of harvesting, the number of plants per pot, and something as to the maturity of the plants are shown in the following outline:

Plant	Date of planting	Date of harvest	No. Plants per pot	Maturity of plants at harvest.
Black Cowpeas	Nov.22,1912	Feb.22,1913	5	Mature pods on many of the plants.
Medium yellow Soy beans	Jan.10,1913	Mar.12,1913	8	Mature pods on many of the plants.
Mammoth red clover (main experiment)	Nov.15,1912	Apr. 5,1913	20	Not yet in bloom.
Mammoth red clover (minor experiment	Dec.13,1912	Apr.19,1913	10	" " "

In the main the laboratory work carried out for the accumulation of data was as follows:

Determinations of nitrates by the phenol-disulphonic colorimetric method were made before the experiment started and at the close in the fallowed pots and in the pots in the main experiment planted to cowpeas and to clover. The dry weights of all the crops, tops and roots separately, were secured. The amount of inoculation was ascertained, by removing, counting, and weighing the nodules of the cowpeas and soy beans and by inspection of the clover. Nitrogen determinations by the Kjeldahl method were made of the cowpeas and clover in the main experiment, tops and roots separately.

Cow peas at harvest.

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CROP YIELDS.

The total dry matter contained in the crops is shown in the following table:*

Table I Grams of Dry Matter in the Tops and Roots of the Crops.

No. Series	Treatment	No. Pot	Clover (oven-dry)	Cow Peas (oven-dry)	Soy Beans (air-dry)
1	None	1	20.48	14.69	6.88
		2	19.93	13.46	6.78
		3	21.28	15.98	6.74
		Av.	20.56	14.71	6.80
2	Medium Limestone	4	17.96	12.61	5.60
		5	20.31	(10.66 Only 4 plants)	6.22
		6	18.25	14.17	5.61
		Av.	18.84	13.39	5.81
3	Heavy Limestone	7	18.98	15.47	4.75
		8	18.23	15.27	5.16
		9	18.49	15.11	6.31
		Av.	18.57	15.28	5.41
4	Heavy L. and Dried Blood	10	15.71	16.30	5.15
		11	17.68	10.22	5.96
		12	14.88	11.11	5.37
		Av.	16.09	12.54	5.49

It is seen that the clover yield was best on the untreated

*Properly the total nitrogen in the crops should be included in this table. The data are omitted for the reason that the effect of the fertilizer treatment was very little different on the total nitrogen from what it was on the total dry matter.

Clover in minor experiment after nine weeks growth.

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pots and decreased appreciably as the treatment increased. A like decrease is evident with the soy beans except that the application of dried blood increased the yield slightly over heavy limestone alone. The yields secured from the cow peas are variable; but the variations likely are not the result of the crop treatment. On the night of February 18th the temperature in the greenhouse dropped almost to freezing. The cow peas were damaged considerably, the best pots seeming to suffer most.

The yields of clover secured in the minor experiment are shown in the following table:

TABLE II - Clover Weights in Minor Experiment.

Treatment	No. Pot	Clover (Roots & Tops, air-dry wts.) gms.
3 tons limestone (in excess of acidity)	1	19.68
	2	25.12
	Av.	22.40
3 tons L. (excess) And 15 tons manure	3	33.72
	4	32.57
	Av.	33.15
15 tons manure.	5	28.61
	6	28.89
	Av.	27.75

Unfortunately the no-treatment pots were omitted in this experiment. It is, therefore, impossible to determine the effect of the lime-alone treatment on the crop yield. The data show the yields here to be less than in the manure-alone pots. However, applied with manure limestone gave a substantial increase. The reason for this is not clear since in the main experiment limestone produced no increase even when applied in connection with large

Clover in main experiment at harvest.

amounts of dried blood. It may be that a deficiency of mineral plant food existed which was corrected by that in the manure, though the heavy applications of bone meal and potassium sulphate render this improbable. Neglecting this point, it may be said that on the whole the influence of limestone was unfavorable on the growth of the legume plants.

The manner of growth of the crops, perhaps, throws some light on this part of the work.

The following statements are taken from the notes on the growth of the clover in the main experiment made from time to time as the experiment progressed. December 24 (one month from time plants were up). The plants in series 1 are noticeably larger than those in the other series.

January 2. Plants in series 1 and 2 are much larger than those in series 3 and 4.

February 11. Plants in series 1 are considerably the best. The other series grade on down in order of treatment. There does not appear to be over half the growth in series 4 as in series 1.

March 13. The plants of the different series are losing their noticeable variations. Series 4 is still clearly the poorest, with series 3 next. There does not appear to be a large amount of difference between series 2 and series 1.

At the close of the experiment the differences between series 1, 2 and 3 were but scarcely apparent to the eye. Series 4 was still inferior in appearance.

Probably the amount of water transpired by the clover plants throughout the experiment is a better index of the relative growth from time to time on the different series. In the following table,

· Soy beans after six weeks growth.

the figures under any date represent the amount of water transpired by the crops from the previous date up to that time. The transpiration figures were obtained by subtracting from the amounts of water lost by the pots the average amount lost by the four fallowed pots. To facilitate comparison between the series, instead of the actual amounts, relative figures are given based on the transpiration in series 1 always equalling 100. The last column is added to show the fairness of the method.

TABLE III

Relative Water Used by Clover Series Throughout the Experiment.

	Dec 21	Jan 18	Jan 31	Feb 8	Feb 14	Feb 22	Mar 1	Mar 10	Mar 17	Mar 24	Total dry Wt. of crop gms.
Series 1	100	100	100	100	100	100	100	100	100	100	20.56
" 2	87	78	81	89	80	88	80	95	74	89	18.84
" 3	72	51	56	54	107	80	85	84	95	81	18.57
" 4	74	31	28	29	67	64	68	78	67	72	16.09

Comparing series 2, 3 and 4 with series 1, the decrease in transpiration of series 2 was slightly greater during the first half of the experiment, the transpiration of series 3 was but little over a half during the early part of the experiment but was almost as much during the latter part, that of series 4 was not quite a third during the first part of the experiment but was almost three fourths at the close.

It is evident that the differences in growth among the series were not the result of a gradual increase or decrease, but were almost wholly due to the fact that some series started rapid growth sooner than others, i. e. the period of no apparent growth characteristic of plants from small seeds was of longest duration in the inferior series.

The clover in the minor experiment afforded similar characteristics of growth. The manure-alone and manure-lime pots began rapid growth first. Later the lime-alone pots apparently made as rapid growth as the former. Likewise with the cowpeas, series 1 during the first part of the experiment was slightly the best.

This much seems to be shown by the manner of growth of the plants. The effect of the limestone at first was to produce changes in the soil detrimental to the crop growth. Later this influence was largely overcome. It is not possible to state definitely just what these changes were. Work to be presented later shows that the limestone greatly stimulated nitrification. Clearly the inferior growths were not due to lack of nitrates.

Some investigators[17] are making much of the using up of available phosphates by the increased bacterial life as a means of explaining the depressing effects of lime sometimes observed on plant growth; but the locking up of phosphorous in this way could exist for only a short time and the process would hardly seem to be of sufficient moment to account for the results in the present experiment.

The most ready explanation is that the lime largely fixed the available phosphates. Laboratory work as to the dissolving effect of water solutions of carbon dioxide on tricalcium phosphate has shown but little action when the solutions are saturated with calcium carbonate, whereas in its absence the amount of phosphorous dissolved is proportional to the strength of the solution.

To secure information as to the effect of the limestone on the availability of phosphates in this experiment, samples of soil were secured on March 12 from the fallowed pots in the agronomy greenhouse. Determinations of available phosphates were made

by the Official fifth normal hydrochloric acid method. The results are as follows:

TABLE IV - Effect of Limestone on Availability of Phosphates.

Series	Treatment	Parts P. per million of oven-dry soil.
1	None	28
2	Medium L.	29
3	Heavy L.	35
4	Heavy L. and dried B.	35

As shown here limestone increased the availability, the medium application very slightly and the heavy application considerably. However, as previously indicated, the first effect of the limestone may have been to lessen the phosphorous availability. It is possible that with some soils the first effect of an application of limestone might be derogatory to plant growth through fixation of a considerable portion of the available phosphates whereas the final result might be beneficial through reduction of the insoluble iron and aluminum silicates.

The tendency of lime to depress crop yields in pot-culture work has been noted by a number of investigators. Two experiments from the New Jersey Station illustrating this were mentioned in the Historical of this thesis. The work of Ellet and Hill [18] also, may be referred to. They found that in sand cultures acid phosphate gave a smaller yield when applied with calcium carbonate than when applied alone.

That a depressing effect of calcium carbonate is scarcely ever noticed in field experiments is perhaps due to several facts* In field experiments the fertilizers are usually applied in much smaller amounts than in pot culture work. Quite likely there is

*Mooers[19] did notice a depressing effect of limestone on the power of floats to increase crop yields.

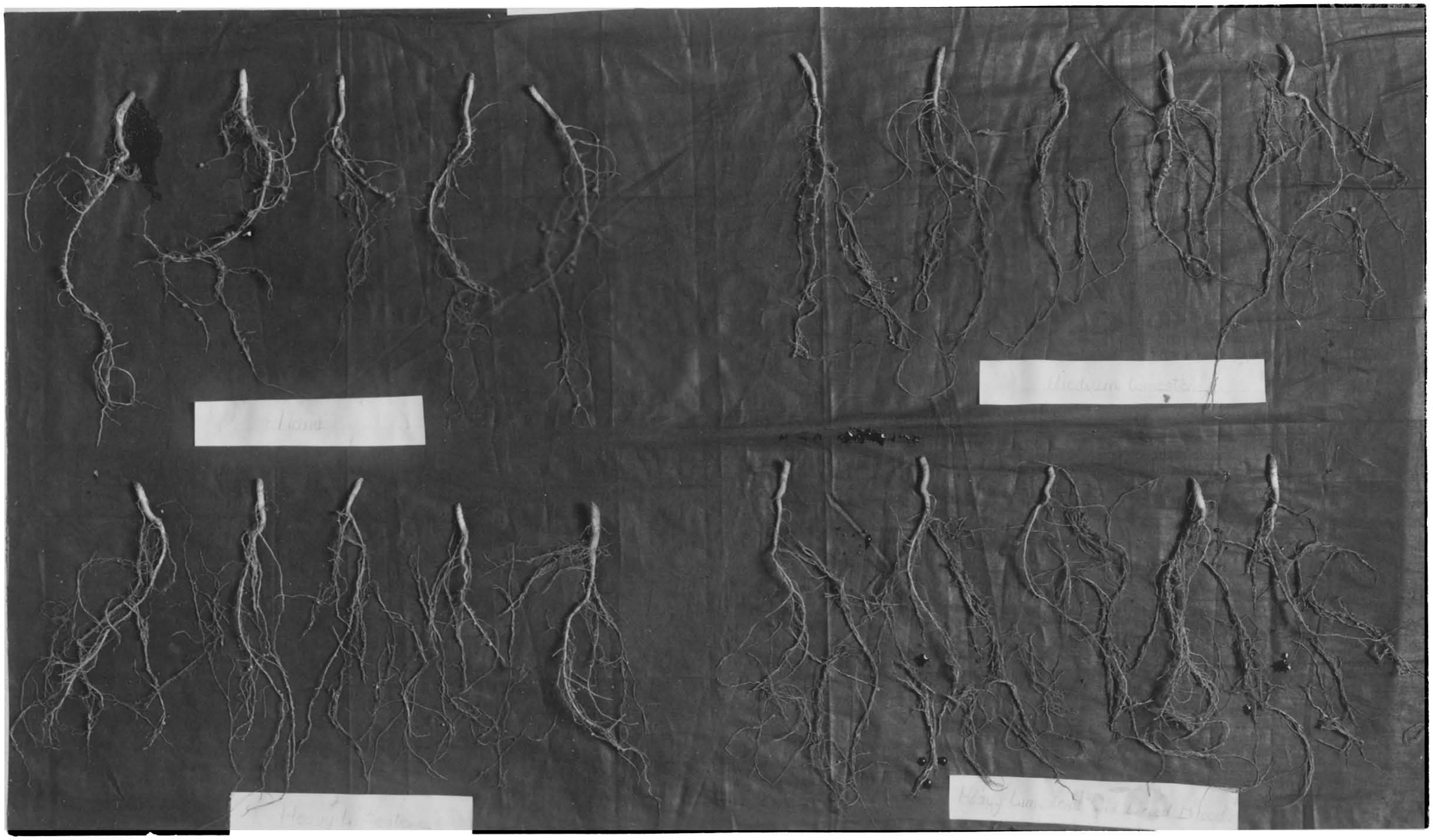
not a very intimate mixing of the fertilizers either among themselves or with the soil. The fertilizers are often applied some little time before planting, and hence the first effect of the limestone in fixing available phosphates is likely to be largely overcome before plant growth. Nevertheless, if in pot culture work an application of less than three tons of limestone per acre produced a noticeable decrease of crop growth, it would seem that heavy applications of lime to the same soil in the field probably would have a depressing effect for a time at least.

INOCULATION, AMOUNT OF NITRATES, andPERCENT OF NITROGEN

The following table shows the inoculation secured with the different crops.

TABLE V - Inoculation, Showing the Effect of the Treatment.

			Cow peas		Soy beans			
No. Series	Treatment.	No. pot	Count of nodules.	Wt.of oven-dry nodules gms.	Count of nodules.	Air-dry wt.of nodules gms.	Clover	Clover in minor experiment
1	none	1	213	.2165	7	.0130	Inoculation best on the no-treatment pots. Thence decreasing in order of treatment thruout the series.	Inoculation best on the manure-alone pots. Second best on the manure-lime pots, & poorest on the lime-alone pots.
		2	201	.2693	17	.0524		
		3	164	.1849	17	.0280		
		Av.	193	.2236	14	.0311		
2	Medium Limestone.	4	164	.1073	23	.0230		
		5	214	.0643	17	.0124		
		6	264	.1136	35	.0160		
		Av.	214	.0951	25	.0171		
3	Heavy Lime-Stone	7	181	.0243	13	.0046		
		8	198	.0187	9	.0034		
		9	167	.0292	20	.0048		
		Av.	182	.0241	14	.0043		
4	Heavy L. & dried blood	10	195	.0265	8	.0024		
		11	94	.0099	3	.0016		
		12	173	.0164	7	.0020		
		Av.	154	.0179	6	.0020		



Cow pea roots, showing inoculation.

The effect of limestone on inoculation was one of marked depression not so much on the number of nodules, at which point the influence was only slight, but on their size, where the influence was very great. The effect was largely independent of the legume used.*

Originally it was planned to count the nodules on the clover roots but the work proved too laborious. However, inspection clearly showed that inoculation decreased as the treatment increased. There was probably most difference between the series 2 and series 3 pots and least between the series 3 and 4 pots.

In the minor experiment the manure alone pots showed clearly the best inoculation. The difference between the lime-alone and manure-lime pots was not great but was in favor of the manure-lime pots.

The count of nodules on the cow-peas and soy beans shows a close agreement in the variation from series to series, increasing in series 2, decreasing the same amount in series 3, and further decreasing in series 4. Inspection of the clover roots afforded no data along this line. From the limited data secured it can not be said that any significance attaches to the behavior of the count of nodules.

In seeking for an explanation of the fact that inoculation, as measured by dry weight of the nodules, decreased as the treatment of limestone increased, two possibilities present themselves: (1) the effect is direct upon the bacteria; (2) the effect is first upon the plant, and thence upon the bacteria.

It is very improbable that the limestone had a direct detrimental or toxic influence upon the bacteria. The limestone used

*This is neglecting the fact that the depression with the cowpeas was greater than with the other legumes. It is not at all certain that this greater depression was due to a more inimical effect of the limestone on the cowpeas or on the cowpea bacteria.

was of a pure grade and the chance that it contained any impurities toxic to the bacteria is very remote. The possibility still remains that the limestone altered the soluble salt content of the soil or stimulated the growth of bacterial life in ways injurious to the tubercle bacteria. The work of this thesis affords no information upon this possibility except in so far as an increased production of nitrates was shown. This point will be discussed later.

The more likely explanation is that the effect was first upon the plants and thence on the bacteria. Work already noted in this thesis by Brown at Iowa and Lipman et al at New Jersey, shows that one of the effects of limestone when applied to soils is a marked increase of the nitrification processes. Results secured in this experiment were in accord with these. In the following table is shown the amount of nitrates in the soil at the end of the experiment both in the fallowed and in the cropped pots.

TABLE VI - Nitrates in the Pots at the Close of the Experiment.

No. Series.	Treatment	No. pot	Clover p.p.m. of ovendry soil		Cow Peas p.p.m. of ovendry soil	
			Fallowed pots.	Planted pots.	Fallowed pots.	Planted pots.
1	None	1		5.15		11.64
		2		5.15		10.75
		3		5.15		15.61
		Av.	92.56	5.15	67.86	12.67
2	Medium Lime-stone	4		6.12		14.20
		5		6.12		14.35
		6		6.12		12.71
		Av.	131.56	6.12	119.71	13.81
3	Heavy Lime-stone	7		72.67		86.02
		8		64.61		67.73
		9		36.45		92.54
		Av.	280.58	57.91	145.22	82.10
4	Heavy L. and Dried B.	10		150.98		170,12
		11		163.50		123.09
		12		153.34		181.11
		Av.	386.64	155.94	271.67	158.11

From the data secured from the fallowed pots it is seen that the limestone greatly increased nitrification. In the fallowed pots in the clover experiment medium limestone gave an increase of 42% over no limestone, heavy limestone an increase of 113% over medium limestone, and the addition of dried blood a further increas of 38% over heavy limestone alone. Doubtless the assumption can safely be made that the amount of nitrates formed in the planted pots was not widely different from that formed in the corresponding fallowed pots and that the increases due to the treatment were

approximately the same in both cases; but it is seen from the table that the increase in amount of nitrates in the planted pots is not nearly equal to the corresponding increases in the fallowed pots. Hence the crops must have secured increasing amounts of their nitrogen from the soil corresponding to increased production of nitrates. The two facts cannot be connected positively in the relation of cause and effect, yet it seems natural to suppose that the increased amounts of nitrates is the fact explaining the depressed inoculation. As stated in the historical part of this thesis present day investigators believe that when legumes can secure sufficient nitrogen from the soil, nodule formation is quite largely suppressed.

However, it will be remembered that in the minor experiment better inoculation was secured with the manure-alone pots than with the lime-alone pots. Unfortunately no determinations of nitrates were made in this experiment but the manure must have increased the supply of nitrates to quite an extent. It would seem that in this case the difference in inoculation could hardly be explained by a difference in the food supply of nitrates. Perhaps the effect is due to the toxic influence of certain forms of nitrates on the tubercle bacteria, but it seems improbable that any more of a toxic influence would arise from the increased nitrates due to limestone than from those added in the manure.

The two effects of limestone, depressed growth and depressed inoculation, are difficult of explanation. It has been stated that the fixation of the available phosphates perhaps explained the depressed crop yields and the increased nitrates the depressed inoculation. But as already mentioned, the behavior of the clover crops in the minor experiment throws some doubt on the latter

part of this statement. If it was known that some other cause than increased nitrates accounted for the depressed inoculation, then it could be supposed that the better inoculation in the no-lime pots was a sufficiently potent factor in placing available nitrogen at the disposal of the crops to more than balance the increased nitrates in the lime-pots. The better crop growths could, in this case, be attributed directly to the better inoculation. The difficulty with this, however, is in finding the "some other cause" for the depressed inoculation.

The growth and inoculation of the crops present problems of a difficult nature and for which a satisfactory solution is not afforded by the data in hand.

It is of interest to notice more in detail the effect of the limestone on the formation of nitrates as shown by the data from the clover fallowed pots. At the beginning of the experiment the soil contained 10 parts of nitrates per million. Subtracting this amount from the nitrates found at the close of the experiment gives the amounts formed during the experiment, as shown in the following table.

TABLE VII - Influence of Limestone on Nitrification.

No. series.	Treatment	Parts NO_3 per M. of soil formed during the expt.	Equivalent to lbs. N. per 2000000 lbs. of soil	Increase, due to treatment, of N. formed per 2000000# soil.	Percent of total N. in soil 3500# made available during the expt.	Bushels of corn increase of N. would grow.
1	None	82.56	37.98	.....	1.08	....
2	Medium L.	121.56	55.92	17.94	1.60	12.0
3	Heavy L.	270.58	124.47	86.49	3.56	58.0
4	Heavy L. and Dried blood	376.64	173.25	135.27	.....	90.0

The data clearly show the important influence limestone had on nitrification.

Hopkins[20] assumes that roughly 2% of the nitrogen in a soil can be made available during a growing season. This is indicated to be attained only under favorable conditions.

The last column is added to show the considerable effect limestone may have in increasing crop yields through increasing the amount of available nitrogen.

The data as to the amount of nitrates formed in the series 3 and series 4 pots offer a means of approximately determining the amount of the dried blood nitrified during the experiment. Assuming that the soil proper yielded the same amount of nitrates in the two pots then the increase of nitrates in the series 4 pot came from the dried blood. This increase was 106.06 parts or 0.215 gms. of nitrogen in the pot. Five grams of dried blood containing 12.84% N. or 0.642 gms. of nitrogen were added to the pot. Then of the nitrogen added in the dried blood approximately one-third was transformed into nitrates in the twenty weeks.

In this connection it will be well to notice whether the treatment influenced to any extent the percent of nitrogen in the plants.

TABLE VIII-Percent of Nitrogen in the Various Parts of the Plants.

No. Series	Treatment	No. pot	Clover Tops	Clover Roots	Cow Peas Tops	Cow Peas Roots		Nodules.
1	None	1	3.378	2.177	3.033	1.774	not inc. nodules.	7.121
		2	2.918	2.197	3.212	2.081		7.531
		3	3.474	2.257	3.003	1.774		7.172
		Av.	3.257	2.210	3.083	1.876		7.274
						2.932	Inc. Nod.	
2	Medium lime-stone	4	3.317	2.143	3.011	2.515		
		5	2.891	1.995	3.142	2.415		
		6	3.086	2.229	2.878	2.316		
		Av.	3.098	2.146	3.010	2.415		
3	Heavy lime-stone	7	3.348	2.223	3.022	2.248		
		8	3.653	2.459	2.907	2.122		
		9	2.847	2.378	3.019	2.084		
		Av.	3.283	2.353	2.983	2.151		
4	Heavy lime & dried blood.	10	3.665	2.412	3.183	2.453		
		11	3.884	2.237	3.467	2.547		
		12	3.114	2.606	3.420	2.804		
		Av.	3.554	2.418	3.357	2.601		

Looking over the table in a general way it is seen that the per cents within any one series vary appreciably. In view of this, the only variation between series to which any significance can be attached is that between series 3 and 4. Here the largely increased amounts of nitrates have appreciably increased the percent of nitrogen in the plants. That the increased nitrates in series 3 or in series 2 did not produce a like change is probably due to the offsetting influence of the decreased inoculation in these instances.

The percent of nitrogen in the clover roots was approximately three-fifths of the percent in the tops. The percent in the cow pea roots was four-fifths of that in the tops. Comparing the two plants, the cow pea tops contained a seventeenth of a percent less nitrogen than the clover tops but the cowpea roots contained a tenth of a percent more than the clover roots.

As a matter of interest within itself, the percent of nitrogen in the cowpea nodules of the series 1 pots was determined. It was found to be over seven percent.

Another point deserving attention is that the average percent of nitrogen in the cowpea roots of series 1 after the nodules had been removed was only 1.876, whereas in series 4 the percent in roots and nodules combined was 2.601; but the weight of nodules in series 4 was very small comprising only a fiftieth of the weight of roots alone. Clearly the percent of nitrogen in the roots alone was much greater in series 4 than in series 1. In view of the fact that but little difference was found in the tops this difference is hard to understand; but in some way heavy inoculation largely decreased the percent of nitrogen in the roots alone.*

RELATIVE WEIGHT OF ROOTS TO TOTAL WEIGHT OF PLANTS.

It has been suggested by Lipman of New Jersey that inoculation decreases the root range of plants. This might possibly be explained either as due to the fact that the nodules supply sufficient nitrates and hence perhaps lessen the root range necessary to

*Smith and Robinson[21] noticed this same effect in some work they did as to the effect of inoculation on the nitrogen content of cowpeas and soy beans. Their results as to the percent of nitrogen in the roots are as follows:

Inoculated	cowpeas	0.89;	not inc. cowpeas	1.97
"	soybeans	1.01;	" " soybeans	1.43

They thought it was due to the greater ease of transportation of the nodule nitrates.

secure needed plant food or as due directly to injury of the roots by the bacteria whereby they become stunted.

In digging the roots of the crops in this experiment some attention was paid to this point to see if by inspection any differences in root range could be discerned among the different series. The cowpea plants especially presented wide variation in inoculation, but it could not positively be stated that any root variation was evident. The fact that the plants were growing in small pots may have prevented appreciable developing of differences that under field conditions might have appeared.

In so far as the ratio of weight of roots to weight of tops is an indication of the relative development of roots, this can be ascertained by reference to the data, which for convenience will be reproduced here.

TABLE IX-Relative percentage Wt. of Roots to Total Wt. of Plants.

No. series.	Treatment	No. pot.	Inoculation	Cow Peas		Soy Beans Incl. Nod.	Clover	Clover in minor Expt.
				Incl. Nod.	Not incl. nodules			
1	None	Av.	Heavy	7.77	6.25	5.54	30.88	36.22 (Manure-alone Ser.
2	Medium L	Av.	Medium	7.75	6.99	6.34	31.74	40.00 (L.&M.
3	Heavy L.	Av.	Light	7.18	7.02	7.10	31.61	39.74 (Lime-alone ser.)
4	Heavy L. & dried blood	Av.	Light	7.19	7.05	6.64	30.83	

Noticing first the cowpeas, when the nodules are included with the roots no stunting of root development due to inoculation is evident, when the nodules are not included a slight stunting is to be seen. The soybeans show an appreciable decrease of root development even when the nodules are included. Clover shows a very slightly decreased root development compared only with series

2 and 3. In the minor experiment clover shows an appreciable decrease of root development in the manure-alone pots, which were the pots having the best inoculation.

In the main the data show a decreased development of roots when inoculation is heavy; but the difference is not sufficient to be of any great significance.

PROPORTION OF DRY MATTER AND NITROGEN OF THE PLANTS IN THE TOPS AND ROOTS.

The proportion of the total nitrogen and dry matter of the plants found in the tops and roots respectively is shown in the following tables.

TABLE X - Percentage of the Total Nitrogen of the Plants Found in the Tops and Roots.

No. Series	Treatment	No. pot	Clover		Cow Peas	
			Tops	Roots	Tops	Roots
1	None	Av.	77.12	22.88	92.97	7.03
2	Medium L.	Av.	75.87	24.13	93.68	6.32
3	Heavy L.	Av.	75.15	24.85	94.72	5.28
4	Heavy L. & Dried Blood	Av.	76.93	23.07	94.84	5.16
		Av. of all pots.	76.27	23.73	94.05	5.95

TABLE XI - Percentage of the total dry matter of the plants found in the tops and roots.

No. Pot.	Clover		Cow Peas	
	Tops	Roots	Tops	Roots.
Average of all pots.	68.71	31.29	92.53	7.47

The series treatments are seen to have had little or no effect upon the percent of nitrogen in either the tops or roots.

Noticing the average of all the pots as to the relative percent of nitrogen and dry matter in the tops and roots, it is seen that about three-fourths of the dry matter and five-sevenths of the nitrogen of the clover plants are in the tops; over nine-tenths of the nitrogen and of dry matter of the cowpeas are in the tops.

AMOUNT OF NITROGEN SECURED FROM THE AIR.

The nitrate analyses of the soils in the fallowed pots and in those which had grown clover and cowpeas offer a means of determining the amount of nitrogen in the crops secured from the air. Assuming that the amount of nitrates formed from the soil was the same in a planted pot as in the corresponding fallowed pot, it is only necessary to determine the difference in nitrates in the two pots at the end of the experiment to ascertain the amount of nitrogen secured from the soil by the crop. The difference, then, between this amount and the total nitrogen found in the crop is the amount of nitrogen secured from the air.

It is not intended that the results thus secured shall be allowed any great weight as a measure of the fixing power of the legumes. It is not likely that the assumption as to equal quantities of nitrates formed in the fallowed and planted pots is strictly correct. The moisture conditions in the pots were not always the same; further it is probable that the plants by removing the nitrates as formed, or perhaps in more direct ways, influenced the production of nitrates considerably.*

*Leather[22] has found some evidence that higher plants depress nitrification. Lyon and Bizzell[23] say this: "The growth of a legume is thus apparently favorable to the process of nitrification"

The results secured from the clover are as follows:

TABLE XII-Nitrogen secured from the air by the clover.

No. Series.	No. pot	p.p.m. NO_3 in fallowed pots at end of Exp.	p.p.m. NO_3 in planted pots at end of exp.	Difference due to plant growth	Gms. of Nitrogen used by plants from soil (9000 gms oven dry) in ea.pot	Total N. in plants gms.	Grams nitrogen secured from air	Percent Nitrogen secured from air.
1	Av.	92.56	5.15	87.41	.1778	.6002	.4224	70.37
2	Av.	131.56	6.12	125.44	.2552	.5243	.2691	51.33
3	Av.	280.58	57.91	222.67	.4529	.5545	.1016	18.32
4	Av.	386.64	155.94	230.70	.4692	.5151	.0459	8.91

It is seen that the amount of nitrogen secured from the air became less as the inoculation decreased. The percent of nitrogen (70.37) secured from the air in series 1 corresponds fairly closely with that (66 2/3) usually considered to be secured from the air under average conditions.

The results from the cowpeas are not so satisfactory:

TABLE XIII - Nitrogen secured from air by cow peas.

No. series	No. pot	p.p.m. NO_3 in fallowed pots at end of expt.	p.p.m. NO_3 in planted pots at end of exp.	Difference due to plant growth	Gms. of N. used by plants from soil (9000 gms. oven dry in ea.pot	Total N. in plants Gms.	Gms. N. secured from air	% N. secured from air	Weight of nodules Gms.
1	Av.	67.86	12.67	55.19	.1144	.4475	.3331	74.44	.2236
2	Av.	119.71	13.81	105.90	.2194	.3686	.1492	40.48	.0951
3	Av.	145.22	82.10	63.12	.1308	.4467	.3159	70.72	.0241
4	Av.	271.67	158.11	113.56	.2305	.4109	.1804	43.90	.0179

Comparing the percent of nitrogen secured from the air with the weight of nodules it is evident that the percent in the series 2 pots is too low and that in the series 3 pots too high. The difficulty arises quite likely from an error somewhere in the nitrate determinations of the series 2 and series 3 fallowed pots. This appears probable from a comparison of the amount of nitrates found in the cowpea fallowed pots with that found inthe clover fallowed pots.

TABLE XIV-Ratio of nitrates in the two lots of fallowed pots.

No. Series	Parts p.m. NO_3 in cowpea fallowed pots.	Parts p.m. NO_3 in clover fallowed pots.	Ratio NO_3 in clover F.P. to that in Cowpea F.P.
1	67.86	92.56	1.364
2	119.71	131.56	1.099
3	145.22	280.58	1.932
4	271.67	386.64	1.432

There is no apparent reason why the ratio should not be practically the same in all the series. If now an average of the ratios for series 1 and 4 pots (1.364 and 1.432) be taken and the nitrates in the series 2 and 3 cowpea fallowed pots be determined by dividing this average ratio into the amount of nitrates found in the corresponding clover fallowed pots then the results for the cowpeas become as follows:

TABLE XV - Corrected amount of N. secured from air by cowpeas.

No. series.	No. pot	Parts p.m. NO_3 in fallowed pots at end of exp.	Parts p.m. NO_3 in planted pots at end of Exp.	Difference due to plant growth	Gms. of nitrogen used by plants from soil (9000 gms oven dry in ea. pot	Total N. in plants gms.	Grams nitrogen secured from air.	Percent nitrogen secured from air.
1	Av.	67.86	12.67	55.19	.1144	.4475	.3331	74.44
2	Av.	94.44 calc.	13.81	80.63	.1671	.3686	.2051	55.64
3	Av.	201.41 calc.	82.10	119.31	.2472	.4467	.1995	44.66
4	Av.	271.67	158.11	113.56	.2305	.4109	.1804	43.90

Comparing this table with table XII it is seen that the cowpeas secured a larger amount of their nitrogen from the air than the clover. This is to be expected since the greater root development of the clover would enable it to secure a greater amount of nitrogen from the soil.

As stated it is not intended that the results given in tables XII and XV shall be considered authoritative; nevertheless it is believed that results secured in this way could be made fairly accurate if a greater number of pots were used both of the fallowed and of the planted and if part of the legumes were grown without inoculation to determine their effect on nitrification.

EFFECT OF ORGANIC MATTER ON THE LOCATION OF NODULES.

The minor experiment, consisting of six clover pots, was planned particularly to supply information as to the effect of lumps of organic matter on the location of nodules.

Fairly well rotted yard manure was added to four of the pots at the rate of fifteen tons per acre in ten lumps per pot. The

plants made rapid growth and were harvested after four months time. Good inoculation was secured in all four pots. The location of the nodules were determined merely by inspection.

The organic matter had very little effect on the location of the nodules. However, there may have been a slight increase in and directly around the lumps. Roots were much more numerous here than elsewhere and the inoculation may have appeared better on this account.

EFFECT OF ONE CLOVER CROP ON THE LIME REQUIREMENT.

It was thought that it would be of interest to determine the lime requirement at the close of the experiment of the three no-treatment clover pots in the main experiment whence by comparison with the original lime requirement the effect of one clover crop could be ascertained.

The Veitch limewater method was used in this work. The results are as follows:

TABLE XVI-Effect of one Clover Crop on the Lime Requirement.

No. pot.	1	2	3
Lime requirement at beginning of Expt. (Comp. Sam. from 3 pots)	2577	2577	2577
Lime requirement at end of Expt.	3221	2706	3479
Increase in Lime Requirement	644	129	902

The table shows that in all three pots the crop increased the lime requirement but in very different amounts. It is not probable that this difference is due to a varying effect of crop growth but

rather to differences somewhere in the analytical work or in the action of the chemicals.

Throughout this experiment the Veitch method was used to determine the lime requirement since it was thought the most reliable of the methods in use, but it has not proven entirely satisfactory. Evidently the amount of lime neutralized by a soil is due in part to other factors than its acidity[24], and capable of rather wide variation through small differences in laboratory technique or in action of the chemicals used.

TRANSPIRATION FACTORS OF COW PEAS AND CLOVER.

The following tables show the amount of water used by the cowpea and clover crops.

TABLE XVII - Water Used By Cow Peas.

No. Series	No. pot	Total water lost Gms.	Average water lost from 4 fallowed pots. gms.	Water transpired by plants gms.	Total weight of plants gms.	Gms. water used per gm. dry matter formed.
1	1	12316	3893	8423	14.691	573
	2	11686	"	7793	13.459	579
	3	12471	"	8578	15.981	537
	Av.					563
2	4	11241	"	7348	12.606	583
	5	10741	"	6848	10.662	642
	6	11431	"	7538	14.173	532
	Av.					586
3	7	11396	"	7503	15.466	485
	8	11916	"	8023	15.267	525
	9	11211	"	7318	15.108	484
	Av.					498

TABLE XVII (Cont'd)

4	10	11678	3893	7785	16.296	478
	11	9366	"	5473	10.222	535
	12	10126	"	6233	11.107	561
	Av.					523
Average of all pots..................						542

TABLE XVIII - Water used by Clover.

No. Series	No. pot	Total water lost Gms.	Average water lost from 4 fallowed pots. gms.	Water transpired by plants. gms.	Total weight of plants. gms.	Gms. water used per gm. dry matter formed.
1	1	10956	3915	7041	20.48	343
	2	11081	"	7166	19.93	360
	3	11206	"	7291	21.28	342
	Av.					348
2	4	9386	"	5471	17.96	305
	5	9898	"	5983	20.31	294
	6	10026	"	6111	18.25	334
	Av.					311
3	7	9156	"	5241	18.98	276
	8	9556	"	5641	18.23	309
	9	9651	"	5736	18.49	310
	Av.					298
4	10	7966	"	4051	15.71	258
	11	8046	"	4131	17.68	233
	12	7406	"	3491	14.88	234
	Av.					242
Average of all pots........................						300

The transpiration factors have only an approximate value since the amount of water lost by the fallowed pots probably corresponded only fairly closely with that lost by evaporation from the cropped pots.

Investigations as to the transpiration factors of various plants have shown clover to have a slightly higher factor than peas*. The much higher factor found for cow peas in the present work is due, no doubt, in part to the fact that their average growing temperature was some 15° C. higher than that of the clover but, for the most part, it is to be ascribed to the fact that the soil under the dense clover foliage probably evaporated considerably less water than under the more open peas.

A comparison of the transpiration factors as averaged by series afford a means of determining the effect of the density of the soil solution on the amount of water used by the plants. Referring to Table VI, (p. 33) it is seen that the nitrates increased very slightly from series 1 to 2, and very markedly through series 3 and 4, both under the clover and the cow peas. It is not known to what extent the special fertilizer treatment influenced the amount of other salts in solution but probably the differences in the nitrate content form the main differences in the densities of the soil solutions of the different series.

Noticing first the results from the cowpeas, it is seen that in the main the transpiration factor decreased as the density of the soil solution increased; however the results here are erratic and afford but little positive information. Turning to the clover: a consistent and appreciable decrease of the transpiration factor is evident throughout the series as the density of the soil solu-

*Cf. Bull. 285, Bur. of Plant Industry. p. 83

tion increased. This is in line with practically all investigations[26] on this point which have shown that any soil treatment whereby the amount of soluble plant food is increased appreciably decreases the amount of water used per unit of dry matter formed.

SUMMARY

In the main limestone exerted an unfavorable influence on the crop growth. In accounting for this it was suggested that perhaps the lime by fixation created for a time a deficiency of available phosphates.

Limestone very markedly decreased the inoculation as measured by dry weight of the nodules. The large increase in nitrification due to the treatment was held as the most probable explanation for this. The fact that manure exercised no depressing effect on inoculation was a disturbing element in this part of the work.

As indicated by determinations from the fallowed pots, limestone greatly stimulated nitrification. It was pointed out that on an acid soil where nitrogen is the limiting plant food this effect of limestone may alone be sufficient to account for the increased yield of cereals due to limestone treatment.

The limestone treatment with the attendant large increase of nitrates produced no change in the percent of nitrogen in the plants. The fact that inoculation greatly decreased as the supply of nitrates increased probably accounts for this.

Heavy inoculation slightly decreased the root development of the plants.

Three fourths of the dry matter and five sevenths of the nitrogen of the clover plants were found in the tops; over nine tenths of the nitrogen and of the dry matter of the cowpeas were in the tops.

As determined by differences in the nitrate content of the planted and fallowed pots at the close of the experiment, in the no-treatment pots the clover secured seventy percent of its

nitrogen from the air and the cowpeas seventy four percent. In the other pots the percent decreased as inoculation decreased and as the treatment increased. The results thus secured were not to be considered authoritative, nevertheless it was indicated that with more care fairly accurate results should be secured by this method.

Masses of decaying organic matter perhaps increased inoculation in their immediate vicinity to a very slight extent.

Work as to the effect of one clover crop on the lime requirement showed the unsatisfactoriness of the Veitch method for determining acidity.

Under the conditions of the experiment the transpiration factor for cowpeas was found to be over a third higher than for clover. It was also found that the increased nitrates in the limed series appreciably decreased the amount of water used per gram of dry matter formed.

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