SOME BIOLOGICAL AND CHEMICAL EFFECTS OF SULFUR
UPON PLANT GROWTH AND UPON THE SOIL.

by

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INTRODUCTION.

It has long been known that sulfur is one of the essential mineral elements in plant nutrition. Only during recent years, however, has any special attention been given to the importance of the specific role of sulfur in plant growth. Probably its presence and functions within the soil itself have been investigated still less than its action within the plant.

The probability of sulfur becoming a limiting element in the soil was formerly thought to be out of the question because of an erroneous idea as to the amount of this element removed by plants. According to the old analyses sulfur was thought to be used in very small quantities. It was, therefore, assumed that the supply of this element in the soil was sufficient to maintain production indefinitely. The error of this conception was seen when quite recent methods of analysis proved that the old method for the determination of sulfur gave results far too

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low. This fact, together with the discovery of the fertilizing value of sulfur by accident in the application of sulfur to plants as a fungicide, has led several investigators both in Europe and America to conduct experiments during the last decade with a view of determining more accurately the action of sulfur in plant nutrition. To date, the work has not, on the whole, been conclusive nor exhaustive, and it was with the idea of studying the role of this element still further that the following work was undertaken.

HISTORY

DISCUSSION OF PREVIOUS INVESTIGATIONS.

Interest in this question of sulfur in plant nutrition was greatly stimulated by the results obtained by Hart and Peterson (1911)* of the Wisconsin Experiment Station in which they showed the old analysis of plants by Wolff to be entirely too low. At the same time, they found the amount of sulfur to compare very favorably with phosphorus. Their work indicates that the amount varies a great deal in different crops, and that plants belonging to the same botanical family have the least variation in sulfur content. A table compiled from their data (1911) shows this fact very clearly:

TABLE I. SULFUR CONTENT OF CERTAIN CROPS.

<u>GRAMINEAE</u>	Per cent Sulfur
Rye	.123
Barley	.153
Corn	.170
Wheat	.170
Oats	.180
Timothy hay	.190

*Dates refer to bibliography and year of publication.

LEGUMINOSAE

Per cent Sulfur

Red Clover	.164
Alfalfa hay	.287
Beans (White)	.232
Soybeans	.341

LILIACEAE

Onions	.568
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CRUCIFERAE

Turnips	.740
Cabbage	.819
Turnip tops.	.900
Rape tops	.988

The same authors also tabulate the amounts of SO_3 and P_2O_5 found in certain common crops, together with a comparison of the old standard for SO_3 in the same crops.

TABLE II. POUNDS SO_3 AND P_2O_5 REMOVED PER ACRE
BY AVERAGE CROPS.

<u>CROP</u>	WOLFF'S SO_3	TOTAL SO_3	TOTAL P_2O_5
Potatoes	- - -	11.52	21.5
Corn	5.35	12.00	18.0
Red Clover	8.20	15.40	24.9
Wheat	3.55	15.70	21.1
Oats	6.20	19.7	19.4
Tobacco	- -	21.0	16.0
Alfalfa	37.80	64.8	39.9
Cabbage	<u>62.80</u>	<u>98.0</u>	<u>61.0</u>
Total	123.80	258.12	220.9

In explanation of the low sulfur content of plants as shown by Wolff's data, it is now known that in the seed of plants sulfur exists mainly in the organic form in the protein molecule and, hence, is partially volatilized when plant tissue is ignited. Consequently, since the first analyses for SO_3 were made on the ash of plants, they were necessarily low.

As to the amount of sulfur in the soil upon which plants can feed, very little is known even today. In the soil surveys thus far made, no analysis for sulfur has been reported, and only a few scattered analyses for this element in soils are available.

About the first work done along this line was an investigation of the forms of sulfur in soils by Barthelot and Andre (1893). According to these men, sulfur exists in the soil as sulfates and sulfides; as ethereal sulfur; and in organic compounds. "Probably the chief forms in all normal soils will be those existing as sulfates and those in the organic form." "Sulfides would rarely exist except in water logged soils where reducing processes are prominent." In view of the probable importance of sulfates in soil, numerous methods have been devised for determining the SO_2 content, but none are entirely satisfactory.

The amount of sulfur in soils seems to vary a great deal and to depend upon several factors. Cultivation very noticeably affects the sulfur content. This is well shown by some results of a recent investigation carried on by the Kentucky Station (1913). They analyzed soils from various geological areas of the state for sulfur and phosphorus in both the virgin and cultivated soils and obtained the following results in pounds per acre for the top eight inches:

TABLE III. SULFUR AND PHOSPHORUS IN VIRGIN AND CULTIVATED SOILS OF KENTUCKY.

GEOLOGICAL AREA	Virgin		Cultivated		Per cent loss due to cultivation.	
	S	P	S	P	S	P
Eastern coal fields	598	1337	371	1031	38	23
Cincinnatian	880	1920	580	2580	34	34*

TABLE III (CONTINUED)

GEOLOGICAL AREA	Virgin		Cultivated		Per cent loss due to cultivation	
	S	P	S	P	S	P
Western coal fields	560	- 907	413	- 787	26	13
Keokuk - Waverly	360	- 540	350	- 555	3	3*
Devonian	1027	-2053	593	- 1687	42	18
River Alluvial	610	-1725	650	- 1430	7*	17

* Indicate gain.

The Wisconsin Station (1911) also has some interesting data on this point. As an average of five soils, they obtained SO_3 in surface eight inches of soil as follows:

TABLE IV.

AMOUNT SO_3

Virgin Soil	.084 per cent
Cropped Soil	.052 per cent
Loss (according to analysis)	640 pounds
Removed by crops	684 pounds
Added in manure	60 pounds
Removed by crop - excess of manure	624 pounds.

They showed the effect of liberal manuring as follows:

Virgin soil	.096% SO_3
Cropped and manured . .	.110% SO_3

These data tend to show that the sulfur content of soils may be greatly decreased by cultivation, and also that the proper balance may be maintained by good handling.

Dymond, Hughes and Jupe (1905), three English investigators, say that the average British soil contains less sulfur than phosphorus. The average of the analysis of twenty-one typical soils showed .020 per cent sulfur and .059 per cent phosphorus in the surface soil. They found that a permeable subsoil may contain more combined sulfuric acid than the surface soil. One analysis showed a boulder clay subsoil to contain .055 per cent SO_3 , while the top soil contained only .028 per cent.

There are several ways in which sulfur is returned to the soil. The foregoing tables show that manure may be a very efficient means of supplying the element. There are also several sulfatic fertilizers, such as potassium sulfate, ammonium sulfate, and calcium sulfate or gypsum, which are used quite extensively. It is believed by some that the sulfur of the excessive sulfuric acid used in the manufacture of acid phosphate may give that fertilizer much of its value. Acid phosphate also contains considerable calcium sulfate, which may account in large part for its value.

Another source of sulfur supply to the soil is that brought down by rain water. The sulfur in the atmosphere comes largely from the combustion of coal and other fuel. Hall reports in 1912 determinations made at Rothamsted, which showed an annual precipitation of about $18\frac{1}{2}$ pounds of SO_3 per acre. A test at Madison, Wisconsin, showed a total rainfall from June 1 to November 1, of 11.14 inches, and a total of 10.7 pounds of SO_3 brought to the surface, - almost a pound of SO_3 per acre for every inch of rainfall.

While there is a considerable gain in the SO_3 from the rainfall, there is also a noticeable loss thru drainage, which will probably totally, and in some cases more than offset the gain. Most rivers and lakes contain considerable quantities of sulfates, which have been dissolved out of the soil. At Rothamsted, the drainage waters from the wheat plots of Broadbalk field were collected and completely analyzed. From the unmanured plots they report the quantity of sulfur trioxide in the drainage water at 24.7 parts per million, while the quantity varied from 41 to 106.1 parts per million in the waters from the plots receiving various fertilizer treatments. Hall assumes a loss of 10 inches of drainage water, at which rate the unmanured plots would lose approximately 50 pounds of SO_3 annually per acre, while the fertilizer plots would lose from 85

to 220 pounds. These data show that the loss of sulfur from the soil far exceeds the amount brought down by rain water. At least it would seem reasonable to say that under average conditions the loss by drainage in general will equal or exceed the gain from rain water.

After getting a more accurate knowledge of the amount of sulfur found in the various crops and the amount of that element lost from the soil under systems of cultivation and thru drainage waters, it seems quite evident that the supply of sulfur in the soil must necessarily decrease and finally become a limiting factor in crop production. With this idea in mind, several investigators during the last decade have made numerous experiments to determine the effect of adding different forms of sulfur to the soil as a fertilizer. In this work the French have been far the most prominent.

Sulfur has been applied to plants as a fungicide or insecticide for many years, but any other effect which it might have had on the plants or soil was long overlooked. About the first data which throws any light on the further effect of sulfur on plant growth was obtained by Halsted (1897) of New Jersey about 1895. He used flowers of sulfur for checking potato scab and soil-root(?) rot, and found that the effect of the sulfur on the soil lasted thru at least the second year. A very interesting part of his work was with peas. He treated plots with sulfur, corrosive sublimate, carbonate of lime,

and copper sulfate; and grew peas on them. The peas were pulled at frequent intervals thruout the growth of the crop. It was found that the tubercles on the roots of the plants from the plot where sulfur had been applied was less than one-tenth of that where the soil had received no treatment. The sulfur was applied at the rate of 1200 pounds per acre.

Wheeler, Hartwell and Moore (1899) tried experiments in 1896 on the after effects of sulfur which had been applied the year before for potato scab. The sulfur was applied in large quantities and was found to be still injurious to oats and millet. The authors say that sulfur employed in considerable quantities upon soils which are acid, or which contain but a limited excess of basic ingredients, is capable of causing much subsequent injury to crops, the extent of the same being greatest upon acid or neutral soils.

A large number of European investigators have worked with the effect of sulfur upon the growth of various crops. Most of them have reported favorable results from sulfur fertilization. Among these investigators are: Bernhard (1912), Berthault (1914), Bogdanoff (1900), Boulanger (1912, 1913) Cercelet (1913), Chancrin and Desriot (1911, 1912, 1913), Degrully (1912), Demolon (1912, 1913), Dugardin (1912), Giannetto (1912), Magnien (1913), Miede (1914), Sabasknikov (1914), Thalau (1913), Tritschler (1913), Urban (1913), Vermorel and Danthony (1913).

Pfeiffer and Blanck (1914) claim to have shown by field experiments that applications of sulfur did not increase either the plant growth or the utilization of the soil nitrogen; in fact, they obtained many negative results. Therefore, they came to the conclusion that the value of sulfur as a fertilizer has been overestimated by some investigators, and they think that many positive results have been due to faulty methods of investigation.

The Americans who have worked on this question are few and their work will be reviewed more in detail, as it offers more of local interest.

The Wisconsin Station reports favorable results from the use of sulfur fertilizers. To determine the effect of sulfur fertilization various plants have been grown by Professor Tottingham (1912) of the Agricultural Chemistry Department, under greenhouse conditions with and without the addition to the soil of sulfur fertilizers. In the case of rape and radishes, both plants high in sulfur, sulfur fertilization has had a marked effect on the yield of dry matter. With rape on soil from their Hill farm, supplying a sulfur fertilizer (gypsum, or land plaster) in addition to nitrogen, phosphorus, and potassium, gave an increase of over 30 per cent in the dry matter of the crop.

The Oregon Experiment Station has obtained good results by the use of gypsum. Some soils of Western Oregon seem to respond very readily to applications of gypsum

especially with vetch and clover crops.

Other men have found opposite results. L. L. Harter (Bul.1, Va. Truck Exp. Sta.) reports: "In all our experiments, gypsum has proven decidedly injurious to Norfolk soils and its use is not to be recommended."

F. C. Reimer (1914) of the Oregon Station, selected a portion of a field where alfalfa had been giving unsatisfactory yields for two years. Two plots were fertilized with flowers of sulfur; one plot with iron sulfate; one with superphosphate; and two plots with ground phosphate rock. Check plots receiving no fertilizer were left alongside the fertilized plots for comparison. The plots receiving the ground phosphate rock gave no increase in yield over the unfertilized plots. The plots receiving flowers of sulfur, iron sulfate, and superphosphate at the rate of 300 pounds per acre, produced an increase of more than 100 per cent over the unfertilized check plots. Later work has still further confirmed these results.

Professor O. M. Shedd (1913) of the Kentucky Station, has conducted greenhouse pot experiments on two types of their soil regarding the use of sulfur as a fertilizer. He found that soybeans, turnips, radishes and mustard respond to sulfur fertilizers, but no positive results were obtained with clover.

CHEMICAL STUDIES:

W. H. Peterson (1914) names Arendt, Ulrich, Shulze, Berthelot and Andre, Fraps, and Thompson, as men who have investigated the amount of sulfate in plants and found it to vary within wide limits according to the amount in the soil, the parts of the plant analyzed, and the stage of growth.

Professor Peterson says, "Where rape, radishes, and clover were grown in ordinary soil, with a good supply of nitrogen, phosphorus, and potassium, and made vigorous growth, there were no sulfates in the tissues of the plants." Evidently the plants were utilizing all the available sulfur to form organic sulfur compounds and to build sulfur-containing tissue, and they had none left to form excess sulfates. Where sulfates were added to the soil, large quantities of sulfur were circulating in the plants as sulfates, and an abundant supply existed for building tissue and forming the volatile compounds of the plant.

Volatile sulfur was obtained from red clover, alfalfa, June grass, and sugar beet tops. The nature of this compound is not known, but in rutabagas it is thot to be a sulfide.

The sulfates in the field samples which were examined varied a great deal. Where large quantities of sulfates were added to the soil, there was a corresponding increase of sulfates in the plant tissue. Where no sulfates were added to the soil, 90 per cent or more of the total sulfur was found in the form of unoxidized sulfur, probably

in proteins. The plant was evidently very economical of its supply and made healthy, vigorous growth where no sulfates could be found in the tissues.

Dymond, Hughes, and Jupe (1905) have shown that the importance of sulfur in crop growth has been overlooked. They have proven that a sufficiency of sulfates in the plant food is necessary to the formation of a high percentage of albuminoid. They found that the value of sulfate manuring is generally confined to heavy yielding crops high in albuminoids. The addition of sulfates increased both the albuminoids and the total weight of the crop.

In two different crops in which the percentage of albuminoids were increased by the application of sulfates, they have found that in these crops the percentages of amides, soluble carbohydrates and oils were increased, and the fiber was decreased, while the ash remained about the same as in the check crop.

PHYSIOLOGICAL STUDIES:

Demolon (1912) found that sulfur had a very marked effect upon the development of chlorophyll. The leaves on the sulfur plots had a much darker green tint than the plots grown in untreated soil.

Sabasknikov (1912) also observed this deep green color on plots previously treated with sulfur. Farmers in Oregon claim to have noted the same thing on alfalfa

under apple trees which were sprayed regularly with lime-sulfur spray mixtures.

BACTERIOLOGICAL STUDIES:

E. Boullanger (1912) used sulfur in connection with sterilized and non-sterilized soil. He found the sulfur to have a marked beneficial effect with the ordinary soil, but it was very feeble with the sterilized soil. From this he concluded that the sulfur acts indirectly to modify the bacterial flora in the soil and encourage the development of certain organisms.

Boullanger working with M. Dugardin (1912) has shown that the favorable influence which very small doses of sulfur exert upon vegetation is due to its activating effect on the bacteria which break down nitrogenous matter in the soil to ammonia, and also to its influence on the nitrifying ferments. Large doses of sulfur, while enhancing the first effect, check the second.

In pure cultures in Omelianski's media with nitrous ferments, they used different amounts of sulfur and found that the complete oxidation of ammonia to nitrite took place in all flasks within 18 days. They concluded that sulfur does not have much influence upon nitrous fermentation.

In a similar pure culture experiment with nitric ferments it was found that small doses of sulfur favored the work of nitric ferments, but larger amounts retarded their action.

In a third experiment they used 200 cubic centimeters of a 5 per cent peptone solution, added different amounts of sulfur, and then inoculated with a soil solution. After five days they found that the amount of ammonia was increased in the sulfur treated cultures, thus showing that sulfur favors the work of ammonifying bacteria.

In soil cultures they found sulfur to affect the ammonia and nitrate content as follows:

TABLE V:

Treatment	NH_3	N_2O_5
	In mg:kg dry soil	In mg:kg dry soil
Check - No sulfur	4.07	523
Sulfur - 20 millograms	6.65	543

This notable increase in the ammonia content of soil by the action of sulfur and slight increase in nitrate seemed quite conclusive.

A similar test was made, but with the addition of nitrogen in the form of dried blood rather than peptone. At the end of ten days the amounts of ammonia, nitrate, and total nitrogen in the soil are as follows:

TABLE VI:

Treatment	NH_3	N_2O_5	Total Nitrogen
	Mg:kg Dry soil	Mg:kg Dry soil	Mg:kg Dry soil
Check - no sulfur	134.6	720	1737
Sulfur - 20 millograms	203.2	712	1737

In the presence of a nitrogenous material certain bacterial activities seem to be increased. The work of the

ammonifying bacteria is also considerably increased by the addition of sulfur. At the same time, however, the nitrates were reduced a little, while the total nitrogen did not vary. This the authors say is because the nitrogen fixing bacteria (*Azotobacter clostridium pasteurianum*) are not influenced by sulfur.

These authors conclude that the favorable role of flowers of sulfur is the active influence which it exercises upon those bacteria whose function it is to reduce the complex nitrogenous materials, and also upon the nitrification ferments producing ammonia.

Demolon (1913) worked with two calcareous soils, adding one per cent of sulfur to each. He found that after forty days part of the sulfur had been transformed to sulfate as follows:

TABLE VII:

		BaSO ₄ 100 gm. Soil	Sulfur trans- formed into H ₂ SO ₄
Soil A	(With sulfur	.970	.124
	(No sulfur	.062	- -
Soil B	(With sulfur	.612	.077
	(No sulfur	.047	- -

The sulfur was here able to become a continuous source of sulfuric acid.

This author also sterilized some soil, part of which after adding sulfur was kept cool, and the other incubated at 25° C. He found that the sterilization slackened the oxidation, while the incubation at 25° accelerated

it.

He found that H_2S was produced when soil was added to a fermentable medium containing sulfur.

Demolon also found the evolution of sulfur in the soil to run parallel with the supply of nitrogenous material. He says further that sulfur may be a very helpful addition to the soil, but its action diminishes and becomes negligible in the presence of a sufficient amount of organic and mineral fertilizers.

He says that the fertilizing action of sulfur may be due:

(a) To its effect upon bacterial activity.

(b) To the formation of sulfate, which may tend to cause the solubility of other mineral elements.

Brioux and Guerbet (1913) found that sulfur increased the amount of sulfates in a soil, but the addition of starch or sugar retards the action, while peptone (nitrogenous material) in addition to sulfur very markedly increases the production of sulfate. Lime used in connection with sulfur caused still greater oxidation to sulfate. With sterilized soil they found the production of sulfate to be very slight as compared with non-sterilized soil.

Dymond, Hughes, and Jupe (1905) experimented with two tubes of sterile soil, one inoculated with washings from a fresh soil and the other untreated. Sterilized air was drawn thru them for 70 hours at the rate of 3 liters per hour. Each soil was then extracted with hydrochloric acid and the sulfuric acid determined. The sterile soil contained .026

per cent SO_4 , the inoculated soil .034 per cent, an increase of .008 per cent by oxidation due to bacterial action. From this it would seem that the SO_4 extracted from a soil by hydrochloric acid would not represent the supply available for a crop during its whole period of growth, but this will depend upon the amount of bacterial activity.

H. Clay Lint (1914) studied the effect of sulfur on the lime requirement of soil. In his experiments he put 100 grams of soil into glass tumblers and added to half of them 33 millograms of sulfur, equivalent to 1000 pounds per acre. One tumbler with sulfur and one check were analyzed each week for acidity. The Jones calcium acetate method of determining acidity was used and gave the following lime requirements in pounds of CaO for 3,000,000 pounds of soil:

TABLE VIII. LIME REQUIREMENTS IN POUNDS
PER ACRE.

Time of Incubation Weeks	No Sulfur	With Sulfur
1	2347	2393
2	2347	2465
3	2250	2655
4	2393	3105
5	2404	3780
6	2538	4000
7	2565	4230
8	2538	4325
9	2465	4270
10	2160	3860
11	2430	4110

These results show clearly that sulfur in this case increased the lime requirement, as there was a steady increase in the acidity for several weeks. The author

thought the sulfur was about all oxidized in the first nine weeks, and as a result, the acidity did not increase after that time.

Lint also tested samples from old potato rows treated with sulfur one year before and obtained the following results:

TABLE IX.

Row	Rate of Sulfur Application	Lime Requirement
1	600 lbs.	3187
2	300 "	2590
3	600 "	3025
4	300 "	2247
5	Check	883

These results seem to show that sulfur may affect the lime requirement for a year after the application and in proportion to the amount used. In his laboratory experiments he showed much more rapid oxidation of sulfur in sandy loam than in a heavy clay loam soil.

OBJECT OF THIS INVESTIGATION.

The object of this research is an attempt to determine some of the effects of an application of sulfur fertilizers, and to explain so far as possible the cause of its action.

Owing to the very limited amount of experimental work that has been done on this subject in America, and in view of the apparent good results of many European investigators, a further study of the question in this country seemed to be amply justified. In accordance with this idea, this investigation was begun in September 1914.

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PRELIMINARY DATA.

Before beginning the experiment it was thought that the best results with sulfur fertilizers would be obtained by the use of a soil low in total sulfur content. Upon investigation it was found that there had been practically no reliable analysis of any Missouri soils for sulfur. It was, therefore, decided to make an analysis of several different types of soil taken from various parts of the state. Samples of nine stock soils in the laboratory, which were collected as representing the important soil types of the state, were analyzed.

The determination of sulfur was made by the strong hydrochloric acid digestion according to the official method of Agricultural chemists.

The results of the analyses are as follows:

TABLE X.

<u>Locality</u>	<u>Soil type</u>	Per cent <u>Sulfur</u>	Sulfur per A. 2,000,000 <u>lbs. soil</u>
Maryville	Marshall silt loam	.053	1062 lbs.
McBaine	Loess-Knox silt loam	.051	1027 "
Centralia	Putnam silt loam	.044	897 "
Bowling Green	Putnam silt loam	.038	778 "
Hurdland	Putnam silt loam	.030	603 "
Sedalia	Oswego silt loam	.025	502 "
Springfield	Crawford silt loam	.016	329 "
Lebanon	Lebanon silt loam	.015	314 "
Carthage	Eldon silt loam	.014	297 "

The Carthage soil was from the Outlying Soil Experiment Field located at that place, and because of the low sulfur content shown by the analysis, it was judged to be a satisfactory soil for sulfur pot experiments. The soil for the experiment, however, was not taken from the experiment plots, but from an adjoining field of the same type of soil.

PLAN OF THE EXPERIMENT

It was thought that more could be accomplished in the time allowed if the problem was attacked from different angles. Therefore, it was planned to start some field work in small plots, but the major part of the problem was

to be worked out in the greenhouse and laboratory. A description of the work under each of these heads follows:

FIELD EXPERIMENT

For the field work it was impossible to use the Carthage soil, so a small piece of ground near the southeast corner of Block J of the Experiment Station Field at Columbia was selected. The land was quite uniform but of rather low fertility. Since time did not permit the growing of a summer or fall crop, it was decided to sow a very early variety of rye, which would probably head out before May 1st, 1915.

Small plots with several replications were thought to be better than larger ones in eliminating differences arising from variation in the soil.

The size of the plots used were 4 feet 2 inches wide by 7 feet long, or 1/1493 of an acre.

The fertilizer treatment of plots is shown below in pounds per acre; the numbers of the treatments correspond to the numbers in the diagram on the next page:

1. Calcium carbonate (CaCO_3) 1000 pounds.
2. Gypsum (CaSO_4) 500 pounds.
3. Sulfur, 275 pounds.
4. Check - no treatment.
5. Potassium Chloride (KCl) 200 pounds.
6. Potassium Chloride (KCl) plus sulfur.
7. Potassium sulfate (K_2SO_4) 200 pounds.
8. Bonemeal, 300 pounds.
9. Bonemeal plus sulfur.

10. Dried blood, 200 pounds.
11. Check - no treatment.
12. Dried blood plus sulfur.
13. Sodium nitrate (200 lbs.) plus bonemeal (400 lbs.) plus Potassium Chloride plus Calcium carbonate (4000 lbs.)
14. Sodium nitrate plus bonemeal plus Potassium Chloride plus Calcium Carbonate plus sulfur.

DIAGRAM SHOWING ARRANGEMENT
OF PLOTS
FIELD EXPERIMENT.

North

7	9	<u>11</u>	13	1
8	10	12	14	2
9	<u>11</u>	13	1	3
10	12	14	2	<u>4</u>
<u>11</u>	13	1	3	5
12	14	2	<u>4</u>	6
13	1	3	5	7
14	2	<u>4</u>	6	8
1	3	5	7	9
2	<u>4</u>	6	8	10
3	5	7	9	<u>11</u>
<u>4</u>	6	8	10	12
5	7	9	<u>11</u>	13
6	8	10	12	14

2 ft. alley

East

Alternations were made in the arrangement of plots so that two plots with the same treatment would not be in a line across the different series. By this means it was hoped to still further eliminate the effects of soil variation. The series was replicated four times, making five series. Two feet alleys were left between each two series of plots so that the fertilizer treatments would not mix by drainage and to afford a passage way in working the plots. A small furrow was run down each alley, which almost perfectly prevented surface washing. This drainage ditch is well shown in the photograph of these plots. (Plate I).

GREENHOUSE WORK

The work in the greenhouse was carried on by growing crops in sand and soil culture. A no-crop series was also run for bacteriological and chemical studies.

The idea in the sand cultures was to find the effect of flowers of sulfur and gypsum when used in a nutrient solution, which contained no other form of sulfur. Corn was the crop grown, one gallon pots being used. The sand was a good grade of quartz, which was washed free as possible from sulfates and chlorides with distilled water.

The soil used in the other pot work was the silt loam soil from Carthage, low in sulfur but with a good supply of organic matter. It was sifted thru a one-fourth inch mesh sieve and thoroly mixed before putting into the pots.

The watering device used was modeled after that devised by Briggs and Shantz. A diagram of this is shown in Plate III.

The rock used in this watering device was broken chert, which was obtained at a rock quarry southeast of Columbia. The pieces of chert were treated with hydrochloric acid to remove all traces of limestone.

The material for the fertilizer treatments was weighed out in the laboratory on a delicate balance. Then a given amount of the thoroly mixed soil was weighed into each jar. The soil from this jar was dumped onto a table, the proper fertilizer added, and the whole thoroly mixed by hand. The fertilized soil was then tamped into the jar and the watering device inserted. Enough water was added to bring the moisture up to the desired amount, 25 per cent, and the pot was ready for seeding.

It was thought that as much diversity of crops should be used as possible, and accordingly three botanical families were chosen: Gramineae, Cruciferae, and Leguminosae, respectively represented by corn (*Zea indentata*), rape (*Brassica napus*), and red clover (*Trifolium pratense*). These three crops were grown with the same fertilizer treatments, as will be shown later.

Another series of soil pots was run for the purpose of studying the effect of sulfur upon nodular development in legumes. Soybeans (*Glycine hispida*) was the crop grown in this instance.

The other series of soil pots contained no crop

and was used for the purpose of studying the content of sulfate, nitrate, lime requirement, and the bacteria count under the different soil treatments. The above determinations were made at the beginning, at intervals, and at the end of the experiment. Four gallon pots were used in this part of the experiment, and they were watered in the same manner as the other soil pots.

In the sand cultures corn (*Zea saccharata*), variety-country Gentleman, was the crop used.

ANALYTICAL WORK

The laboratory analytical work consisted of making a preliminary analysis of a number of soils as shown in the preliminary data, and in periodic analyses of the soil from the no-crop series. Similar analyses of the sand cultures were made at the conclusion of this part of the experiment. The harvested crops were air dried in the laboratory and the weights accurately taken.

Determinations for nitrates and for sulfates were made by the colorometric method as described by Schreiner and Failyer (1906). Lime requirement determinations were made by the official Veitch method. In the bacteriological work a modified synthetic agar, as described by P. E. Brown (1913), was used. The bacteria were grown on petri dishes and incubated in the greenhouse at an average temperature of 20° C. for a period of one week.

During the latter part of the work some acidity tests were made by the new method devised by E. Truog (1914).

EXPERIMENTAL DATA

SAND CULTURES

The sand culture work was carried on in one gallon glazed stone pots. These were filled with pure quartz sand. An equal amount, 6500 grams, was put into each pot. It was decided from a determination of the pore space that 900 cubic centimeters of nutrient solution would be sufficient to keep the sand moist near enough the surface satisfactorily to feed the crop.

Pfeffer's nutrient solution was used in this part of the experiment. It was modified in the following manner: In the check pots which were to receive no sulfur at all, magnesium chloride was substituted for magnesium sulfate. Two other pots received this modified solution, but with the addition of one gram of flowers of sulfur thoroly mixed thru the sand before it was put into the pots. Another pair of pots received the modified nutrient solution with 4.37 grams of gypsum (CaSO_4) to supply the sulfur. The gypsum contained one gram of the element sulfur, and was, therefore, equivalent in that respect to the amount of flowers of sulfur added to the pots just described.

The following table shows the various treatments, together with the weights of the pots:

TABLE XI. TREATMENT AND WEIGHT OF POTS.

Pot	Treatment	Wt. pot empty	Wt. pot and 6500 gm. sand	Total Wt. with 900 cc. of solution
I	No Sulfur-Modified Pfeffer's	2165	8665	9565
II	No Sulfur-Modified Pfeffer's	2459	8959	9859
III	Normal Pfeffer's	2505	9005	9905
IV	Normal Pfeffer's	2482	8982	9882
V	Sulfur-Modified Pfeffer's	2277	8777	9677
VI	Sulfur-Modified Pfeffer's	2557	9057	9957
VII	CaSO ₄ -Modified Pfeffer's	2445	8945	9845
VIII	CaSO ₄ -Modified Pfeffer's	2363	8863	9763

The water used in making up the nutrient solutions and also for watering the pots was taken direct from the distilling apparatus in the laboratory and kept in bottles with glass stoppers. Chemical tests showed the water to be free from sulfates, sulfides, and chlorides, but it contained some ferrous iron and a small amount of ammonia.

The crop used in this test was sweet corn (*Zea saccharata*), variety-Country Gentleman. It was planted October 23, 1914. The seedlings came up in about six days. The plants in pots VII and VIII were about twelve hours later than the others in coming up. This was due to the sand in the bench underneath these pots being more moist and, therefore, cooler. By November 11th, slight differences could be noted in growth. The magnesium sulfate and the flowers of sulfur treatments were a little ahead of the others. The corn with the flowers of sulfur treatments began to show a

little greener color. At this time the plants were thinned, leaving two in each pot. Both plants in the respective pots and in the duplicate pots showed as much uniformity as could reasonably be expected. Plate II will show something of the character of growth and the relative size of plants during this early part of the period.

At about the middle of the growing period the plants showed several marked differences. The leaves on the sulfur treatments showed a much greener color (estimated 10 per cent), and a much more uniform color than the plants in any of the other pots. The corn leaves under all the other treatments were striated and showed alternating light and dark green lines running the full length of the leaves. The sheathes of the leaves and also the stalks under the sulfur treatments showed a very much greater amount of chlorophyll than did any of those in the other pots. The leaves of the plants with the no sulfur treatment had a very light more or less translucent or whitish color. Further, the plants with the gypsum treatment showed a very large amount of red color on the stems and sheathes, which finally extended over the entire length. The plants with the magnesium sulfate treatment were intermediate as regards color. The stems and sheathes were rather green, but not so much so as the plants in the flowers of sulfur treatments. They later showed a great deal of red color and the leaves were about as striated as on the plants in the gypsum pots.

The relative heights of the plants at different stages of growth are shown in the following table:

TABLE XII. HEIGHT OF PLANTS AT VARIOUS STAGES OF GROWTH.

POT	TREATMENT		*STALK * NOV.17, '14-NOV.24, '14-DEC.7, '14-DEC.30, '14-FEB.8, '15.					MATURE PLANTS
			cm.	cm.	cm.	cm.	cm.	cm.
I	No Sulfur-Modified Pfeffer's	1	6.0	8.5	15.5	27.5	75.0	
		2	8.5	13.5	17.5	38.5	124.0	
II	No Sulfur-Modified Pfeffer's	1	9.0	13.0	20.0	21.5	86.0	
		2	6.0	7.5	13.0	37.5	130.0	
	Total.....		29.5	41.5	66.0	125.0	415.0	
III	Normal Pfeffer's	1	8.0	13.0	18.5	68.0	123.0	
		2	10.0	15.0	21.5	35.0	139.0	
IV	Normal Pfeffer's	1	10.0	13.0	19.0	35.0	115.0	
		2	10.0	13.5	20.5	33.5	96.0	
	Total.....		38.0	53.5	79.5	171.5	473.0	
V	Sulfur-Modified Pfeffer's	1	7.5	10.5	17.0	28.0	122.0	
		2	10.0	14.0	18.5	43.5	124.0	
VI	Sulfur-Modified Pfeffer's	1	9.0	14.5	21.0	22.0	58.0	
		2	7.5	10.0	16.5	28.0	121.0	
	Total.....		34.0	49.0	73.0	121.5	425.0	
VII	CaSO ₄ -Modified Pfeffer's	1	8.0	10.0	17.0	41.0	119.0	
		2	7.5	11.5	17.5	30.5	129.0	
VIII	CaSO ₄ -Modified Pfeffer's	1	7.0	10.5	17.0	30.0	135.0	
		2	8.0	12.5	18.0	31.0	123.0	
	Total.....		30.5	44.5	69.5	132.5	506.0	

*Stalk number corresponds to the plant nearest the front of the pot as shown in Plate II.

In the foregoing table the figures in the last column represent the heights of the plants to the top of the tassel; all other measurements are taken to the base of the top leaf. It will be noticed that during the early part of the growing period the total height of the plants ranked as follows for the different treatments: 1st, magnesium sulfate; 2nd, flowers of sulfur; 3rd, gypsum; and 4th, check, the latter making the least growth. During the latter part of the period one notable change took place, namely, the plants with the calcium sulfate treatment reached a greater total height than any of the others. This striking relative gain in the gypsum pots is due partly to the fact that there was less individual plant variation, there being no extremely dwarf plants, as will be noticed by a study of the data for all the other treatments. This, however, does not entirely explain the phenomenon, for observation showed that the plants with the gypsum treatment became much more stocky and the leaves broader. An effort was made at the end of the experiment partially to explain this phenomenon by an analysis of the sand. These results will be given later. However, the differences in total height is possibly due mainly to individuality in plants, because it will be noted that the tallest plants in any of the various treatments do not vary a great deal.

Some interesting things are shown in the following table giving the comparative leaf areas of the different plants. Since some of the lower leaves were dead, these measurements begin with the leaf at the ear node and include the leaves above it.

TABLE XIII. LEAF MEASUREMENTS AND AREA FOR EACH STALK IN THE
SAND CULTURE SERIES.

Pot	Treatment	* Stalk	Av. Length Leaves cm.	Av. Width Leaves cm.	Leaf Area sq. cm.	Total Each Pot sq. cm.	Total each Treatment sq. cm.
I	No Sulfur-Modified Pfeffer's	1	26.4	2.54	589.3	2021.4	
		2	40.1	3.58	1432.1		
II	No Sulfur-Modified Pfeffer's	1	36.0	3.17	988.9	2134.6	4156.0
		2	34.3	3.32	1145.7		
III	Normal Pfeffer's	1	36.3	2.84	1025.6	2643.5	
		2	40.6	3.48	1617.9		
IV	Normal Pfeffer's	1	39.3	3.53	1586.9	2954.5	5597.0
		2	38.3	3.17	1367.6		
V	Sulfur-Modified Pfeffer's	1	35.5	2.66	775.4	1799.8	
		2	36.8	2.79	1024.4		
VI	Sulfur-Modified Pfeffer's	1	33.5	3.32	702.5	2228.8	4028.6
		2	49.2	3.22	1526.3		
VII	CaSO ₄ -Modified Pfeffer's	1	35.5	3.75	1290.5	2563.9	
		2	38.3	3.22	1273.4		
VIII	CaSO ₄ -Modified Pfeffer's	1	41.4	3.53	1646.9	3568.0	6131.9
		2	45.2	4.49	1921.1		

*Stalk number corresponds to the plant nearest
the front of the pot as shown in Plate II.

The foregoing table shows that the plants with the flowers of sulfur treatment have slightly less leaf area than the check plants. It may be noted, however, that the average length of leaves in the sulfur pots is 4.34 centimeters greater, and the average width .152 centimeters less than in the check pots. These long, narrow leaves, together with their deeper green color, were very noticeable.

The total leaf area of the plants fertilized with gypsum was much the greatest of any, being considerably above those with the magnesium sulfate treatment.

A very striking effect of the flowers of sulfur was noted on the growth of algae in these sand cultures. During the early part of the period absolutely no algae developed on the sulfur treated pots, while all of the others were practically covered with it. Neither did the sulfur pots form a crust on top of the sand as did all of the others.

The pots were kept up to normal moisture content by adding water about twice each week. The nutrient solution was applied about once each week, the same amount being given the various pots at each period. The total amount of nutrient solution and the total amount of water used by each pot will be shown in the following table. No allowance is made in this for surface evaporation.

TABLE XIV. WATER AND NUTRIENT SOLUTION USED BY PLANTS.

Pot	Treatment	Nutrient Solution cc.	Water cc.	Total Wt. dupli- cates cc.	Total water and Nut. Sol. cc.	Rank
I	No Sulfur Modified Pfeffer's	3500	7270			
II	No Sulfur Modified Pfeffer's	3500	7450	14720	21720	1
III	Normal Pfeffer's	3500	8430			
IV	Normal Pfeffer's	3500	6520	14950	21950	3
V	Sulfur Modified Pfeffer's	3500	7463			
VI	Sulfur Modified Pfeffer's	3500	7317	14780	21780	2
VII	CaSO ₄ Modified Pfeffer's	3500	8745			
VIII	CaSO ₄ Modified Pfeffer's	3500	9745	18490	25490	4

The above table compared with Table XV on the following page will show that the amount of solution used is directly proportional to the amount of crop produced.

HARVEST

The crop was harvested March 4th, 1915. The time from planting to harvest was 131 days. The weights of the plants from each pot are shown in the following table:

TABLE XV. AIR DRY WEIGHTS OF PLANTS FROM SAND CULTURES

Pot	Treatment	Wt. Tops gms.	Wt. Roots gms.	Wt. Ears gms.	Total Wt. Plant gms.	Total Wt. Duplicates gms.	Rank
I	No Sulfur Mod. Pfeffer's	20.3	1.85	10.35	32.50		
II	No Sulfur Mod. Pfeffer's	32.1	3.05	9.80	44.95	77.45	1
III	Normal Pfeffer's	39.8	4.90	11.75	56.45		
IV	Normal Pfeffer's	26.9	4.50	1.40	32.80	89.25	3
V	Sulfur Mod. Pfeffer's	31.0	2.70	12.90	46.60		
VI	Sulfur Mod. Pfeffer's	32.6	3.90	4.40	40.90	87.50	2
VII	CaSO ₄ Mod. Pfeffer's	38.6	3.90	12.40	54.90		
VIII	CaSO ₄ Mod. Pfeffer's	37.3	4.10	8.10	49.50	104.40	4

The check plants lost more weight after cutting than any of the others. This was due to the fact that they did not ripen so quickly as did the plants with the other treatments. At cutting time the plants in Pot I had four green leaves, those in Pot II had eleven ^{green} leaves, while those in Pots V and VII had one each; all other leaves were dead.

After the harvest, samples of the sand were taken for analysis by making two one-inch borings in each pot. These samples were immediately dried at 55° C. to stop bacterial action. Then the roots were washed and photographed. They were then incinerated, the ashes washed off, and the weight of the sand particles which adhered to the roots was subtracted from the total weight, which gave the actual air dry weight of the

roots.

Some very striking differences were noted in the character of the root growth. The roots on the checks showed the least development. Those on the sulfur pots were next, but the chief difference was in the small amount of fibrous roots found on the plants with the flowers of sulfur treatment. Some of these, however, had decayed more than the others, but it was still evident that the root system had been less fibrous, and the main roots were larger than in the other pots.

In Plate II is shown the plants when they had reached their maximum growth, together with one of the root systems taken after harvest. This shows quite well the differences in the development of both tops and roots already described.

ANALYTICAL TESTS

The samples of sand taken from the pots were brought to the laboratory and dried at 55° C., as stated before, in order to stop bacterial action. Analyses were then made for nitrates, sulfates, lime requirement, and total number of bacteria. The determinations of nitrates and sulfates were made by the colorometric method of Schreiner and Failyer (1906). The lime requirement was determined by the official Veitch method. The bacterial count was made in the same manner as was described in the plan of the experiment for the soil cultures.

The results of these analyses are summarized in the following table:

TABLE XVI. DETERMINATION OF NITRATE, SULFATE, LIME REQUIREMENT AND BACTERIAL COUNT ON SAND CULTURES.

<u>Determination</u>	<u>Treatments</u>			
	No Sulfur	MgSO ₄	Sulfur	Gypsum
Nitrates	None	None	None	Trace
Sulfates	None	37.5*	135.5*	117.5*
Lime Re- quirement	None	None	None	None
Bacteria	8,850,000	6,300,000	6,950,000	7,350,000

*Parts per Million.

These data seem to indicate that the nitrates which were added in the nutrient solution were used up by the plants. There was a very marked increase in the production of sulfate in the cultures treated with flowers of sulfur. In the case of the gypsum, while part of the sulfate is readily soluble, a smaller amount of sulfate was found in solution at the end of the experiment than where flowers of sulfur had been used. A considerable amount of the gypsum was noticed in the sand at the end of the experiment, showing that part of it had come thru the test unchanged. The small amount of sulfate in the magnesium sulfate treatments may be due to one of two causes. In the first place, because of its solubility only a small amount of sulfate (.75 gm. of MgSO₄) was added to these pots in all feedings. Then, too, there was no nutrient solution added for about two weeks prior to the harvest and the plants probably had sufficient time to use up most of the sulfate in the solution, as they evidently did the nitrates.

Tests were made by putting flowers of sulfur in nutrient solution and in distilled water to see if sulfate was produced in a solution from flowers of sulfur. At the end of three weeks the solutions were tested, but no sulfate was found. Since large amounts of sulfate were found in the sand cultures which contained sulfur, it was concluded that the production of sulfate must be due in this case to aerobic sulfur bacteria, which could live in the sand cultures but would have been unable to have performed their functions in the water cultures above described. An experiment attempting to prove this point will be described later.

SUMMARY

This phase of the work shows clearly that in sand cultures flowers of sulfur, in the presence of nutrient solution minus sulfur, ^{partially} was changed to sulfate. This sulfate is beneficial to plant growth as shown by the increased yield over the check plants. The sulfur can, therefore, partly take the place of a sulfate in the nutrient solution.

Sulfur is probably oxidized to sulfate by means of the aerobic sulfur bacteria present in the sand, as it was shown that no such results were obtained in water cultures having no plants growing in it.

Calcium sulfate proved more effective in the nutrient solution than did magnesium sulfate.

Flowers of sulfur had a very marked effect upon the production of chlorophyll in the leaves.

MAIZE SERIES

The maize (*Zea indentata*) series was carried on in three gallon stone pots. The amount of soil (8820 gms.) and the fertilizer treatments were the same as in the clover and rape series. Pride of the North, a small early variety of corn was used.

Considerable trouble was encountered in getting a stand of corn. Two plantings were destroyed by mice and the last planting was December 26th, 1914. The crop was harvested April 19th, 1915, making the growing period 113 days. The greenhouse was fumigated February 3rd, 1915, with hydrocyanic acid for the purpose of killing the greenhouse white flies. The corn plants were badly damaged by this gas. The leaves were burnt and the stems of the plants became slightly whitened, as did also the parts of the leaves which were not killed. The plants never seemed to recover completely from this shock and the small amounts of crop produced are undoubtedly due largely to this cause.

In setting up the experiment, one mistake was made in the application of fertilizers. Pot 11, which should have received complete fertilizer with lime and gypsum, appeared during growth and at the end of the experiment to have had only lime applied. The acidity test on the soil at the end of the experiment showed the presence of lime. Since it is impossible to tell definitely the treatment of Pot 11, it will be omitted in the consideration of the results. The yield of pot 12 will,

therefore, be considered for that treatment, but since it is not duplicated, the results are, of course, of less value.

The plants were thinned to four to each pot February 2nd, 1915. Plate IV will show the character of growth at that time.

The fertilizers applied to all the soil pots were of a good grade of chemicals. The sulfur was a very pure quality of flowers of sulfur. The lime used was precipitated calcium carbonate. The nitrogen was added in the form of sodium nitrate, the phosphorus as calcium hydrogen phosphate ($\text{CaH}_4(\text{PO}_4)_2$), the potassium as potassium chloride (KCl), and the gypsum as finely precipitated calcium sulfate (CaSO_4).

In this series, and also in the clover and rape series, sulfur was applied at the rate of 150 pounds to the acre, lime at the rate of one ton to the acre, and gypsum 600 pounds to the acre. Sodium nitrate, calcium phosphate, and potassium chloride were made up into a 3-10-4 fertilizer and added at the rate of 600 pounds to the acre.

The soil treatments, together with the amount of water used by each pot, are shown in the following table:

TABLE XVII. SOIL TREATMENTS AND AMOUNT OF WATER USED BY EACH POT.

Pot	Treatment	Water used cc.	Total each Treatment cc.	Rank
1	No treatment	5645		
2	No treatment	6090	11735	2
3	Sulfur	6320		
4	Sulfur	6220	12540	5
5	Lime and gypsum	5575		
6	Lime and gypsum	5895	11470	1
7	Lime, NaNO_3 , $\text{CaH}_4(\text{PO}_4)_2$, KCl	8540		
8	Lime, NaNO_3 , $\text{CaH}_4(\text{PO}_4)_2$, KCl	7460	16000	8
9	Lime, NaNO_3 , $\text{CaH}_4(\text{PO}_4)_2$, KCl, Sulfur	8045		
10	Lime, NaNO_3 , $\text{CaH}_4(\text{PO}_4)_2$, KCl, Sulfur	7480	15525	6
11	Lime, NaNO_3 , $\text{CaH}_4(\text{PO}_4)_2$, KCl, Gypsum	6200	- - -	
12	Lime, NaNO_3 , $\text{CaH}_4(\text{PO}_4)_2$, KCl, Gypsum	7880	7880	7
13	Lime and sulfur	5955		
14	Lime and sulfur	6100	12055	3
15	Lime	5955		
16	Lime	6390	12345	4

* Probably lime alone.

The foregoing table shows that the least amount of water was used by the plants with the treatment of lime and gypsum. It will be shown later that this treatment produced the smallest crop. In general, the amount of water used corresponded fairly well with the amount of crop produced.

NOTES ON GROWTH OF CROP

As already stated, the amount of crop produced in this series was not as much as could be expected under more favorable conditions. The plants in all the pots were consid-

erably dwarfed, but where the more complete fertilizer was used the growth was very markedly increased. This will also be found to hold true for both the clover and the rape.

The leaves on the plants treated with nitrogen, phosphorus, and potassium were of a reddish-brown color for sometime before harvest, yet they did not die and dry up. This gave them an unhealthy appearance. The leaves in the other pots, for the most part, remained green until harvest.

A few shoots with silks were produced as follows: Lime and gypsum treatment, two shoots; lime, N, P, K, six shoots; lime, N, P, K, S, six shoots; lime, N, P, K, CaSO_4 , one; and lime and sulfur, two shoots. These shoots produced no mature corn and were weighed with the stalks and leaves.

Measurements of the height of the plants were taken March 30th, 1915, before the plants had tasseled. Another measurement was taken at the time of harvest. The sum of the height of all plants in each pot and under each treatment will be given for the two periods. The measurement was taken to the base of the top leaf the first time, and to the top of the tassel, when present, at the last measurement. A table showing this data and the rank at harvest time follows. The abbreviations for the treatments are given:

TABLE XVIII. HEIGHT OF PLANTS AT TWO DIFFERENT PERIODS.

Pot	Treatment	March 30		April 19		Rank
		Height cms.	Total duplicates cms.	Height cms.	Total duplicates cms.	
1	No treatment	39		74		
2	No treatment	61	100	92	166	1
3	Sulfur	87		116		
4	Sulfur	56	143	99	215	5
5	Lime and CaSO_4	59		76		
6	Lime and CaSO_4	59	118	97	173	2
7	Lime, N, P, K,	167		178		
8	Lime, N, P, K,	183	350	193	371	7
9	Lime, N, P, K, S	198		221		
10	Lime, N, P, K, S	182	380	200	421	8
11	Lime, N, P, K, CaSO_4 *	70	-	95	-	
12	Lime, N, P, K, CaSO_4	161	161	182	182	6
13	Lime and sulfur	79		107		
14	Lime and sulfur	67	146	101	208	3
15	Lime	65		95		
16	Lime	58	123	117	212	4

*Probably lime alone.

These data show that sulfur has materially increased the height of plants over those receiving no treatment, and that it has also given an increase in height where used with fertilizers. It did not give an increase where used with lime. The lime and gypsum gave only very little more than the checks. The rank of the treatments as to height is not exactly the same as when ranked according to the weights of crop produced. The weights of the plants are given in the following table:

TABLE XIX. AIR DRY WEIGHTS OF PLANTS AND ROOTS.

Pot	Treatment	Wt. Tops gms.	Total dupli- cates gms.	Wt. Roots gms.	Total dupli- cates gms.	Total Wt. Plants gms.	Rank
1	No treatment	6.35		2.65			
2	No treatment	6.55	12.90	2.95	5.60	18.50	2
3	Sulfur	8.90		2.40			
4	Sulfur	6.90	15.80	3.40	5.80	21.60	5
5	Lime, CaSO ₄	6.30		2.40			
6	Lime, CaSO ₄	6.40	12.70	2.55	4.95	17.65	1
7	Lime, N, P, K	24.75		5.40			
8	Lime, N, P, K	19.70	44.45	3.70	9.10	53.55	7
9	Lime, N, P, K, S	22.55		4.45			
10	Lime, N, P, K, S	19.70	42.25	4.20	8.65	50.90	6
11	Lime, N, P, K, CaSO ₄ *	6.95	- -	3.20	- -		
12	Lime, N, P, K, CaSO ₄	21.90	21.90	5.35	5.35	27.25	8
13	Lime and sulfur	7.45		2.90			
14	Lime and sulfur	7.65	15.10	2.80	5.70	20.80	4
15	Lime	7.30		2.55			
16	Lime	7.70	15.00	2.10	4.65	19.65	3

* Probably lime alone.

The above table shows that the treatment of lime and gypsum has given the poorest results. The pots receiving no treatment come next in yield. Lime alone, and also lime with sulfur, does not seem to have reduced the yield as in the case of the rape crop. It is, therefore, hard to account for the reduction in yield where lime is used with gypsum.

The effect of flowers of sulfur has been considerable in this series. Where used alone it gave 22 per cent increase over the checks in the amount of aerial parts of the plants. The root growth, however, was about the same in both cases.

Sulfur used with lime also gave a slight increase over lime alone. Where used with the heavy fertilizer applications, the sulfur did not give an increase. No satisfactory reason has been found why flowers of sulfur should give an increase when used alone and not when used with heavy applications of fertilizer. Practically opposite results were obtained with gypsum. It gave an increase when used with heavy applications of fertilizer, but decreased the yield when used with lime.

Acidity tests by the Truog (1914) qualitative method showed that flowers of sulfur had slightly increased acidity and that gypsum with lime decreased acidity slightly more than lime alone. Altho this decrease in acidity by the use of gypsum is small, it seems to hold true in all of the cropped series and give similar results by the Veitch method in the no-crop series.

SUMMARY

Flowers of sulfur slightly increased the yield where used alone, or with lime, but did not when used with a more complete fertilizer.

Gypsum increased the yield when used with a complete fertilizer, but did not when used with lime.

Flowers of sulfur seemed slightly to increase acidity.

Gypsum when used with lime apparently decreased the acidity to some extent.

None of these results from sulfur or gypsum upon the growth of corn were very striking, and further work is needed to determine their effects accurately.

CLOVER SERIES

Red Clover (*Trifolium pratense*) was the crop grown in this series. The soil treatments and the amount of soil were the same as in the corn and rape series. The clover was sown November 24th, 1914, and harvested April 26th, 1915, making the growing period 152 days.

The pots were started with 25 per cent water, but this was later reduced to 22.7 per cent. The pots were weighed once a week and enough water added to bring them up to weight. They were also watered once a week without weighing, and were thus kept near the proper moisture content thruout the experiment. During the latter part of the growing period when the plants were larger, three waterings per week were required. The plants were thinned down to twenty plants per pot, which proved to be about the right number, since they did not have time to fully mature.

NOTES ON THE GROWTH OF THE CROP

The plants started off well and evenly, but after a few days the complete fertilizer treatments began to show an advantage over the other pots. This difference became still more marked as growth progressed. The pots with the complete fertilizers showed but little differences until about two months after planting, when the fertilizer without sulfur appeared to be a little better than the others. Later the fertilizer and

gypsum treatment seemed to be making the most vigorous growth. About two weeks before harvest the fertilizer and sulfur treatments began to gain on the treatment of fertilizer alone in the use of water. This was a good indication of a relative increase in growth.

The pots not having a supply of the complete fertilizer showed but little differences until three months after seeding. At that time the comparative growth in these pots was as follows: The lime and gypsum treatment was the poorest, the lime and sulfur slightly better, with lime, check, and sulfur following in the order named. These differences, however, were slight, as may be seen from Plate VI and from Table XXI. The rank of the treatments in the use of water is given below:

Pot	Treatment	Water used cc.	Total duplicates cc.	Rank
17-No treatment		8425		
18-No treatment		7415	15840	4
19-Sulfur		8140		
20-Sulfur		8885	17025	5
21-Lime, CaSO ₄		7360		
22-Lime, CaSO ₄		7565	14925	3
23-Lime, N, P, K		19070		
24-Lime, N, P, K		17720	36790	8
25-Lime, N, P, K, S		18075		
26-Lime, N, P, K, S		16750	34825	6
27-Lime, N, P, K, CaSO ₄		18185		
28-Lime, N, P, K, CaSO ₄		17880	36065	7
29-Lime and Sulfur		7330		
30-Lime and Sulfur		7385	14715	2
31-Lime		7240		
32-Lime		7250	14490	1

The clover crop was harvested when about half mature. This fact will, of course, reduce somewhat the value of the results, because it cannot be said with certainty how the growth under the different treatments would continue as the plants approached maturity. However, some results were obtained which may be of value. Plate VI will show the condition of growth at harvest time.

The most striking result noted in this series was the increase in the number of nodules on the clover roots where sulfur was applied. This was most noticeable where sulfur was used alone, and where used in addition to a complete fertilizer. In both cases the number of nodules was about seven times as many as where sulfur was not used. When sulfur was used in connection with lime the number of nodules was not materially increased over the treatment of lime alone. This may be due to the fact that the plants in the lime and sulfur pots made less growth than did the plants with only the lime treatment.

In this experiment lime alone slightly increased the number of nodules over the checks. This is opposed to the results obtained on a different soil at this station by P. E. Karraker (1914).

A table will show the number of nodules and weight of plants for the different treatments. Where the heavy applications of fertilizer were used, the nodules were so numerous and the roots so fibrous that it was decided to be impractical to attempt to get an exact count so the number was simply estimated.

TABLE XXI.

NUMBER OF NODULES AND AIR DRY WEIGHT OF CLOVER CROP.

Pot	Treatment	Number Nodules	Wt. Tops gms.	Wt. Duplicates gms.	Wt. Roots gms.	Wt. Duplicates gms.	Total Wt. Plants. gms.	Rank
17	No treatment	8	1.77		1.88			
18	No treatment	12	1.70	3.47	1.63	3.51	6.98	4
19	Sulfur	62	2.30		2.70			
20	Sulfur	80	2.65	4.95	2.80	5.50	10.45	5
21	Lime, CaSO ₄	5	1.17		1.30			
22	Lime, CaSO ₄	15	1.46	2.63	1.40	2.70	5.33	2
23	Lime, N,P,K	1000*	21.76		13.95			
24	Lime, N,P,K	1000*	20.80	42.56	12.32	26.27	68.83	8
25	Lime, N,P,K,S	8000*	21.00		12.40			
26	Lime, N,P,K,S	7000*	19.95	40.95	12.10	24.50	65.45	6
27	Lime, N,P,K, CaSO ₄	800*	22.24		12.61			
28	Lime, N,P,K, CaSO ₄	800*	20.90	43.14	12.30	24.91	68.05	7
29	Lime, Sulfur	35	1.41		1.44			
30	Lime, Sulfur	28	1.16	2.57	1.18	2.62	5.19	1
31	Lime	17	1.48		1.60			
32	Lime	44	1.40	2.88	1.30	2.90	5.78	3

* Estimated.

The foregoing data show that the yields from the treatments of lime and sulfur, lime and gypsum, and lime alone were very much the same and were lower than the check treatments. This seems to emphasize the fact so often found, that lime has a depressing effect upon the vegetative growth for some time after application.

The treatment of flowers of sulfur very markedly increased the growth, as well as the number of nodules, over the plants receiving no treatment. Where heavy applications of fertilizer were applied, the sulfur did not increase either the top or root growth. Under the same conditions, the gypsum slightly increased the top growth, but did not increase the root growth. These plants had a more succulent appearance and lost more moisture upon drying than did those from Pots 23 and 24, which lacked gypsum in the treatment.

Acidity tests by the Truog method showed about the same results as were described under the Maize Series, except that the slight decrease in acidity from the use of gypsum was a little more marked. This effect of gypsum in slightly reducing acidity may possibly be due to the fact that the CaSO_4 used contained traces of magnesium, barium, sodium, and iron.

SUMMARY

Flowers of sulfur increased the yield of clover over no treatment.

Lime alone depressed the yield and when used with sulfur or gypsum, the yield was still further depressed.

Complete fertilizers very markedly increased the yield of clover on this soil.

Flowers of sulfur increased the number of nodules on the roots about seven times. This was the most striking effect of sulfur noted in the whole experiment. The effect was not very marked where sulfur was used with lime.

RAPE SERIES

The rape (*Brassica napus*) series was set up in a similar manner as was the corn and clover series, and was given the same soil treatment. Three gallon pots were used, which held 8820 grams of dry soil. The water content was kept up to 25 per cent for a while, but this was thought to be too high on account of fungi which attacked some of the plants. The water supply was finally reduced to 20 per cent. Water was applied twice a week, but the pots were brought to optimum moisture content only once a week. The rape was planted November 25th, 1914, and harvested March 27th, 1915, making the period of growth 121 days.

During the first part of the period the plants grew briskly and gave a stand of good healthy plants. This was especially true of the pots where complete fertilizers were added. The plants were finally thinned to twenty-five to each pot, which proved to be about the right number. The plants reached their maximum growth about January 20th, 1915, or fifty-five days after planting. Plate VII will show the condition and relative growth under the different treatments at that time.

The leaves began to fall about the time the photograph was taken and continued to do so thruout the period of growth. These leaves were all saved and weighed at the end of the experiment.

Water was added thru the watering apparatus and

none was applied on top of the soil. The amount of water used was in most cases directly proportional to the weight of crop produced, except in the case of the check pots which were on the south end of the series most of the time. The location of the checks probably hastened evaporation from the soil. A table showing the treatments and amounts of water used by each pot is given below:

TABLE XXII. TREATMENTS AND AMOUNTS OF WATER USED BY RAPE CROP.

Pot	Treatment	Water used cc.	Total each treatment cc.	Rank
33	No treatment	6015		
34	No treatment	6555	12570	5
35	Sulfur	5870		
36	Sulfur	6275	12145	4
37	Lime and Gypsum	5990		
38	Lime and Gypsum	5430	11420	3
39	Lime, N, P, K	11955		
40	Lime, N, P, K	11645	23600	7
41	Lime, N, P, K, S	11325		
42	Lime, N, P, K, S	10905	22230	6
43	Lime, N, P, K, CaSO ₄	11930		
44	Lime, N, P, K, CaSO ₄	11750	23680	8
45	Lime and Sulfur	5490		
46	Lime and Sulfur	5870	11360	2
47	Lime	5245		
48	Lime	4905	10150	1

As in the corn and clover series, the complete fertilizers gave remarkable increases in the amount of growth, showing that for rape, as well as for the other crops, this soil is greatly in need of nitrogen, phosphorus and potassium. Where the complete fertilizer was used, the addition

of flowers of sulfur did not increase the growth, altho the addition of gypsum had a very beneficial effect. Flowers of sulfur without other fertilizers gave a slight increase over the check pots. This increase was all in the top growth, the root growth being slightly depressed by the sulfur. Both gypsum and flowers of sulfur when used with lime failed to give an increase over the checks, yet both increased the growth over the treatment of lime alone. A table showing the actual dry weights of the yields will make these points clear.

TABLE XXIII. WEIGHTS OF TOPS AND ROOTS OF
RAPE CROP, AIR DRY.

Pot	Treatment	Wt. Tops gms.	Wt. dupli- cates gms.	Wt. Roots gms.	Wt. dupli- cates gms.	Total Wt. plants gms.	Rank
33	No treatment	2.22		1.22			
34	No treatment	2.63	4.85	1.45	2.67	7.52	4
35	Sulfur	2.70		1.26			
36	Sulfur	2.51	5.21	1.22	2.48	7.69	5
37	Lime, CaSO_4	2.25		1.15			
38	Lime, CaSO_4	2.08	4.33	0.87	2.02	6.35	2
39	Lime, N, P, K	8.62		2.40			
40	Lime, N, P, K	7.55	16.17	2.40	4.80	20.97	7
41	Lime, N, P, K, S	8.60		2.43			
42	Lime, N, P, K, S	6.60	15.20	2.15	4.58	19.78	6
43	Lime, N, P, K, CaSO_4	8.76		2.30			
44	Lime, N, P, K, CaSO_4	8.49	17.25	2.65	4.95	22.20	8
45	Lime and Sulfur	2.20		0.74			
46	Lime and Sulfur	2.32	4.52	1.26	2.00	6.51	3
47	Lime	1.96		0.89			
48	Lime	1.93	3.89	1.20	2.09	5.98	1

One of the most notable things brought out in this table is the fact that lime has materially decreased the yield. This depressing effect of lime upon the first crop has been observed many times and by several different investigators at the Missouri Experiment Station, but no one has offered an entirely satisfactory explanation of the phenomenon. Neither sulfur nor gypsum were able to overcome the depressing effect of lime when used in connection with that substance. Flowers of sulfur alone, however, increased the yield over the checks, and gypsum in connection with a complete fertilizer also increased the yield over the treatment with complete fertilizers alone.

Sulfur used in a complete fertilizer did not increase the yield as did the gypsum, nor did it give as large a yield as did the same treatment without sulfur. This fact can hardly be explained from the data at hand for the following reasons: (1) It can hardly be on account of slight increased acidity, because sulfur alone under more acid conditions gave an increase in growth over the no-treatment pots. (2) It can scarcely be a matter of the variation in sulfate content because the analyses of the soil under these treatments showed that the sulfate content did not vary a great deal under the gypsum and flowers of sulfur treatments.

Another noticeable result shown by these data is the depressing effect of sulfur upon root development. It may be noted that the sulfur treatment gave quite an increase over the checks in the growth of the aerial parts of the plants, but the root development was less than that produced

without sulfur. The same holds true where lime and sulfur were used together. Where sulfur was used in the complete fertilizer it caused a depression in the root development, but the tops were also less. This depressing effect of sulfur upon root growth is also shown in the sand cultures and in the soybean series.

Samples of soil were taken from the pots when the crop was harvested. This soil was tested for acidity by the Truog method. The results showed that the soil treated with sulfur was slightly more acid than the soil receiving no treatment. This fact has been brought out by tests on the other series and by the Veitch method on the No-crop series. Where sulfur and lime were used together the acidity was not reduced so much as where lime alone was used.

Gypsum when used with lime probably had a very slight tendency to reduce acidity over a treatment of lime alone, but the effect was not marked.

SUMMARY

Lime has slightly depressed the yield of rape during the short period of its growth in this experiment. Neither sulfur nor gypsum could fully overcome this depressing effect of lime.

Flowers of sulfur alone slightly increased the yield, but did not when used in connection with a complete fertilizer.

Gypsum slightly increased the yield when used with a complete fertilizer.

Flowers of sulfur slightly increased soil acidity.

The effect of sulfur in the various forms used was very slight upon the growth of rape crop and no concordant results were obtained.

SOYBEAN SERIES

The soybeans (*Glycine hispida*, variety-Medium Yellow) were grown in two-gallon pots which held 8557 grams of soil.

In order to eliminate so far as possible individuality in the plants, the seed for planting were all taken from a single selected plant, thus insuring by means of a "pure line" plants of good uniformity.

The method of setting up the experiment was similar to that used with the other soil culture pots. The treatments, however, were somewhat different, as is shown in the following table:

TABLE XXIV. TREATMENTS AND AMOUNTS OF
WATER USED BY SOYBEAN POTS.

Pot	Treatment	Water used cc.	Total for duplicates cc.	Rank
49	No treatment	4535		
50	No treatment	4255	8790	1
51	Sulfur-300 lbs per A.	4005		
52	Sulfur-300 lbs per A.	4145	8150	6
53	Sulfur-150 lbs per A.	4145		
54	Sulfur-150 lbs per A.	3935	8080	7
55	Sulfur-150 lbs.-inoculation	4260		
56	Sulfur-150 lbs.-inoculation	4205	8465	4
57	Gypsum	4185		
58	Gypsum	4075	8260	5
59	Gypsum and inoculation	4325		
60	Gypsum and inoculation	4165	8490	3
61	Inoculation	4300		
62	Inoculation	4405	8705	2

In the foregoing table no allowance was made for surface evaporation from the soil. Therefore, the figures

show the comparative amounts used by the different treatments rather than actual water transpired.

NOTES ON THE GROWTH OF THE CROP

The seed was sown November 26, 1914. The plants came on evenly and rapidly. When about six inches high they were thinned down to eight plants per pot. In about thirty-five days after seeding the plants had practically reached their full height (about 13 inches) and the flowers were beginning to appear. The beans set on the check pots about one day earlier than on the plants under treatments.

A photograph was taken of the plants January 21st, 1915, fifty-six days after planting. Plate VIII will show the condition of the plants and character of growth on that date.

It was noted shortly after the first photograph was taken that the plants in the inoculated pots were holding their leaves better and were greener than the plants with the other treatments. Next to these, the plants with the treatment of sulfur at the rate of 300 pounds an acre showed the most green color in the leaves. A count of the green and partly green leaves was made January 30th, 1915, and the results are tabulated in the following table:

TABLE XXV. GREEN AND PARTLY GREEN LEAVES
ON SOYBEAN PLANTS.

<u>Pot</u>	<u>Treatment</u>	<u>Green Leaves</u>	<u>Partly Green</u>	<u>Green and Partly green</u>	<u>Total</u>
49	No treatment	16	35	51	
50	No treatment	10	24	34	85
51	Sulfur-300 lbs. per A.	28	28	56	
52	Sulfur-300 lbs. per A.	22	34	56	112
53	Sulfur-150 lbs. per A.	11	40	51	
54	Sulfur-150 lbs. per A.	13	27	40	91
55	Sulfur-150 lbs.-inoculation	45	18	63	
56	Sulfur-150 lbs.-inoculation	27	32	59	122
57	Gypsum	5	30	35	
58	Gypsum	14	32	46	81
59	Gypsum and Inoculation	35	17	52	
60	Gypsum and Inoculation	22	37	59	111
61	Inoculation	27	23	50	
62	Inoculation	20	30	50	100

Plate IX shows the way the leaves were held by the plants under different treatments at the time the foregoing data was taken. It may be noted from this Plate, or from the foregoing table, that the plants with the heavy sulfur treatments retained the greenness of their leaves as well as did the plants with the gypsum and inoculation treatments, and slightly better than those where inoculation alone was used. Gypsum caused the leaves to fall earlier than any of the other treatments, and the plants seemed to mature earlier. Nearly all the leaves on the different treatments were dropped within a period of about ten days. A few, however, still persisted at harvest on the inoculated plants and on those that received flowers of sulfur (300 lbs.), as well as on the check plants. These few leaves tended to keep the stems green, and the plants in the check pots were more succulent

at cutting time and lost more weight upon drying than any of the others. The fertilizers apparently made the plants ripen up more quickly even though they did not begin to ripen, except with the gypsum treatments, quite as early as did the checks. In this way, the fertilizers shortened the time for complete maturity. This fact is further substantiated by results reported for the sand culture series.

HARVEST OF CROP

The leaves of the plants were nearly all harvested during the course of the experiment, that is, all the leaves that ripened early were collected and saved. These were weighed with the rest of the plant at the end of the test. After harvesting the crop, the roots from each pot were washed, the nodules counted and the air dry weight of the roots taken. These data are summarized on the following page. It will also be noticed from this table that the increase in the number of nodules due to inoculation is very striking. Another obvious result is the reduction in the number of nodules on the plants where sulfur or gypsum were used alone. This reduction, however, was partly offset by the larger tubercles on the fertilized pots. Where gypsum was used with inoculation, the number was practically the same as where inoculation alone was used. Where sulfur was used with inoculation, the number was slightly reduced, but not enough to be conclusive evidence that the smaller number was due to the sulfur; because it will be noted that pot 56 with the sulfur and inoculation treatment produced forty-seven nodules,

while Pot 61 with inoculation alone produced but forty-one. Therefore, the difference in the total number of nodules on the duplicate pots where sulfur was used with inoculation, and where inoculation was used alone, is easily within the limit of reasonable variation. Hence, we must conclude from this that neither sulfur nor gypsum had any marked effect upon the number of tubercles produced ^{on soybeans} when the bacteria are present in large numbers.

TABLE XXVI. NUMBER OF NODULES AND AIR DRY WEIGHTS OF SOYBEAN CROP.

Pot	Treatment	Num- ber nod- ules	Wt. leaves, stems gms.	Wt. seeds gms.	Wt roots gms.	Total Wt. plant gms.	Wt. dupli- cates gms.
49	No treatment	14	2.85	1.30	0.95	5.10	
50	No treatment	8	2.71	1.22	1.04	4.97	10.07
51	Sulfur-300 lbs. per A.	10	2.65	1.17	0.90	4.72	
52	Sulfur-300 lbs. per A.	3	2.70	1.20	0.88	4.78	9.50
53	Sulfur-150 lbs. per A.	4	2.77	1.15	0.75	4.67	
54	Sulfur-150 lbs. per A.	3	2.46	1.10	0.60	4.16	8.83
55	Sulfur-150 lbs.-inoc.	37	2.53	1.26	0.73	4.52	
56	Sulfur-150 lbs.-inoc.	47	2.40	1.20	0.66	4.26	8.78
57	Gypsum	8	2.73	1.25	0.88	4.86	
58	Gypsum	5	2.70	1.15	0.70	4.55	9.41
59	Gypsum and inoculation	43	2.54	1.27	0.88	4.69	
60	Gypsum and inoculation	52	2.73	1.30	0.81	4.84	9.53
61	Inoculation	41	2.51	1.21	0.78	4.50	
62	Inoculation	53	2.64	1.31	0.86	4.81	9.31

As to the total weight of the plants, the data show the checks to be greater than any of the treatments. This can hardly be explained. It seems, however, that the fertilizer treatments have reduced the weight thru some chemical effect, and the inoculation may have hindered growth a little while

the bacteria were producing the pustules on the roots, and since the growing period was so short the plants did not have time to regain the loss. These differences, however, were so slight that they were hardly noticeable during the period of growth. In one of the check pots one or two plants were partly broken over, and these put out small side branches which partly accounted for their increased weight, and also kept them from maturing quite so early as the others.

The dry matter produced by the heavier application of sulfur is about the same as where gypsum and inoculation were used. A strange fact is that the light application of sulfur depressed the growth more than the heavy application, and the sulfur and inoculation gave still lower returns than sulfur alone.

The gypsum treatment produced slightly more than inoculation, but where the two were used together, the yield was greater than where either was used alone.

The sulfur and gypsum seemed to depress seed production and favor vegetative growth. The yield of grain was greater in each case where inoculation was used in connection with the fertilizer than where the sulfur or gypsum was used alone. On the other hand, the weight of leaves and stems was greater in each case where the fertilizer alone was used.

SUMMARY

Sulfur and gypsum slightly reduced the number of nodules formed on soybeans when no artificial inoculation was supplied; when well inoculated these fertilizers did not show

a depressing effect upon the formation of nodules.

Sulfur at the rate of 300 pounds per acre made the plants able to retain their leaves as well as did inoculation.

Gypsum without inoculation caused the plants to mature and drop their leaves earlier than the other treatments, altho the weight of dry matter was slightly more than where gypsum and inoculation were used together.

Sulfur and gypsum seemed to favor vegetative growth rather than seed production.

NO-CROP SERIES

As explained in the plan of the experiment, this series was conducted in the same manner as the others where soil was used and plants grown, save that the soil was kept fallow during the experiment. The soil was analyzed at the beginning of the experiment (December 1, 1914) for content of nitrate, sulfate, lime requirement, and bacteria count. After forty-five days samples were taken and the analysis repeated. The second and third periods were thirty days each, the last samples being taken March 15th, 1915.

METHOD OF SAMPLING

The samples for analysis were taken with a one-inch auger and the borings were made to the full depth of the pots. Three borings were taken from each pot for a sample. The auger holes were filled with paraffin which was only slightly above the melting point, thus excessive heating of the soil was avoided. The soil in these pots was kept up to 23.6 per cent moisture with distilled water.

The results of the laboratory analyses will be shown in the following tables:

TABLE XXVII. PARTS PER MILLION OF NITRATE
AT DIFFERENT PERIODS.

<u>Date</u>	<u>Checks</u>	<u>Sulfur-150 lbs.</u>	<u>Sulfur-300 lbs.</u>	<u>Gypsum</u>
December 1914	20	20	20	20
January 1915	48.5	39.0	33.7	38.7
February 1915	29.7	28.3	23.5	24.1
March 1915	38.0	42.0	44.0	41.0

These results seem to indicate that sulfur at first had a depressing effect upon the nitrate content, and the amount of depression is proportional to the amount of sulfur added. This depressing effect checks with Boullanger's⁽¹⁹¹³⁾ results in France. However, the last analysis showed that after the sulfur had been applied for about 105 days, the nitrate content of the sulfured soil came up above the check; and the increase was in proportion to the amount of sulfur applied.

The gypsum occupied a position between the two sulfur treatments until the last period, when the nitrate content fell slightly below that of the latter, but was still slightly above the checks.

The variation in sulfate content under the different treatments is shown in the following table:

TABLE XXVIII. PARTS PER MILLION OF SULFATE
AT DIFFERENT PERIODS.

<u>Date</u>	<u>Checks</u>	<u>Sulfur-150 lbs.</u>	<u>Sulfur-300 lbs.</u>	<u>Gypsum</u>
December 1914	36.4	36.4	36.4	36.4
January 1915	21.2	32.4	33.8	37.5
February 1915	54.3	50.8	61.5	60.4
March 1915	25.3	40.8	53.8	49.8

The January results seem to be too low in all cases, but are in the same relative positions that we should expect judging from the results of subsequent analyses. The heavy application of sulfur gave higher results in every case than did the light application. The gypsum gave a higher per cent of sulfate at first because of the soluble sulfate which it

contains. It later fell below the heavy treatment of sulfur in its production of sulfate. This would tend to show that after the soluble part of the gypsum is used, its ability to produce available or soluble sulfate is less than that of the element sulfur in the no-crop series.

Excepting the very high content of sulfate shown by the check pots in February, the analyses show a much smaller amount of sulfate in these than in the pots treated with sulfur. This seems to be conclusive evidence that the oxidation of sulfur to sulfate in the soil is considerable.

The variation in lime requirement under the different treatments is shown in the following table:

TABLE XXIX. PERIODIC LIME REQUIREMENT
IN POUNDS PER ACRE.
(Veitch Method)

<u>Date</u>	<u>Check</u>	<u>Sulfur-150 lbs.</u>	<u>Sulfur-300 lbs.</u>	<u>Gypsum</u>
December 1914	3389	3389	3389	3389
January 1915	2600	2600	2850	2600
February 1915	4800	5525	6200	5100
March 1915	4852	5314	4967	4390

These results are consistent in showing that sulfur increases the acidity of a soil, but the increase is not very great. This result checks with the work of Lint (1914) in a general way, but the increase in acidity is much less than he obtained. This may be due, however, to the fact that a more acid soil was employed in this experiment than Lint used.

It was further shown by the Truog (1914) method, a qualitative test for soil acidity, that the acidity in the

sulfured pots was slightly higher than in the check pots, but again the difference was not great. This simply confirms the results obtained by the Veitch method.

Gypsum had little effect upon the lime requirement in this series, but apparently did reduce it slightly at the last determination. It has already been described how qualitative acidity tests were made on the other soil series, which showed that where a crop is growing sulfur increased the acidity to about the same extent as where there was no crop. The tests also showed that the effect of gypsum in reducing acidity was very slight in both cases.

The bacteria counts were made on petri dishes, using modified synthetic agar, as described in the plan of the experiment. The results are shown in the following table:

TABLE XXX. PERIODIC BACTERIA COUNT
IN NO-CROP SERIES.

Date	Check	Sulfur-150 lbs.	Sulfur-300 lbs.	Gypsum
December 1914	3,783,333	3,783,333	3,783,333	3,783,333
January 1915	3,300,000	3,850,000	3,900,000	4,050,000
February 1915	4,500,000	3,920,000	5,750,000	5,550,000
March 1915	6,850,000	5,550,000	4,850,000	7,850,000

It is a noticeable fact that during the course of the experiment the total number of bacteria increased over what it was at the beginning. During the first part of the period the sulfur treatments showed a slight increase in bacteria count over the checks, but later this was not the case. The sulfur seemed to stimulate the number of bacteria for a while, but after considerable sulfate had been produced and the soil

became slightly more acid, the total number of bacteria was noticeably less than in the check pots.

The gypsum, on the other hand, consistently increased the number of bacteria, the total number being more than doubled during the course of the experiment. This result is possibly due to the fact that gypsum tended to reduce slightly the amount of acidity, or else the gypsum must have had some other stimulating effect upon the bacterial flora.

The correlation between the content of nitrate, sulfate, lime requirement and number of bacteria may be graphically represented by a number of curves, which will be shown on the pages following.

A glance at these curves, which represent the results of all the quantitative analyses made on the soil from the No-crop series, will reveal the positive correlation existing between the sulfate content and the lime requirement. This shows that as the quantity of active sulfate increases, the soil becomes more acid and the lime requirement also increases. When the amount of sulfate was reduced, the lime requirement was also less in every case, except in the checks at the last determination when the sulfate content was decreased and the lime requirement remained practically the same.

The nitrate content showed a marked negative correlation with the sulfate content and lime requirement. That is, where the sulfate or acidity showed an increase, the amount of nitrate was decreased, and vice versa.

The bacteria count showed no consistent correlation with any of the other determinations, but did show that it was

CURVES SHOWING ANALYTICAL RESULTS

NO-CROP SERIES

Relation of the Different Determinations for Each Soil Treatment.

Numbers in first column represent the bacteria in hundred thousand, the lime requirement in hundred pounds, the nitrate and sulfate content in parts per million.

Black line represents bacteria count
 Red line represents lime requirement
 Brown line represents nitrate content
 Violet line represents sulfate content

Curves Showing Relation of Various Soil Treatments to Each Constituent Determined

Black line represents checks
 Red line represents gypsum
 Brown line represents sulfur, 150 lbs per A.
 Violet line represents sulfur, 300 lbs per A.

markedly affected by the fertilizer treatment. This fact is brought out by the last series of curves, and was also explained in the foregoing discussion on the bacteria count.

The effect of the treatments upon the different analytical determinations has already been discussed, but curves will be added to make the results readily understood.

SUMMARY

Sulfur produced sulfate, which evidently tended to lower the nitrate content of the soil.

Sulfur was oxidized to sulfate in this soil, the amount of sulfate formed being in proportion to the amount of sulfur added.

Flowers of sulfur furnished soluble sulfate more readily than gypsum, at least after the soluble part of the latter was used.

Flowers of sulfur slightly increased the lime requirement and decreased the total number of bacteria in the soil.

Gypsum increased the total number of bacteria in the soil.

FIELD EXPERIMENT

The only field work carried on in connection with this experiment was a test of the effects of flowers of sulfur alone and in connection, or combination with other fertilizing elements. A very early variety of rye was the crop used in the test.

The different soil treatments used, together with a diagram of the arrangement of plots, was given on pages 24 and 25. The land used for this work proved much less uniform than had been anticipated. This variation will, of course, make the results less reliable. However, part of this error will be eliminated by the fact that each treatment was placed in five different parts of the plat.

The rye used was a very early variety. It made a good start in the fall and withstood the winter well. Plate I will show the winter condition. It grew very rapidly in the early spring and began to head about April 20th, 1915. Observations and notes regarding the date of heading, color, and height at heading were taken. Some variations were noted, but when each treatment is considered thruout the five series, no marked differences seem to stand out for any treatment.

Since the plots appeared to show but little variation so far as the immature crop was concerned, it was decided to cut only half of each plot in time to record results ⁱⁿ this

thesis. This part of the crop was harvested May 3rd, 1915. Plate I will show the condition of the rye at this time. The remainder of each plot will be allowed to mature and any differences which may develop will be noted at that time.

The following table will show the green weights for each plot under the different treatments. The weights are given in order of the series, ranging from east to west. The exact location may be noted from the diagram of the plots on page 25.

TABLE XXXI. GREEN WEIGHTS OF RYE FOR EACH TREATMENT.

Plot	Treatment	Green Weight gms.	Total Weight Replicates gms.	Rank
1	Limestone	1570 1530 1585 1585 1790	8060	8
2	Gypsum	1605 1670 1465 1480 1587	7807	7
3	Sulfur	1800 1260 1196 1715 1613	7584	3
4	No treatment	1625 1250 1730 1520 1593	7718	5
5	Potassium Chloride	1375 1105 1690 1830 1613	7613	4

TABLE XXXI.

Continued:

Plot	Treatment	Green Weight gms.	Total Weight Replicates gms.	Rank
6	Potassium Chloride, Sulfur	1305 1580 1770 1800 1730	8185	9
7	Potassium Sulfate,	1335 1200 1577 1510 1240	6862	1
8	Bone Meal	1725 1480 1610 1705 1755	8275	10
9	Bone Meal, Sulfur	1675 1485 1640 1845 1685	8330	11
10	Dried Blood	1625 1705 1430 1575 1440	7775	6
11	No treatment	1345 1700 1525 1545 1315	7430	2
12	Dried Blood, Sulfur	1730 1627 1775 1835 1800	8767	12
13	Sodium nitrate, bone meal, potassium chlor- ide, lime	2145 1750 1615 1860 2130	9500	13
14	Sodium nitrate, bone- meal, potassium chlor- ide, lime, sulfur	1715 1985 1865 2000 1990	9555	14

These data show no very striking returns upon the growth of rye from the use of sulfur. When used alone, flowers of sulfur decreased the yield slightly as compared with the adjacent no-treatment plot, No.4. It was, however, a little above the average of the two no-treatment plots, Nos. 4 and 11. Where sulfur was used with other fertilizers it increased the green weight in every case, altho not very greatly. With potassium chloride and ^{with} blood meal, the increase from flowers of sulfur was quite marked, as may be noted from the foregoing table. Where sulfur was combined in the fertilizer, as in potassium sulfate on plot No.7, a marked decrease in yield occurred. No reason for this is apparent.

When used with heavy applications of other fertilizing elements, flowers of sulfur gave but a slight increase in growth. This, together with the greenhouse tests under similar applications of fertilizers, seems to indicate that sulfur does not necessarily give any marked response when large amounts of other plant foods are supplied which would necessarily tend to throw the balance of sulfur low. This may be due to the fact, as found by Peterson of Wisconsin, that the plants become very economical of sulfur and make a good growth even tho the total amount of sulfur is small.

SUMMARY This field work has not progressed far enough to justify any definite conclusions at present, but indications are that sulfur when used with other fertilizers is having some effect in increasing the vegetative growth of rye. Other differences may develop as the rye matures which might tend to change the final results.

EFFECT OF BACTERIA UPON THE
PRODUCTION OF SULFATE.

As stated on Page 40, flowers of sulfur was placed in water cultures and in distilled water and found to produce no sulfate, as indicated by the barium chloride test. Other cultures, using the same nutrient solution in a sand medium, showed that sulfate was produced from flowers of sulfur. These facts seemed to indicate that the bacteria, which would have a much better chance to work in sand than in water because of greater aeration, must have been the direct cause for the production of sulfate in the sand cultures.

When bacteria counts were made on the sand cultures, it was found that from the pots fertilized with flowers of sulfur more red colored colonies appeared than on the other plates. On the culture plates made from the magnesium sulfate treatments there were more light colored gelatinous colonies than on the other treatments.

It was thought that the bacteria forming these special colonies might be connected with the formation or existence of sulfate in these cultures. The organisms forming the red colonies are cocci forms about four microns in diameter. The gelatinous colonies are made up of bacilli organisms about six microns in length.

The apparatus used for determining the effect of these bacteria upon the formation of sulfate consisted mainly

of six pint fruit jars. Five hundred grams of well washed quartz sand was placed in each jar. The sand was moistened well with modified Pfeffer's nutrient solution, as described under the sand culture work. Before putting into the jars, .15 grams flowers of sulfur was added to the sand for each jar.

In order that the cultures might be aerated, intake and outlet tubes were inserted thru holes in the lids of the jars. The air was filtered thru absorbent cotton and then thru water in a small bottle before entering the culture jar. The air was drawn thru the apparatus by means of an aspirator. Plate X will show this apparatus very clearly.

The whole apparatus was sterilized in the autoclave at 15 pounds pressure for thirty minutes. When cool, two of the jars were inoculated with fresh plate cultures of the red organisms and two were inoculated with the light colored organisms. The other two jars were kept as checks and not inoculated.

At the end of one week samples were taken from each culture and an attempt was made to determine the amount of sulfate by precipitating with barium chloride and weighing the barium sulfate. The amount of precipitate was so small that it was thought to be within the limit of error in weighing, so no weights were made. The qualitative test, however, indicated the presence of a small amount of sulfate in the inoculated jars and showed none in the checks.

Two weeks after the cultures were started, a second sample was taken from each jar and the amount of sulfate determined. This time the colorometric method of Schreiner

and Failyer was used. Altho not as accurate as a gravimetric determination, this method was found to be the most satisfactory for this work. Fair results were obtained, except for the second determination, when the water blanks averaged as high as any of the treatments. No good reason could be assigned for this, and evidence of its incorrectness is shown by later determinations.

A table showing the results of the determinations for sulfate expressed in parts per million follows:

PLATE XXXII. PARTS PER MILLION OF SULFATE
IN INOCULATED AND UNINOCULATED SAND
CULTURES.

Jars	Inoculation	1st De- termina- tion	2nd De- termina- tion	3rd De- termina- tion	4th De- termina- tion	Average
1,2	None-checks	63.7	---*	76.4	108.3	82.8
3,4	Red Bacteria	197.4	---*	152.8	133.7	161.3
5,6	White Bacteria	172.0	---*	191.1	152.8	171.9

These results show that according to this method of determination the amount of sulfate produced in the inoculated cultures was about twice as great as that in the uninoculated. The only way that seems reasonable to account for the production of sulfate in the uninoculated cultures is the possibility of an incomplete sterilization. The fact that the amount of sulfate gradually increased in the checks would

* There was plainly some error in this determination, because the water blanks which were run along with the analyses of the different treatments showed more sulfate than the treatments themselves. The cause of this error could not be ascertained, and so no results can be reported for this determination.

indicate that the bacteria were increased in their activity. In the inoculated cultures the limit of sulfate production seemed to have been reached within the first two weeks.

No appreciable difference existed between the two forms of bacteria as to their ability to produce sulfate.

SUMMARY

Sulfate was produced from flowers of sulfur in sand cultures. The rapidity of sulfate production was increased when the cultures were heavily inoculated with either of the two kinds of bacteria described in the first part of this test.

GENERAL SUMMARY

From the results obtained in these experiments, the following general summary may be given:

Flowers of sulfur partly took the place of a soluble sulfate in a nutrient solution when used in a sand medium.

In a nutrient solution, flowers of sulfur had a marked effect upon the production of chlorophyll in corn plants.

When used alone flowers of sulfur was slightly beneficial to the yield of corn and rape, and still more beneficial to clover on the type of soil used in these experiments.

Gypsum had no beneficial effect upon the growth of corn, rape, or clover when used with lime. It had a beneficial effect when used with more complete fertilizers.

Lime had a depressing effect upon the vegetative growth on this soil during the progress of these experiments.

Fertilizers containing nitrogen, phosphorus and potassium gave a marked crop increase on this soil in every case.

Flowers of sulfur very markedly increased the production of nodules on the roots of red clover.

Neither sulfur nor gypsum showed any marked effect upon the formation of nodules on soybean roots.

Flowers of sulfur was oxidized to sulfate in both

sand and soil cultures.

Flowers of sulfur slightly increased soil acidity. The lime requirement was directly correlated with the amount of soluble sulfate.

The nitrate content varied inversely as the amount of soluble sulfate in the soil.

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PLATE I

FIELD EXPERIMENT.

February 28, 1915.

May 5, 1915.

Half of Plots Harvested.

PLATE II.

SAND CULTURES.

Forty-six Days After Planting.

At Maturity.

No Sulfur

Pfeffer's
Solution

Mod. Pfeffer's
Flowers of Sulfur

Mod. Pfeffer's
Gypsum

PLATE III.

WATERING DEVICE USED IN ALL
SOIL CULTURE POTS.

WATERING DEVICE IN JAR

PLATE IV.

MAIZE SERIES THIRTY-SEVEN DAYS

AFTER PLANTING.

Odd Numbered Pots, 1-15.

Even Numbered Pots, 2-16.

PLATE V.

MAIZE SERIES AT TIME OF
HARVESTING.

Odd Numbered Pots, 1-15.

Even Numbered Pots, 2-16.

PLATE VI.

CLOVER SERIES AT TIME
OF HARVESTING.

Odd Numbered Pots, 17-31.

Even Numbered Pots, 18-32.

PLATE VII.

RAPE SERIES AT ABOUT MAXI-
MUM GROWTH.

Odd Numbered Pots, 33-47.

Even Numbered Pots, 34-48.

PLATE VIII.

SOYBEAN SERIES - MAXIMUM GROWTH

Odd Numbered Pots, 49-61.

Even Numbered Pots, 50-62.

PLATE IX.

SOYBEAN SERIES - MATURE

Odd Numbered Pots, 49-61.

Even Numbered Pots, 50-62.

PLATE X.

APPARATUS FOR DETERMINING THE EFFECT
OF
BACTERIA UPON SULFATE FORMATION.

UNIVERSITY OF MISSOURI
COLUMBIA

DEPARTMENT OF BOTANY

5-26-1915.

Dean Walter Miller,
211 Academic Hall.

My dear Dean Miller:-

I herewith return the thesis of Mr.
Frank L. Duley with my approval.

Yours very truly,

GMR:H

A handwritten signature in cursive script, reading "George M. Reed". The signature is written in dark ink and is positioned to the right of the typed name "GMR:H".

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