ACTIVATION OF IRON IN PLANTS BY MANGANESE AND OTHER CHEMICALS IN A LIME-INDUCED CHLOROSIS

A THESIS

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Introduction

A great deal of attention has recently been given to the role of minor elements in soil fertility and plant nutrition. At present, investigation is being directed not only to the determination of the quantities of these elements present or required for crop production, and to the factors affecting their availability to the plant, but also to their utilization by animals which will ultimately feed upon these plants. Manganese is one of the more prominent of the elements belonging to this group, to which much consideration is being paid, both in nutritional and fertility studies. A lack of sufficient manganese is likely to cause a chlorotic condition in the plant. Manganese-deficiency symptoms vary greatly among different species of plants, due to the inherent characteristics of the plant. It appears very doubtful if there are any specific symptoms which will definitely characterize manganese deficiency, unless it be some of the malnutritional diseases such as "grey speck disease" in oats.

In Michigan, manganese deficiency occurs on certain neutral to alkaline organic soils* which alkalinity has

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* Since the definition of organic soils varies from region to region, it becomes necessary to define certain terms as they are used in Michigan. Organic soils of Michigan are divided into two groups, mucks and peats. They are differeniated on the basis of decomposition and not upon their mineral and organic matter composition. Muck is a well decomposed organic soil in which the original material from which it was derived cannot be easily recognized. Peat refers to the organic soil in which the original material is readily recognizeable.

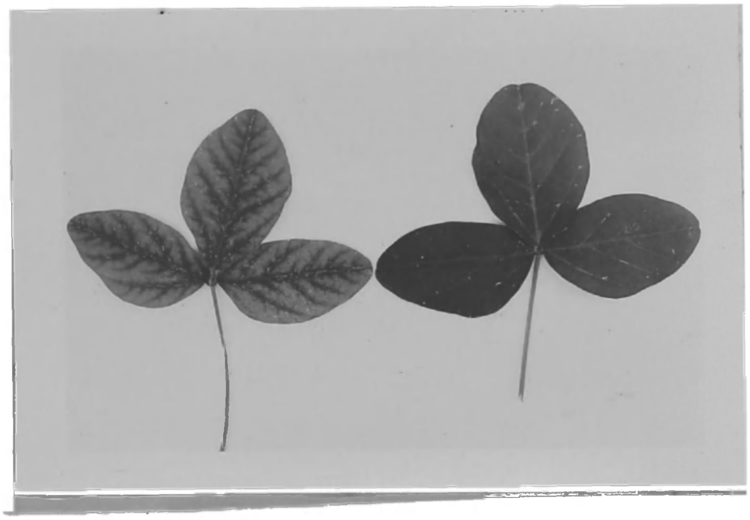
often been produced by burning. These alkaline organic soils are decidedly less productive than are the acid organic soils. The productiveness of these soils can be restored, either by the application of manganese salts or by restoration of the soil acidity by the application of sulphur. The distribution of such alkaline organic soils in Michigan is considerable and they may occupy the whole or only portions of the organic soil areas in practically any part of the state. These manganese-deficient soils present a real problem to the farmer, vegetable grower and the fertility specialist as to what is their best management. This study was made with the purpose of securing a better understanding of these problems, which information might lead to a more profitable and economical management of these areas.

Historical.

Manganese deficiency in organic soils of Michigan has produced a lime-induced chlorosis in many crops similar to that of the soybean leaves in Plate 1. It has also produced the characteristic "grey speck disease" in oats which is shown in Plate 2. This chlorosis has varied from crop to crop, so no definite description is possible which would cover all cases.

Chlorosis was first recognized by Gris (25) in 1844, when he caused yellow plants to become normal by the application of soluble iron to the soil. In 1895, Degrully (16)

Plate 1.



A. Soybeans leave exhibiting the lime-induced chlorosis.
B. A normal soybean leaves from a plant grown on a soil receiving manganese sulfate.

Plate 2.

Grey Speck Disease of Oats.

A. A leaf showing early stages of Grey Speck disease.
B. A normal leaf from a plant growing on soil receiving manganese sulfate.
C. Intermediate stage of Grey Speck.
D. Advanced stage of Grey Speck.

discovered that sulphuric acid, applied to the soil, would also cure this chlorotic condition.

The actual use of sulphur for this purpose was not made until 1911, and then by Bertrand (8). The first applications of manganese sulfate were made on rice plantations in Japan, in 1902, by Aso (4) and in 1903, by Nagoaka (56). The first application of manganese to organic soil probably was made in Sweden by von Feilitzen (75) in 1907. In 1909, Sjollema and Hudvig (68) found that manganese salts would cure "grey speck disease" in oats.

Many theories had been advanced by 1916, regarding the function of manganese, both as to its use as a fertility element and to the part it plays in plant growth. Bertrand was perhaps the first to claim that manganese is indispensable for plant growth which theory of its essentiality as a plant nutrient has been generally accepted. Gilbert (23) stated that it was essential since iron did not cure the chlorotic condition. Culture solutions have presented ample proof of its necessity as a plant nutrient. In recent years, some workers (13) have suggested that the benefit of manganese to the plant lies in its control of the intake of other ions or in its making possible the utilization of some of the other essential ions by the plant. Kelley (41) reported that the addition of manganese fertilizers resulted in a marked increase in the availability of calcium and magnesium. Sullivan and Reid (71) found that manganese additions increased the oxidative power of the soil and that it could be associated with the productivity of the soil.

Many workers held that the function of manganese was one of regulating the biological oxidation and reduction reactions within the plant. Bertrand (7) considered that manganese increased the oxygen-carrying power of oxidase enzymes. Hopkins (34) believed that the function of manganese was one of activation of iron, in that iron was in the reduced form in the organism and manganese served to reoxidize it. Willis (79) considers that there is a relationship between manganese and iron which theory is also supported by Scholz (65) who considers that a relationship existing between manganese, iron and calcium in plant nutrition.

"Grey speck" disease has been recognized in Europe, Australia and Canada for some time and is known in certain localities as dry speck, dry spot or halo blight. It attacks principally oats and barley but there are reports of similar disturbance in wheat and rye. Although known for some time, there is still doubt as to whether it is a bacterial disease or is due to an abnormal soil condition. For 30 years or more, the application of manganese sulfate has been recognized as a corrective of this condition. It occurs on soils that have a pH of 6.3 to 7.8, usually organic soils or sands high in organic matter. The majority of workers, including Davies and Jones (15) of Wales; Maschhaupt (51) of the Netherlands and Samuel and Piper (62) and Wild of Australia(77) are quite positive that grey speck is due to manganese deficiency in soils.

Gerretsen (19) is of the opinion that grey speck is caused by bacteria. He further states that the precipitation of insoluble manganic oxides in the soils seems to be caused by specific organisms which are active between the pH 6.5 and 7.8, which corresponds to the limits of grey speck in oats. This has been substantiated by Leeper and Swaby (44).

Because of the rather widespread distribution of manganese-deficient organic soils and the large acreage of organic soils in Michigan, it was considered that this problem warranted this detailed study regarding (1) the chemical nature of the soils before and after application of manganese salts and elemental sulphur; (2) the changes in chemical composition in the plants due to these treatments; and (3) the role played by manganese in plant nutrition.

Soil Factors.

The literature relating to soils which have exhibited manganese deficiency reveals many contradictory opinions as to the kinds of soil in which this problem is manifested. Greater still are the various opinions as to the nature and properties of manganese compounds in the soil. Manganese deficiency has been reported from many parts of Europe, Australia, Japan and the United States. Leeper (43), in his recent paper, states that this deficiency occurs on certain

Australian soils which have naturally a neutral or alkaline reaction or soils which originally had a strongly acid reaction but which have been made neutral or alkaline by the application of lime.

The question of availability of soil manganese is a very live one. Leeper (43) concludes that, in general, the total quantity of manganese in the soil has little bearing on the occurrence of the deficiency. This opinion naturally brings up the question of availability. Piper (59) believes that manganese is absorbed by the plants as the manganous ion and that, in the form of MnO_2, it is unavailable to plants. He holds further that, under decidedly alkaline conditions, manganese exists as the bivalent exchangeable form or in the form of MnO_2. The equilibrium which exists between these forms swings towards the bivalent form under conditions of reduction and towards MnO_2 if oxidation prevails. Steenbjerg (70) has a similar opinion and states that the available manganese is controlled by reaction and oxidation-reduction conditions. Mann (48) holds that lime represses the solubility of manganese to such an extent that it becomes unavailable. He worked with soils similar to those which Gilbert et al, (23) investigated, in that they were strongly acid and leached soils which had been limed. According to Leeper, manganese occurs as the bivalent form in strongly acid soils and in this form may be leached from the soil. He holds that liming a soil until it has an alkaline reaction might reasonably cause a manganese deficiency.

Not all of the work reported, however, has dealt with manganese deficiency. Considerable attention has been given to soils that have a highly soluble manganese content, which situation quite often causes a chlorotic condition or other metabolic disturbance in the plant. Johnson (38) and others (41) attribute the chlorosis in Hawaiian pineapple to a very high pyrolusite (MnO_2) content in the soil which, he holds, results in the oxidation of the iron in the soil to the ferric condition, a form very unavailable to plants. Bortner (10) cites a similar condition with tobacco in Kentucky; as did also Jacobson and Swanbeck (37) in Connecticut. The brown spotting disease of potatoes found in Russia is reported by Arkhangelskaya (3) not to be due to soil acidity but rather to a high content of soluble manganese in the soil.

In Michigan, according to Harmer (31) manganese deficiency appears on high-lime organic soils which have been burned; on those which are fed by alkaline spring water; on those which have a marl deposit near the surface and on those which originally were acid but have been made alkaline by the application of lime. It should not be construed that every alkaline or burned organic soil in Michigan is manganese deficient. Occasionally, decidedly alkaline organic soils have never given any crop response to the application of manganese salts. Results with these Michigan soils agree quite favorably with those from soils in other countries in that the reported deficiency generally occur at a pH above

6.7, although in Michigan, benefits have sometimes been noted down to 6.2. A strongly acid organic soil deficient in manganese has not been encountered, even though some acid soils show very little manganese upon total analysis.

Harmer, in his efforts to study this very interesting problem, laid out an experimental area in 1934, on the alkaline portion of the Michigan State College Muck Experimental Plots at East Lansing. On these plots a series of treatments were made with the following purposes in view: (1) to determine the effect of the application of manganese salts on crop growth on an alkaline organic soil. (2) to determine the comparative results secured by acidifying the soil with an application of sulphur flour, both with and without the added application of manganese. Twelve plots were laid out and the annual treatments applied as shown in Table 1.

During the period of this experiment, Harmer has used many different crops. The crops can be divided into two general groups, namely, those that are definitely responsive to manganese fertilizers and those that show little or no response. In the first group, such crops as barley, rye, oats, potatoes, alfalfa, onions, peas, radishes, spinach, leaf lettuce and many other crops gave very marked response to the application of manganese salts. If these salts were absent, a chlorotic condition developed in the plant. There were some plants, however, which did not develop this chlorosis in the absence of manganese, namely, sugar beets, peppermint, sweet clover, Swiss chard, strawberries and rhubarb. Either these plants have a greater power to extract manganese from the soil, or the nature of their physiological processes are such that the need of manganese is not acute. There were instances, however, when even these crops showed some improvement during growth due to these treatments. From Harmer's unpublished data (31), the yields of the several years indicate that the highest yields generally were obtained on the plots receiving manganese salts in combination with sulphur. The highest application of sulphur has at times given the highest yield, which usually was in the neighborhood of the yield secured with a combination of sulphur and manganese. Manganese salts alone did not give as high yields as did sulphur or the two in combination. In general, it may be stated that manganese applications gave yields which are from 50 to 85 per cent of those of the sulphur or the combination applications. The nature of the prevailing weather during the growing season of the several years of this experiment has influenced the response to these treatments. A cold wet season has resulted in the greatest response to manganese, with the control and copper sulfate treated plots producing a near crop failure. Under high temperature and near drouth conditions, the yields from the controls and copper sulfate treated plots may nearly equal those of the treated plots with certain crops and in the case of spinach may sometimes be even better.

Table 1, showing arrangement of plots of the Manganese-Sulphur Series of the College Muck Experimental Fields on which the bulk of the studies presented in this thesis were made. All applications were made broadcast in the spring of each year and prior to seeding of crops.

Plot	1934	1935	1936	1937	1938	1939	Total
1	Mn - 100	Mn - 100	Mn - 100	Mn - 100	Mn - 200	Mn - 200	Mn - 800
2	0	0	0	0	0	0	0
3	Mn - 100 S - 500	Mn - 100	Mn - 100 S - 500	Mn - 100	Mn - 200 S - 500	Mn - 200	Mn - 800 S -1500
4	Mn - 200	Mn - 200	Mn - 200	Mn - 200	Mn - 400	Mn - 400	Mn -1600
5	0	0	0	0	0	0	0
6	Mn - 200 S - 500	Mn - 200	Mn - 200 S - 500	Mn - 200	Mn - 400 S - 500	Mn - 400	Mn -1600 S -1500
7	S - 500	0	S - 500	0	S - 500	0	S -1500
8	0	0	0	0	0	0	0
9	S - 2000	0	S -1000	0	S - 500	0	S -3500
10	S - 1000	0	S -1000	0	S - 500	0	S -2500
11	0	0	0	0	0	0	0
12	Cu - 50	Cu - 50	Cu - 50	Cu - 50	Cu - 50	0	Cu - 250

In view of the numerous conceptions as to the part played by manganese in the soil, it appeared advisable to study the various soil factors affecting the yields. This study began with the total analysis of the soil followed by investigations of the various soil equilibriums, such as the salts extractable by successive leachings with water, and a determination of exchangeable bases. This in turn was followed by a study of the soil manganese in the various horizons of the soil, with special attention to the amounts extractable by various reagents. The results of these experiments and a consideration of work of Leeper (43) and Piper (59) led to a seasonal trend study to follow the transformation of the soil manganese from its manganous to manganic form.

Total Analysis.

The 12 plots of the manganese-sulphur series of the College Muck Experimental Plots were sampled in November of 1937. Each plot was sampled to a depth of 6 inches and at 8 sites. The samples from the 8 sites were thoroughly mixed to form a composite sample. Portions of these samples were dried at 100°C and a 10-gram sample taken from each for analysis. These were ashed in an electric furnace and the following constituents determined; insoluble ash, soluble ash, organic matter, R_2O_3 group, and ferric, calcium, magnesium and manganese oxides. Soluble ash included the portion of total ash soluble in aqua regia. The sesquioxides

(R_2O_3) were determined as the ammonium precipitate. Ferric iron was determined by the titration of the ferric ion in a thiocynate solution with a standard titanous chloride; calcium volumetrically as the oxalate by the standard titration with permanganate solution. Magnesium was determined as the pyrophosphate and manganese, colorimetrically as the permanganate by a method described by Williard and Greathouse (78).

The data (Table 2) show very little variation in determined constituents as a result of soil treatment, except in the case of Mn_3O_4 which was markedly increased when manganese sulfate was applied. The plots receiving manganese sulfate are higher in most constituents, which may be due to the higher mineral composition of the soil on these plots. There is a suggestion in the data that the application of sulphur has caused a loss of MgO from the soil.

Successive Extractions.

The total analysis was followed by studies for the purpose of securing an insight as to the various soil equilibriums which might exist. A series of successive extractions were made to determine the ability of the soil to give up various ions. The procedure was as follows; a 25-gram sample of soil was weighed out and transferred to a 500 ml. Erlenmeyer flask. 250 ml. of carbon dioxide-free distilled water was added and flasks were tightly stoppered. These were shaken at frequent intervals for 72 hours at which time the soil-water mixture was filtered through a Büchner funnel.

Table 2, showing total per cent of various constituents in soils from Manganese-Sulphur Series on the College Experimental Muck Plots at East Lansing.

Plot	Treatments to 1937	Insoluble ash	Soluble ash	Organic matter	CaO	MgO	R_2O_3	Fe_2O_3	Mn_3O_4
1	$MnSO_4$-400	19.21	15.63	65.16	6.63	2.26	4.82	1.04	.023
2	Control	17.03	14.68	68.29	5.94	1.96	4.37	.94	.016
3	S-1000 $MnSO_4$-400	14.85	12.11	73.04	5.32	1.32	3.50	.79	.035
4	$MnSO_4$-800	14.57	15.22	70.21	6.70	1.82	4.14	.87	.056
5	Control	15.22	14.75	70.03	6.58	1.82	3.99	.88	.010
6	S-1000 $MnSO_4$-800	13.72	14.38	71.90	6.16	1.58	3.90	.83	.059
7	S-1000	13.56	13.78	72.66	6.21	1.55	3.76	.80	.011
8	Control	11.90	13.54	74.56	6.18	1.42	3.53	.74	.009
9	S-3000	13.72	13.36	72.92	6.03	1.28	4.07	.97	.010
10	S-2000	15.26	15.41	69.33	6.67	1.28	4.59	1.00	.013
11	Control	14.95	14.59	70.46	6.37	1.50	4.03	.90	.010
12	$CuSO_4$-200	16.70	17.26	66.04	7.54	1.78	4.81	1.10	.013

Averages in Per Cent

Treatments	Insoluble ash	Soluble ash	Organic matter	CaO	MgO	R_2O_3	Fe_2O_3	Mn_3O_4
Controls	14.77	14.38	70.84	6.27	1.68	3.98	.87	.0112
$MnSO_4$	16.89	15.42	67.68	6.66	2.04	4.48	.96	.0395
S + Mn	14.28	13.24	72.46	5.74	1.45	3.70	.81	.0470
S	14.18	14.18	71.64	6.30	1.37	4.14	.92	.0113

The soil was washed with four 50 ml. portions of carbon dioxide-free distilled water and allowed to filter quite dry between each wash. The filtrate was transferred to a 500 ml. volumetric flask and made up to volume. The soil was returned to the original flask and allowed to stand 72 hours, at which time the whole procedure was repeated. In aliquots of the filtrade, CaO, MgO and SO_4 were determined by methods described previously. Attempts were made to determine manganese and iron, but in such a water extract they were in such small concentrations that accurate analysis was impossible with the technique employed. K_2O was determined gravimetrically as the chloroplatinate salt. The same soil samples were used for this study as were used in the total analysis.

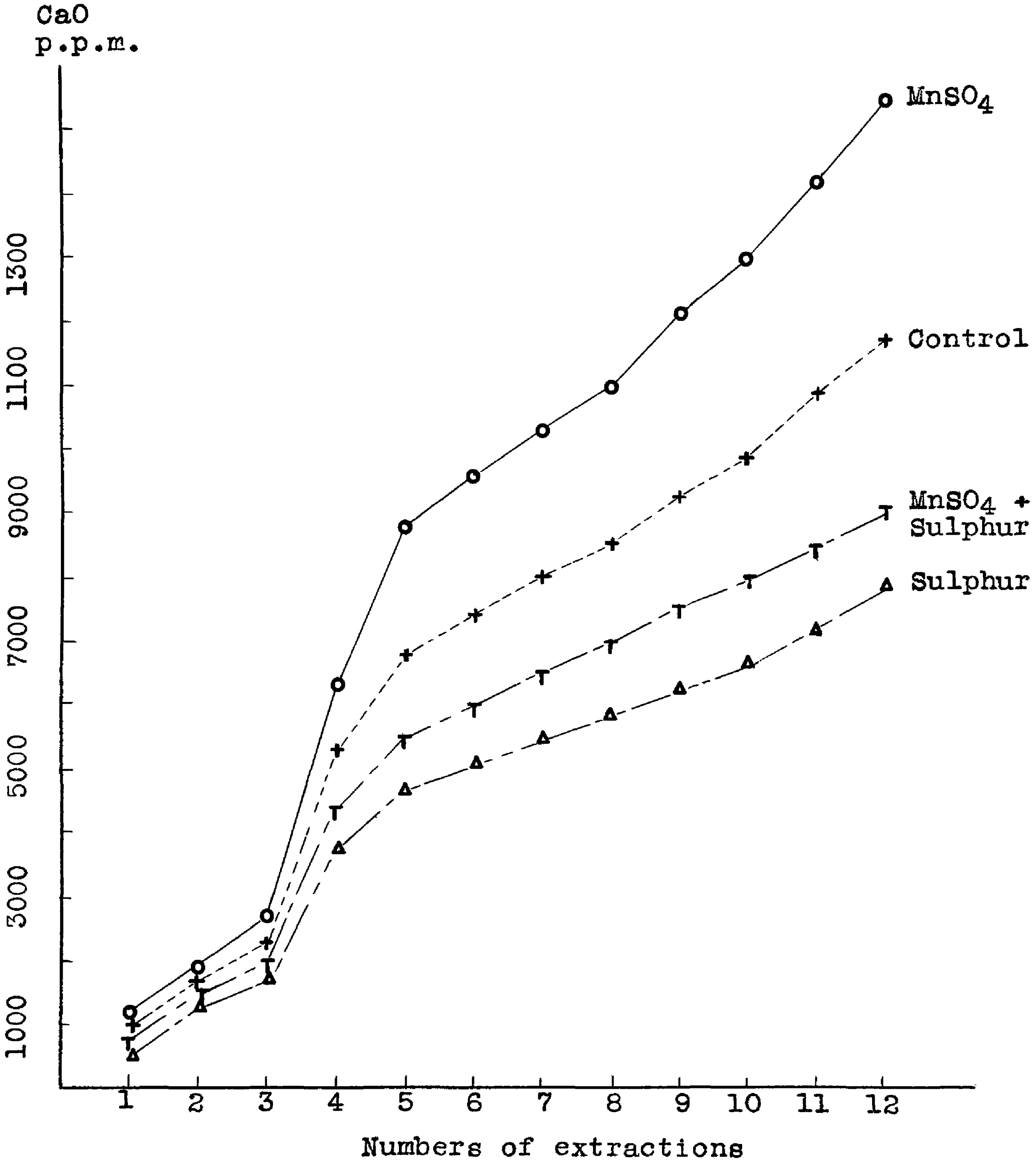
The most striking feature of these data is the behavior of calcium. Figure 1 shows that in the first three extractions, there appears to be a repression of the solubility of the calcium ion, followed by a tremendous release on the fourth extraction. A possible explanation lies in the common ion effect, as the fourth extraction shows the last measureable quantity of the sulfate ion. Since a soil is a very heterogensis substance, a definite explanation is quite difficult and such a repression of solubility might be the result of several factors. The calcium data show that the treatments have affected its solubility in that, although the total analysis shows practically no difference in CaO content of these samples, the sulphur as shown in the graph, has markedly decreased the solubility of calcium, even after the disappearance of water-soluble sulfate. This point becomes difficult to explain, since there is an apparent conversion of calcium to gypsum and the data show no difference between control plots and those treated with sulphur in the amount of sulfate ions. Leaching would explain it if there were some indication in the total CaO content of these plots that such leaching occurred. The data show that the water-soluble calcium content was lowered almost two-fifths by the application of sulphur. On the other hand, the manganese applications apparently increased the solubility of calcium.

Magnesium gave curves which were in general more uniform but which have some points of interest. A small jump in the totals extracted came in most cases on the fifth extraction. The application of sulphur tended to lower the soluble magnesium, with very little difference existing between control plots and those plots which received manganese sulfate.

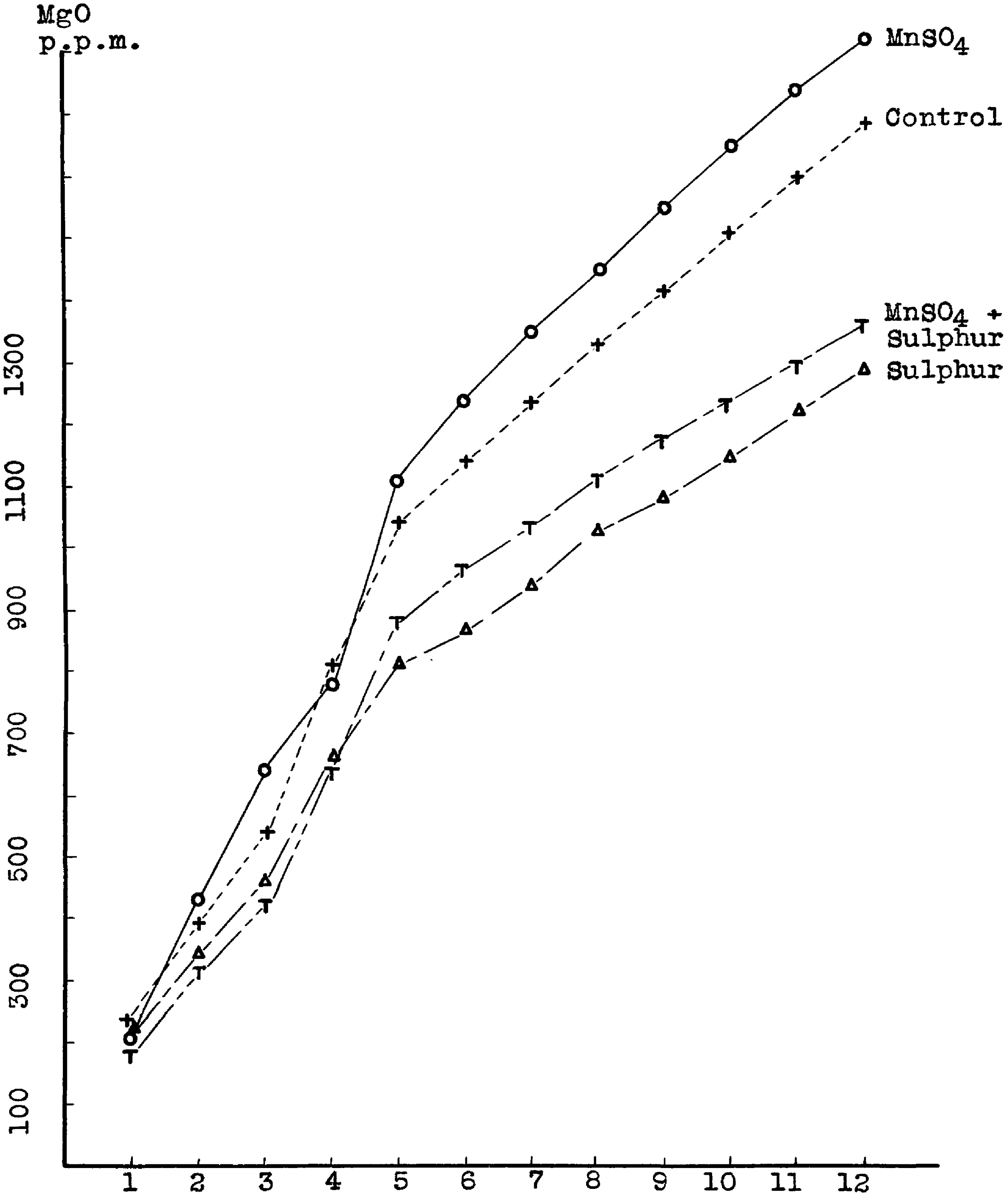
Figure 3, presenting the quantities of water-soluble K_2O shows that a large quantity was obtained in the first extraction and then it dropped to a low constant value. The plots receiving manganese gave the highest values and the sulphur applications the lowest value of K_2O.

The value of successive extractions can be shown better later in this work. If the effect of the various treatments has had a bearing on the solubility of any one element, that

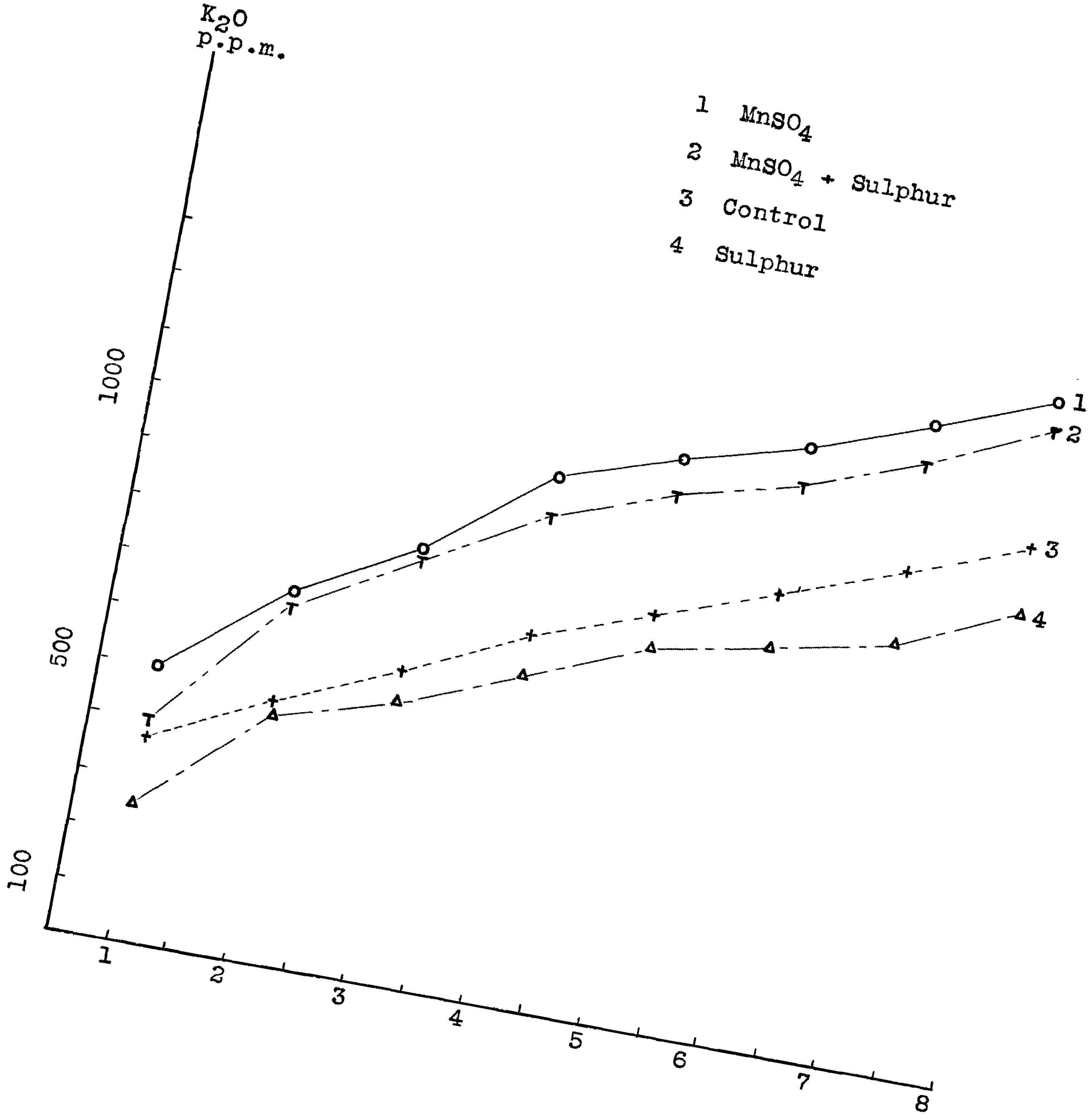
Graph 1, showing accumulated totals of CaO extracted by successive (1-10) soil-water leachings.



Graph 2, showing accumulated totals of MgO extracted by successive leachings.



Graph 3, showing accumulated totals of K_2O extracted by successive leachings.



element is calcium. This would strongly indicate that the soil equilibriums are dominated by calcium. Ligon (46) in his work on the effects of overliming has shown that, in periods when soils were recently drenched with rains, the quantity of calcium in solution was greatly increased. With a high content of calcium in solution the solubilities of the other ions are greatly influenced. The bases capable of taking part in base exchange reactions are repressed by calcium under such conditions. Lastly, it is not unreasonable to suspect that the nutrition of plants is greatly affected even to the extent that a physiological disturbance may be the result.

Exchangeable Bases.

Any study of soil equilibrium should include a study of exchangeable bases. It is to be realized that, in alkaline organic soils, the method of analysis employed will influence the values obtained. Because of the low calcium carbonate content of these soils, it was considered unnecessary to use an alcoholic solution of the replacing reagent, avoidance of which is desirable whenever possible. Although it was realized that the method has some deficiencies, the neutral normal ammonium acetate was chosen as the replacing agent. The method used was essentially the one proposed by Schollenberger and Dreibelbis (63). In aliquots of the extract, the following exchangeable bases, calcium, manganese, magnesium, iron and potassium, were determined by methods previously described. Exchangeable sodium was determined by

Table 3, showing exchange capacity and milliequivalents of exchangeable bases. Values are expressed as milliequivalents per 100 grams of soil.

Plot	Treatments applied in 1938 in lbs. per acre	pH	Exchange-capacity	Ca	Mg	K	Na	H	Mn
1	200 $MnSO_4$	7.5	118	100	15.6	.94	.15	1.18	.13
2	Control	7.8	123	105	15.5	.83	.31	1.29	.07
3	200 $MnSO_4$, 1500 Sulphur	7.4	128	110	14.0	.53	.18	3.07	.22
4	400 $MnSO_4$	7.8	138	121	15.1	.69	.17	.81	.23
5	Control	7.2	130	109	15.6	.56	.39	4.35	.10
6	400 $MnSO_4$, 1500 Sulphur	6.8	128	108	14.0	.41	.23	4.40	.96
7	1500 Sulphur	6.4	126	105	14.1	.34	.23	6.26	.07
8	Control	7.2	125	107	15.5	.47	.22	1.71	.10
9	Sulphur-3500	5.1	123	99	10.3	.92	.29	12.37	.12
10	2500 Sulphur	6.7	113	93	9.3	.41	.29	9.88	.12
11	Control	7.6	122	105	15.1	.56	.25	1.00	.09
12	50 Cu SO_4	7.6	123	105	15.1	.73	.32	1.77	.08

Averages

Controls	125	106	15.4	.60	.29	2.09	.09
$MnSO_4$	128	110	15.3	.81	.16	1.00	.18
$MnSO_4$ + Sulphur	128	109	14.0	.47	.20	3.73	.59
Sulphur	121	99	11.2	.56	.27	9.50	.10

the zinc uranyl acetate method proposed by Barber and Kolthoff (5) and adapted by Bray (11).

The data show very little difference in the base exchange capacity of soils from the various plots, with the exception of that from plot 10, which appears slightly lower. The average for all the samples shows that they have a base exchange capacity of slightly over 120 milliequivalents. It may be said that no essential difference between the capacity of the soil from various plots exists.

The outstanding feature of the exchangeable-base data is the high saturation of calcium and magnesium in the soil complex, which in most cases make up more than 100 milliequivalents of the exchangeable bases. The calcium ion accounts for more than 90 milliequivalents, which is not surprising when one considers the high quantity of soluble calcium obtained in the successive leaching data. The change of reaction, due to the application of sulphur, appears to have reduced both calcium and magnesium, the latter being reduced as much as a third. Their reduction is represented in an approximately equal gain in exchangeable hydrogen.

The exchangeable ions, sodium, potassium, manganese and iron, together, make up less than a milliequivalent of the exchange capacity. The application of manganese salts causes an increase in exchangeable manganese. Exchangeable iron occurred in such small quantities that it was impractical to measure it. The evidence points to the low affinity for exchangeable potassium or sodium ions in organic soils. This contradicts some of the work of Enfield and Conner (17) who attempted to show a fixation of potassium in organic soils. In these soils, the highly soluble calcium content may be a prime factor in the low values for these ions. The organic soils demand great quantities of these monovalent ions and, due to the great annual need for crop production, it is quite apparent that these ions are rapidly leached from the soil.

Extraction of Manganese from Soil.

To study the relationship of manganese to soil fertility, it is necessary to investigate its solubility in soils under various conditions. To accomplish this end, a series of extractions were designed to determine the quantities of manganese soluble in water, weak sulphuric acid, strong nitric acid, a saturated solution of carbon dioxide, and the replaceable and the easily reducible manganese in the soils of the various plots of the manganese sulphur series of the College Muck Experimental Field. The samples were collected December 6, 1938, from layers representing 0 - 5 inches, 5 - 10 inches and 10 - 16 inches. The samples were air dried and passed through a 4 mns. sieve.

Methods of Extraction.

Water soluble manganese. A 25-gram sample of air dry soil is taken and placed in a 500 ml. Erlenmeyer flask; 250 ml. of carbon dioxide-free water is added and the flask stoppered tightly to prevent entrance of air. The flask is shaken at intervals and after 48 hours the contents filtered through a Büchner funnel. The filtrate is evaporated to a small volume and it is then transferred to crucibles and evaporated to dryness. The soil is returned to the Erlenmeyer flask to use in further extractions. After the filtrate has reached dryness, the crucibles are placed in an electric furnace and are ignited over night, after which time they are removed from furnace, allowed to cool; aqua regia is added and is evaporated to dryness on a steam bath. The residue is then taken up with sulphuric acid (1-1) and evaporated to dryness to volatilize the chlorides. The residue is taken up with a nitric acid (1-4), filtered and 20 ml. of concentrated nitric acid is added with approximately 0.3 gram of potassium periodate and the solution boiled until color of the permanganate developes. The solution is then cooled and is compared in a colorimeter with previously prepared standards. The manganese is reported in parts per million.

Exchangeable Manganese. Exchangeable manganese is the manganese which can be replaced in the soil complex by cation exchange. The exchangeable medium used is a normal neutral ammonium acetate solution adjusted to pH 7.0.

To the soil sample used in determining water-soluble manganese, which was returned to the original flask, is added 250 ml. of neutral normal ammonium acetate. The flask is tightly stoppered and then shaken at frequent intervals.

At the end of 24 hours, it is assumed that equilibrium has been attained. The mixture is filtered through a Büchner funnel, the soil washed with portions of ammonium acetate solution and the soil again returned to the original flask. The filtrate is treated exactly as in the determination of water-soluble manganese.

Easily Reducible Manganese Dioxide. Easily reducible manganese is the quantity of manganese dioxide that can be reduced by a 0.2 per cent solution of hydroquinone in a buffered solution of neutral, normal ammonium acetate after the water-soluble and replaceable manganese have been extracted. The theory of this extraction was proposed by Leeper (43), in 1935, when, in his work on calcareous soils of Australia, he found he could pick out the manganese deficient soils by this method. His contention is that this represents the quantity of manganese dioxide that the plant roots can reduce and which becomes available for their nutritional processes.

To the soil in the flask from which both the water-soluble and replaceable manganese has been extracted, is added 250 ml. of normal ammonium acetate solution containing 0.2 per cent of hydroquinone and buffered to the pH 7.0. The flask is tightly stoppered and shaken at frequent intervals. At the end of 24 hours, the content is filtered through a Büchner funnel, and the filtrate is treated in the same manner as the filtrate in the water-soluble manganese determination.

Total Manganese in Organic Soils. The method here is applicable only to organic soils and really expresses the quantity of manganese that is soluble in aqua regia. A 5-gram sample is weighed into a crucible. It is then ignited in an electric furnace at red heat. After complete ignition, the crucible is cooled and then placed on a steam bath and digested with aqua regia. The residue is taken up with sulphuric acid (1-1) and chlorides are expelled by heating on a hot plate. If much calcium is present, it is better to filter before expelling the chlorides. After the removal of the chlorides, 20 ml. of nitric acid (1-4) is added and the solution digested a short time. If a residue or any cloudiness is present, cool and filter. To the filtrate is added 20 ml. concentrated nitric acid and 0.3 gram of potassium periodate and the solution boiled until color of the permanganate appears. After cooling, the color comparison is made with previously prepared standards.

Discussion of Experimental Results

Very few tests have been proposed for the determination of the manganese available for plant growth in organic soils. Muckenhirn (55) proposed the use of .005 N sulphuric acid for such a determination while Steenbjerg (69) used a normal solution of magnesium nitrate as a means of measuring the exchangeable manganese, since he considered the magnesium ion a better replacing agent. The so-called quick-test methods are of little value in determining available manganese in organic soils, as the organic matter in the extracted solution prevents the development of the required permanganate color.

Leeper (43) proposed the use of 0.2 quinol solution buffered with neutral normal ammonium acetate, which, according to his theory would extract the easily reducible manganese dioxide by reducing it to the bivalent form. All of these methods measure three forms of soil manganese, namely:

(1) The portion soluble in weak acids.
(2) The portion taking part in the cation exchange.
(3) The portion in the form of easily reducible manganese dioxide.

The various extractions listed above were made on the soils being studied and it was found, as shown in Table 4, that the amounts of water-soluble manganese, of the manganese soluble in .005 N. sulphuric acid and of that soluble in a saturated carbon dioxide solution, were so small as to be of no value. The data obtained, when five per cent nitric acid was used, are interesting in that they show a marked increase in soluble manganese with the application of manganese sulfate. The plots receiving sulphur show, in general, slightly less nitric acid-soluble manganese than do soils from the controls. This difference becomes more marked in the 5-10 inch layer, while in the 10-16 inch layer the manganese is almost half that of the control. The manganese soluble in .05 N. sulphuric acid is about one-half that of the nitric acid-soluble and bears approximately the same relationships as the nitric-acid soluble does in respect to treatments.

Many investigators ascribe a low exchangeable manganese supply as the cause of insufficient manganese for plant growth.. Steenbjerg (70) states that it is not only the amount of exchangeable manganese that is important but also the tenacity

Table 4, showing amount of manganese extracted by various reagents from different soil depths and varying soil treatments, together with the soil pH and the Mn_3O_4 content of these legume crops produced on the plots.

			Mn Extracted by Various Reagents in p.p.m.									% Mn_3O_4 Content of Crops		
Plot	Depth	Treatment	Total aqua regia	H_2O Sol-uble	.005 N H_2SO_4	5N H_2SO_4	Ex-change-able	Easily reduc-ible	Sat. with CO_2	5% HNO_3	pH of soil	Alfal-fa	Al-sike clover	Sweet clover
1	0-5	200 $MnSO_4$	334	0.2	0.3	128	18	27	—	281	7.5			
	5-10	"	182	tr	0.1	94	11	14	0.3	166	7.8	.0025	.0031	.0014
	10-16	"	83	—	tr	14	3	2	—	33	6.0			
2	0-5	Control	138	0.1	0.2	59	11	10	—	110	7.8			
	5-10	"	110	tr	0.1	50	9	8	0.1	96	8.0	.0014	.0017	.0011
	10-16	"	80	—	tr	20	7	6	—	40	7.1			
3	0-5	200# $MnSO_4$	420	0.2	0.2	190	17	61	—	290	7.4			
		+ 1500# S	146	tr	0.1	67	16	16	0.2	144	7.3	.0034	.0044	.0017
		"	48	—	tr	16	4	3	—	36	6.8			
4	0-5	400# $MnSO_4$	808	0.6	1.9	320	22	111	—	640	7.8			
	5-10	"	286	tr	0.6	132	12	25	0.3	286	7.7	.0034	.0045	.0018
	10-16	"	22	—	tr	8	3	1	—	16	7.2			
5	0-5	Control	152	0.1	0.3	59	9	8	—	111	7.2			
	5-10	"	84	tr	0.1	33	7	7	0.2	81	7.2	.0027	.0022	.0018
	10-16	"	18	—	tr	10	3	2	—	18	6.6			
6	0-5	400# $MnSO_4$+	776	0.7	1.6	340	44	99	—	699	6.8			
	5-10	1500# S	251	tr	0.8	114	23	20	0.5	250	7.0	.0069	.0090	.0032
	10 -16	"	12	—	tr	4	3	2	—	7	6.2			
7	0-5	1500# S	93	0.1	0.2	32	8	8	—	66	6.4			
	5-10	"	48	tr	0.1	22	7	5	0.1	42	6.8	.0024	.0025	.0011
	10-16	"	14	—	tr	4	4	2	—	11	6.3			
8	0-5	Control	98	0.1	0.3	32	7	7	—	70	7.2			
	5-10	"	73	tr	0.1	31	6	6	0.1	66	7.3	.0020	.0028	.0011
	10-16	"	10	—	tr	5	3	2	—	10	6.8			
9	0-5	3500# S	128	0.1	0.5	42	9	15	—	87	5.1			
	5-10	"	84	tr	0.2	35	9	6	0.2	63	6.7	.0017	.0020	.0011
	10-16	"	13	—	tr	2	2	2	—	4	6.7			
10	0-5	2500# S	100	0.1	0.3	39	9	11	—	80	6.7			
	5-10	"	76	tr	0.1	34	8	5	0.1	66	7.0	.0017	.0022	.0011
	10-16	"	23	—	tr	4	5	4	—	9	6.4			
11	0-5	Control	118	0.1	0.4	41	6	10	—	80	7.6			
	5-10	"	80	tr	0.1	34	9	7	0.1	71	7.4	.0018	.0022	.0012
	10-16	"	57	—	tr	9	3	7	—	19	6.4			

of retention of the exchangeable manganese by the soil colloids. He states further that the exchangeable manganese is decreased by liming to a high pH; by excessive aeration and by deficiency of moisture and is increased by chemical reducing agents as readily decomposable organic matter; starch and waterlogging. He holds that heavy soils have a high exchangeable manganese content and a low retention of the manganese by the soil colloids, which is perhaps the reason why "grey speck" disease in cereals never occurs on this type of soil. In a later paper, he states that, on soils having a total exchangeable manganese content of 0.5 milliequivalents per 27.5 kg. of air dry soil, "grey speck" disease would occur but that, if there were 2.0 milliequivalents, there would be no signs of the disease.

A recent paper by Ruether and Dickey (61), working in Florida on mineral soils, states that two pounds per acre of exchangeable manganese was found insufficient for normal growth of tung trees, that 2.3 to 3.4 pounds per acre gave normal trees and that an excess would be toxic. This does not hold true for the manganese-deficient soil on the College muck plots, since a very conservative figure shows that the soil of the control plots contain at least three to four pounds of exchangeable manganese per acre in the surface five inches.

The determination of exchangeable manganese shows that, where manganese sulfate has been added, the exchangeable manganese is increased, while a sulphur application tends to decrease it in respect to the total manganese of the soil and, in actual quantities, it is only slightly higher than the exchangeable manganese in the controls. The data of the 10-16 inch layer are not entirely reliable, inasmuch as the underlying clay enters this layer section in plots 1, 2, 3, 11 and 12.

Leeper (43) in his work in Australia, found that a low content of easily reducible manganese dioxide gave rise to the so-called manganese deficient soils. The soils that he worked on were very calcareous and he considers that the water-soluble and exchangeable manganese in these soils are so low that they would be of little value to the plant. His theory is that the plants can reduce colloidal manganese dioxide, a reasonable assumption, since other workers (36) have reported benefits from the application of manganese dioxide. He suggested the use of a 0.2 per cent solution of quinol in a buffered solution of neutral normal ammonium acetate for the extraction of reducible manganese. This extraction is made after the exchangeable manganese has been removed. He found that soils having 100 parts per million or more of manganese grew healthy plants; those having 15 or less had a very severe chlorosis. Leeper apparently holds that the range between these figures is the range of recovery. It is also interesting to note that he states the best yields were not obtained on those plots which received manganese but on those which received sulphur which correlates with conditions that occur on the plots under consideration in this work.

The results of the extractions using 0.2 per cent hydroquinone in neutral ammonium acetate are very interesting. Those plots receiving 200 pounds of manganese sulfate per acre showed 27 and 61 parts per million of manganese by this test, while those receiving the 400-pound application gave 111 and 99 parts per million. The controls yielded only 9 and the sulphur treated plots 12 parts per million. In the 5 - 10 inch layer, the plots receiving manganese sulfate still showed more than 15 parts per million while the controls and sulphur contained 7 and 6, respectively. The two mineral soils from Minnesota, high in manganese, gave 98 and 492 parts per million, the latter being the soil high in pyrolusite. It is very likely from these results that this reagent can be used to measure the active manganese dioxide content of the soil.

Considering Steenbjerg's (70) work again, the difference in retention of manganese by the soil colloid may be the very factor as given here. In Table 5, it will be noticed that a Minnesota soil high in pyrolusite, when extracted with the 0.2 per cent hydroquinone reagent, yielded a quantity of manganese approximating that soluble in the five per cent nitric acid. It is apparent, then, that the pyrolusite content of a soil might be a factor in the quantity of manganese available to plants.

Table 5, showing the analysis of two mineral soils from the Red River Valley in Minnesota.

Soil Type	Depth of sample	.005N H_2SO_4	.05N H_2SO_4	Exchangeable	Easily reducible	5% Nitric acid
ⓧ 1. Semi-hydromorphic associate of Barnes	0-6	2	148	50	98	625
2. Dolomite Sand (Averill Area, Clay Co.)	22-38	---	---	32	492	522

ⓧ 1. Semi-hydromorphic associate of Barnes Series. Very calcareous containing 44% $CaCO_3$, also some $CaSO_4$. The pH of this soil is 7.89. Plants growing on this soil do not respond to applications of manganese salts up to 1250 pounds per acre and above this figure yields are depressed. Copper Sulfate and Gypsum also depress yields. The application of ferrous sulfate has shown beneficial results with such crops as red clover and spinach.

2. A glacial lake sand in which the carbonates have been altered to dolomite by the action of ground water rich in magnesium. This sample is taken from the C horizon of the Ulen Series 3 miles southeast of Averill, Clay County, Minnesota. This subsoil has from 25 to 30 per cent carbonates of which 90 to 95 per cent is in the form of dolomite. The pH of this soil ranges from 7.4 to 7.6. This subsoil has many pyrolusite concretions and upon heavy mineral separation, some carbonates in the form of ankerite are found with the heavy minerals. Ankerite is the double carbonate dolomite in which either the calcium or magnesium ion has been replaced by either iron or manganese. No minor

element studies have been conducted on this soil but, from the analysis, it undoubtedly has sufficient manganese to meet the requirement of plants under these conditions.

Table 6, showing the Mn extracted from various alkaline mucks by the different reagents.

Location of muck	Reaction	Total aqua regia	5% nitric acid	Water soluble	Exchange-able	Easily reduci-ble
Boysen	Alkaline	1070	715	0.6	33	53
Calhoun	"		---	trace	34	40
Richmond	"		500	trace	69	22
Hubbard	"	3234	1667	0.6	80	302
Wyn	"	150	111	trace	9	trace
Born	"		217	0.4	35	16
Rich	Very acid		6	trace	none	none

Table 6, showing the analysis of various mucks from different parts of Michigan, is very interesting in respect to these various extractants. In field and greenhouse experiments in past years, Harmer found that all of these alkaline mucks, except the Hubbard, responded to the application of manganese salts. The Hubbard and Boysen mucks are both very high in iron content, the chief difference between them other than the above analyses being that the Boysen muck was very high and the Hubbard very low in carbonates.

The Hubbard muck has three times the manganese content of the Boysen, yet they show little differences in water-soluble manganese. The Hubbard has 80 parts per million of exchangeable manganese against 33 for the Boysen and the difference in the reducible is still greater, 302 against 53. The data support both theories that the exchangeable or reducible manganese is the important factor but, when one takes into account the results of Richmond's muck which proved very responsive in Harmer's trials to both manganese or sulphur applications; this muck has almost as high an exchangeable manganese content as the Hubbard muck, but has a lower reducible manganese content than Boysen. This fact gives a great deal of weight to the reducible manganese dioxide theory but it appears to be only applicable to alkaline conditions. Rich's muck, which is very acid shows no exchangeable or reducible manganese. Coupling this with Harmer's field experiments, it appears definite that the plants demand for manganese is much less on acid organic soils than on alkaline soils. This is in agreement with trials on mineral soils and with Martin's work (50) on sugar cane with nutrient solutions which showed that manganese was beneficial at pH of 6.0 or higher.

Table 4, which presented the various quantities extracted by various reagents, showed a much higher concentration of manganese near the surface. This may be indicative of a migration of manganese in the soil, or it may be due to a negative enrichment. The work of McCool (53) will lend some support to the former theory that, during reducing conditions, the bivalent manganese is set free and during drouthy conditions may migrate to the surface, where it is oxidized into colloidal pyrolusite. If such a process takes place the surface soil will show a higher manganese content in the colloidal fraction. This theory is supported by the following table from the work of Alexander, Byers and Edgington (1).

Table 7, showing total MnO in soils derived from limestone.*

Per cent MnO

Soil Hori- zon	1 Hagerstown silty clay loam	2 Hagerstown silt loam	3 Fred- erick silt loam	4 Maury silt loam	5 Dewey silt loam	6 Decatur clay loam	7 Greenville sandy loam	8 Fullerton gravelly loam	9 Hagerstown silt loam	10 Lebanon silt loam
A_1	.82	.30	.03	.39	.32	---	.12	---	.26	---
A_2	.54	.12	.02	.39	.35	.21	.12	.04	.27	.09
B_1	.20		.02	.35	.17	---	.05	.02	.17	.05
B_2	.10	.04	.02	.37	.15	.12	.04	.02	.20	.03
B_3				.18	.39			.02	---	.03
C	.02	.04	.03	.20	.49	.15	.05	.03	.20	.02

MnO content in per cent of colloids extracted from above limestone soils.

Hori- zon	Hagerstown silty clay loam	Hagerstown silt loam	Fred- erick silt loam	Maury silt loam	Dewey silt loam	Decatur clay loam	Greenville sandy loam	Fullerton gravelly loam	Hagerstown silt loam	Lebanon silt loam
A_1	1.02	.52	.07	.87	.48	---	.39	---	.38	---
A_2	.73	.17	.08	.92	.34	.12	.38	.08	.46	.13
B_1	.31	---	.05	.56	.15	---	.12	.02	.18	.06
B_2	.13	.05	.04	.57	.14	.07	.08	.01	.16	.06
B_3	---	---	---	.13	.07	---	---	.01	---	.05
C	.09	.06	.04	.11	.09	.06	.06	.01	.15	.04

* Data taken from U.S.D.A. Tech. Bul. 678, by Alexander, Byers and Edgington.

Soils.

1. Hagerstown silty clay loam, State College, Pennsylvania.
2. Hagerstown silt loam, Hagerstown, Maryland.
3. Frederick silt loam, Fairfield, Virginia.
4. Maury silt loam, Ashwood, Tennessee.
5. Dewey silt loam, Russellville, Alabama.
6. Decatur clay loam, Russellville, Alabama.
7. Greenville sandy loam, Pretoria, Georgia.
8. Fullerton gravelly loam, Lawrence, Alabama.
9. Hagerstown silt loam, Hazel Run, Missouri.
10. Lebanon silt loam, St. James, Missouri.

Seasonal Trend of Replaceable, Total Manganese and of Reducible Manganese Dioxide

The apparent enrichment in manganese of the upper part of the soil profile leads one to suspect that a change in the chemical state of manganese is taking place in the soil. The question naturally arises as to what becomes of the manganese sulfate after its application. What climatic factors appear to play a part in the chemical changes of the manganese? Of special interest would be the effect upon the equilibrium between the exchangeable manganese and the active manganic oxides. This is of considerable importance, since there are two schools of thought as to which of these two forms of manganese are most important to plant growth.

Beginning with May 9, 1939, samples of muck were collected every fifteen days until September 22. The area sampled was that portion of the plots seeded to alfalfa in the spring of 1938. The samples were rapidly air dried so as to keep chemical changes after sampling to a minimum.

The application of manganese sulfate to plots 4 and 6, was made May 1, 1939. The treatments of these plots including the 1939 application are as follows:

Plot	1938 Treatment	1939 Treatment	1934-1939 Treatment
4	$MnSO_4$-400	$MnSO_4$-400	$MnSO_4$-1600
6	$MnSO_4$-400 Sulphur-500	$MnSO_4$-400	$MnSO_4$-1600 Sulphur-1500
8	Control	Control	Control
9	Sulphur-500	No treatment	Sulphur-3500

The samples were analyzed for total manganese, exchangeable manganese and reducible MnO_2 by methods described previously in this paper.

Figure 4 shows the results obtained from such a study in respect to total manganese. The uniformity of the total manganese curve suggests that manganese is fixed rather firmly in the soil. This supports the results shown in the horizon study in which there was very little evidence of enrichment of the lower horizons due to manganese sulfate applications to the surface. The decided uniformity of the curve of the total manganese on the control plot and on the plot receiving sulphur is in general what one would expect. Any enrichment due to migration from a lower layer would have very little effect upon total content, due to the small amount present.

The curves representing the seasonal changes in exchangeable manganese are of an entirely different nature. In general, as the season progresses, the exchangeable manganese becomes less and less. It would appear that the tendency during the season is for the manganese to be oxidized apparently to the manganic forms. The treatments indicate that the

Table 8, showing the seasonal trend of the manganese existing as reducible MnO_2 and exchangeable cation and the total manganese in the soil at each sampling date during the season of 1939. Manganese sulfate applied approximately a week before first sampling.

Form of Mn	May 9	May 25	June 9	June 24	July 9	July 24	Aug8	Aug23	Sept. 7	Sept. 22
Plot 4. 400# $MnSO_4$ -- 1939.										
Total	1315	1250	1311	1250	1315	1298	1276	1300	1335	1258
Exchangeable	133	194	110	105	133	51	41	21	33	8
Reducible MnO_2	120	90	120	143	178	134	132	160	178	155
Plot 6. 400# $MnSO_4$ -- 1939 and 1500# Sulphur -- 1934-1939.										
Total	1110	1304	1177	1212	1135	1150	1218	1135	1216	1160
Exchangeable	154	167	143	125	143	87	44	32	33	15
Reducible MnO_2	90	120	120	133	166	122	113	134	145	146
Plot 8. Control										
Total	61	68	66	71	66	69	80	69	65	62
Exchangeable	6	5	6	4	4	3	3	3	1	0.6
Reducible MnO_2	3	5	5	4.5	2	6	8	8	4	5
Plot 9. 3500# Sulphur -- 1934-1939										
Total	56	67	64	66	54	49	55	56	50	62
Exchangeable	8	7	8	6	6	4	4	3	2	0.6
Reducible MnO_2	4	5	5	5	2	7	8	8.5	5	5

Figure 4, showing seasonal trend of total manganese in 1939 of certain plots of Mn-S Series.

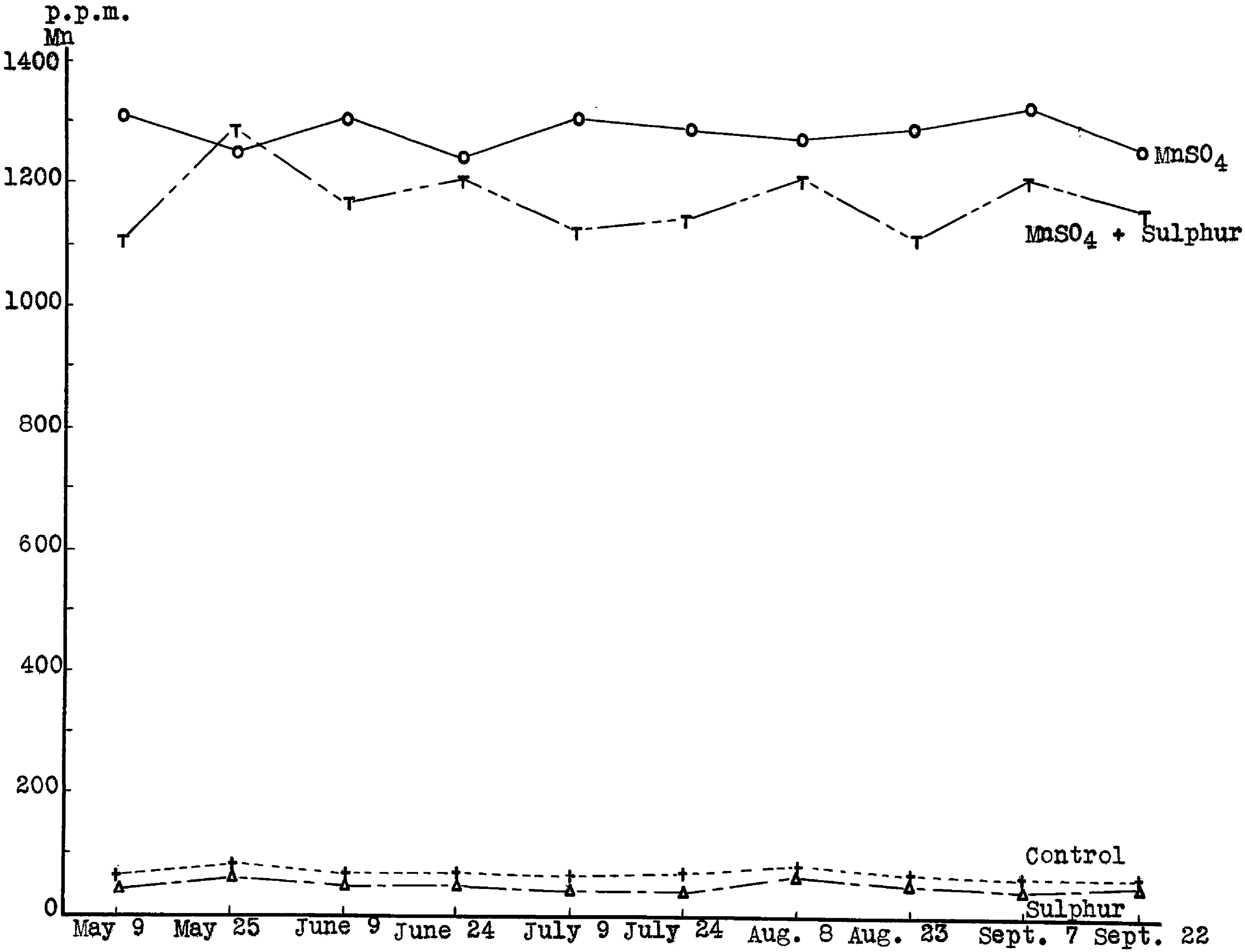


Figure 5, showing seasonal trend of exchangeable manganese in 1939 of certain plots of Mn-S Series.

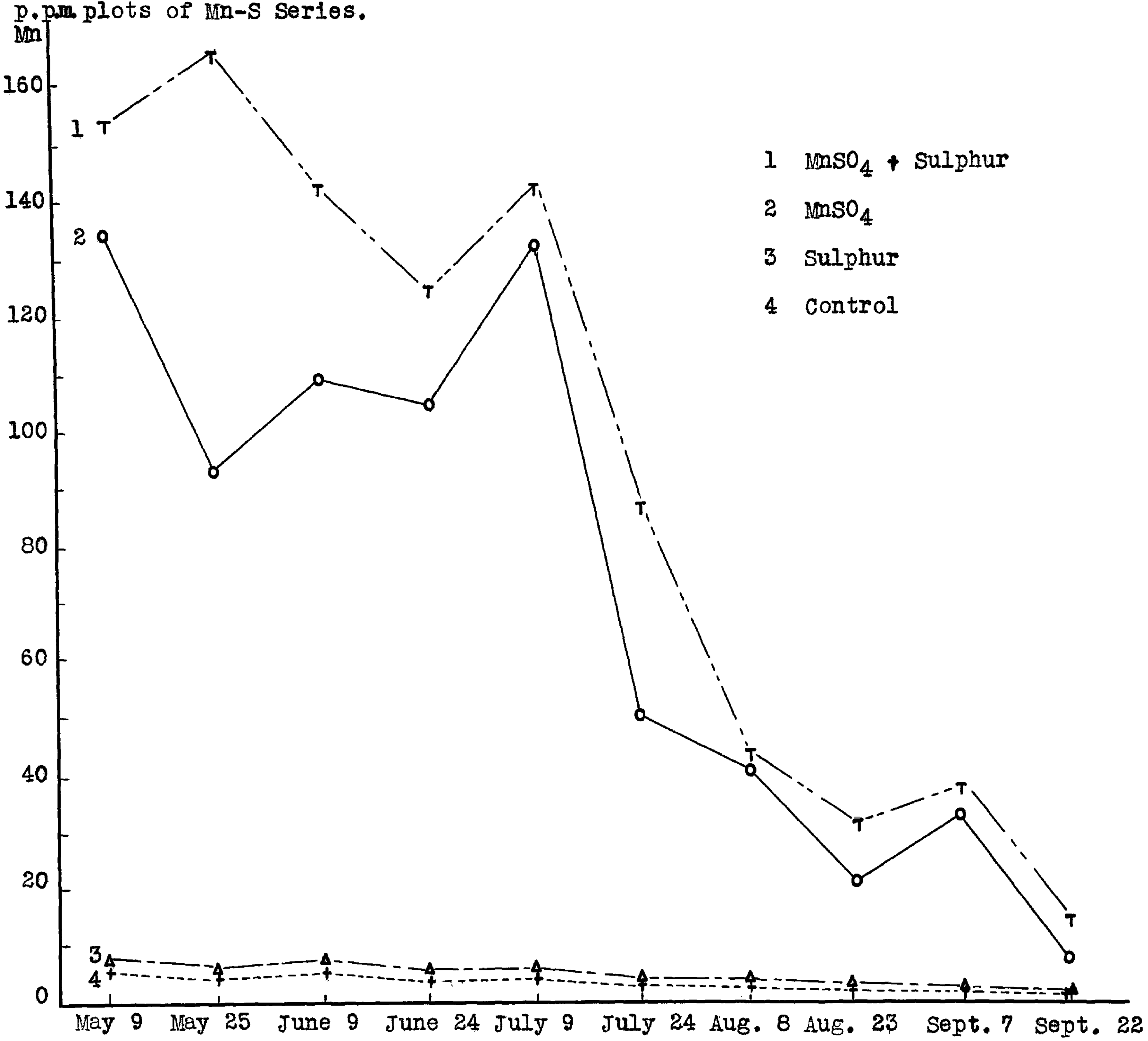
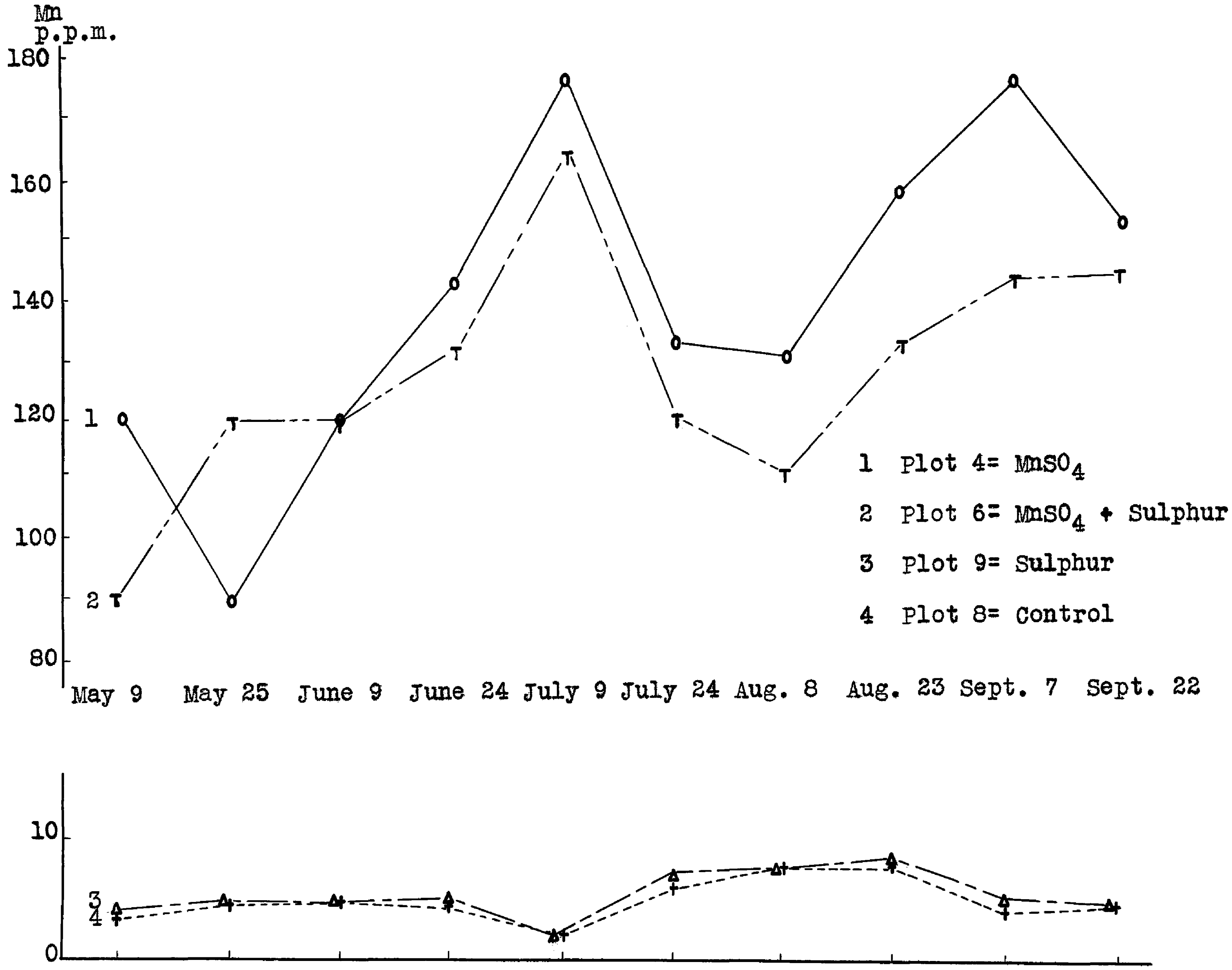


Figure 6, showing seasonal trend of reducible MnO_2 during 1939 of certain plots of Mn-S Series.



application of sulphur tends slightly to increase the exchangeable manganese or rather it tends to hold it at a slightly higher level. During the season of increasing soil temperatures and lowering soil moisture content, the exchangeable manganese falls from 6 parts per million to 0.6 part per million. It is reasonable to assume that, during the winter season, the reverse action will take place and that the level of exchangeable manganese on the controls from one spring to the spring of any following year will be reasonably constant.

There is a gain during the summer in the amount of active MnO_2, as measured by Leeper's method. In other words, the conclusion can be drawn that, as the exchangeable manganese decreases, the active MnO_2 increases. It seems probable, then, that during the winter part of the season the reverse of this reaction is possible. It appears that the manganese sulfate application gives the highest level in these treatments and that sulphur treatments give values which are practically comparable with the control. This contradicts in a measure Leeper's theory since, in this case, the crop yields do not correlate with his test. At this point it might be well to consider Leeper's theory more seriously. Fundamentally, he has divided the soil manganese into several groups and, in a broad sense, they are exchangeable and manganic compounds. He states (page 24): (43)

> "It appears that manganic oxides may form a continuous series, from the most active to the most inert; for convenience one may separate these oxides into four classes -

(a) extremely active, capable of oxidizing quinol at pH 7 and of oxidizing the organic matter of the soil rapidly in sulphuric acid suspension (pH 1.5 to 2.0);

(b) also very active, capable of oxidizing quinol at pH 7, but only slowly attacking organic matter at pH 1.5 to 2.0;

(c) moderately active, capable of oxidizing quinol at about pH 2 or $Na_2\ S_2\ O_4$ at pH 7;

(d) inert (including all the remaining manganic compounds)."......."analogous to the most resistant residues of pyrolusite, which dissolve in oxalic and sulphuric acids only after prolonged boiling."

He believes that the various categories of manganese are in a dynamic equilibrium as he expresses it:

"The dynamic equilibrium, ... Exchangeable Mn $\rightleftarrows$ unavailable MnO_2 of former workers, however, may now be replaced by some such series as Exchangeable Mn $\rightleftarrows$ MnO_2 (a) $\rightleftarrows$ MnO_2 (b) $\rightleftarrows$ MnO_2 (c) $\rightleftarrows$ inert MnO_2 (d)"

This equilibrium will be shifted from right to left or vice versa by the conditions existing in the soil. Piper (59) has shown that water logging will shift it from right to left and, in the seasonal trend of soil manganese presented here, we have shown that, under increasingly drouthy conditions, the equilibrium is shifted in the reverse direction. The relationship which exists between exchangeable and active MnO_2 certainly substantiates this theory of dynamic equilibrium in the soil. The rapidity of the oxidation and the lack of leaching shows that the direction of this equilibrium is strongly in the direction of the inert MnO_2. This perhaps explains the low values received when this soil was extracted with water or weak acids.

It is interesting to note that, if one were to compare the total manganese in acid organic soils and alkaline organic soils in Michigan, one would at once be struck by the great difference in the two types of soils. Harmer's unpublished data (31) show that a majority of the acid mucks of Michigan have a total manganese content which is very small, in most cases, much lower than Leeper's critical values of active MnO_2 and even lower than some of the critical values given for exchangeable manganese. A great deal has been attributed to the soil reaction, since, at a low pH, the organic matter will reduce MnO_2 easily, with the result that an increase occurs in bivalent Mn which is subject to leaching. Leeper (43) states in mineral soils,

> "since reserves of "active MnO_2" fall off rapidly with a fall in pH, one might forecast that, other things being equal, it is more dangerous to lime a highly acid than a moderately acid soil. It may be remarked that many of the soils that have become "deficient" after liming have naturally a very acid reaction".

This perhaps explains some of the observations in the past in which it is possible to have a medium acid soil under conditions which subject it to long periods in which reduction is predominating. Under such a condition the manganese can be leached from the soil. However, as long as this soil is acid in reaction, no deficiency of manganese will appear, but, whenever it is limed, this deficiency will appear as soon as the active MnO_2 becomes too low. It is also possible to have a medium acid soil which does not have periods of reduction or, if it does, the manganese is not leached from the soil and, under such a condition, overliming will not be possible.

THE EFFECT OF MANGANESE FERTILIZER ON THE MINERAL COMPOSITION OF THE CROP

A large amount of work has been done on the effect of the application of certain fertilizers upon the mineral composition of the crops grown on mineral soils. Very little work has been done on the composition of the crops produced on organic soils. Recent studies of Thomas and Mack (73) working with mineral soils, have shown that considerable more attention should be paid to the time of season that samples are collected for analysis. Further, the work of McCool (53) has demonstrated that light intensity and kinds of light determines to a great extent the quantity of ions present. Thus, it is evident that there are many factors playing a part in the utilization of soil nutrients other than availability of the nutrients themselves.

The difference between the composition of normal and chlorotic tissue will be considered very thoroughly in this work. As has been pointed out, plants growing on the area under investigation are chlorotic unless they either receive manganese or the soil is made acid by the application of sulphur. The assumption has prevailed in the latter case that a change in soil acidity will cause the manganese to go into solution and be available to plants. In order to secure definite information regarding this point, it was considered desirable to make mineral analysis of plants receiving these respective treatments. It was deemed of equal interest to see if there were any effects upon the other mineral constituents other than the contents of manganese. Scholz (65) states in his work with nutrient solutions on lupines, that manganese appears to be essential and that there exists some relationship between calcium, manganese and iron. Hoffer's work (33) indicates a possible association between iron and potash and Willis (79) intimates that there may be a relationship between manganese and iron.

It was believed that chemical analysis may give valuable information as to the validity of these points. For this study samples of plant material were taken for analysis during the seasons of 1937 and 1938. The samples taken at the date of harvesting of the crops, should shed some light on amounts of the various materials removed in the crop. Early in the summer of 1938, a crop of canning peas offered excellent material for a comparative study of chlorotic and normal tissue. Since there are seasonal changes in composition due to various seasonal factors, a seasonal trend study was considered advisable. During the season of 1938, samples of barley plants likewise were taken, at ten-day intervals during their growing season. Samples also were gathered at fifteen-day intervals on a legume experiment which included alfalfa, alsike clover and sweet clover. Determination of CaO, MgO, Fe_2O_3, Mn_3O_4, S, P_2O_5, K_2O, and Na_2O were made upon the crops sample in 1937. The seasonal trend study included only Mn_3O_4, Fe_2O_3, CaO and MgO. In some cases a few analyses were made to determine its possible influence on still other constituents. The plants were sampled, weighed immediately, and then dried rapidly at 60°C. The dried plant tissue was ground in a Willey Mill. A portion of the mixed ground material was then placed in a drying oven and dried approximately six hours at 105°C. At this point, samples were taken for analysis.

Calcium was determined volumetrically by titrating the oxalate with a standard permanganate solution, and magnesium gravimetrically as the pyrophosphate. The iron determination was made volumetrically by dissolving the ash by hydrochloric acid and then converting the iron to the ferric state by cautiously adding a weak permanganate solution. Ten milli-leters of a ten per cent solution of ammonium thiocyanate solution was added and the ferric iron reduced by titrating with a freshly standardized solution of titanous chloride. Manganese was determined by the method described for the determination of total manganese in the organic soils. Sulphur and phosphorous were determined in a magnesium nitrate ignition, sulphur gravimetrically as barium sulfate and phosphorous volumetrically by dissolving the yellow ammonium phospho-molybdate precipitate with an excess of a standard alkali and titrating the excess base with a standard acid. Sodium and potassium determinations were made by methods similar to those employed in exchangeable ions.

The following crops were analyzed by these methods: onion bulbs, potato tubers, potato vines, sugar beets, sugar beet tops (including crowns), strawberry leaves, and raspberry leaves. Strawberries and sugar beets are crops non-responsive to manganese and sulphur applications. The following tables will show the data which were obtained in these analyses.

Table 9, showing the mineral composition of mature onions produced in 1937 under varying applications of manganese and sulphur on various plots of the Mn-S Series at the College Mck Plots.

	Total			Percentage calculated on field weight basis*							
Plot	applications 1934 - 1937	H_2O	Ash	CaO	MgO	Fe_2O_3	Mn_3O_4	K_2O	Na_2O	S	P_2O_5
1	(a) $MnSO_4$ -400	91.34	6.59	.083	.022	.00117	.00017	.176	.0023	.046	.287
3	$MnSO_4$ -400 S - 1000	91.32	6.67	.083	.024	.00111	.00012	.212	.0034	.041	.289
4	$MnSO_4$ -800	91.15	6.39	.086	.023	.00112	.00020	.206	.0039	.038	.284
5	Control	90.01	6.62	.073	.024	.00140	.00008	---	---	.058	.320
6	$MnSO_4$ -800 S-1000	90.29	6.03	.079	.024	.00096	.00024	.194	.0022	.039	.239
7	S - 1000	90.51	5.74	.078	.022	..00109	.00013	.212	.0024	.037	.245
8	Control	90.59	6.43	.079	.029	.00236	.00007	.245	.0023	.050	.285
9	S - 3000	90.58	6.19	.091	.026	.00102	.00011	.223	.0034	.044	.276
11	Control	90.00	6.27	.062	.030	.00137	---	---	---	---	.335

Average composition in per cent.

Treatment	H_2O	Ash	CaO	MgO	Fe_2O_3	Mn_3O_4	K_2O	Na_2O	S	P_2O_5
Control	90.20	6.44	.071	.028	.00171	.00007	.245	.0023	.054	.313
$MnSO_4$	91.24	6.49	.084	.022	.00114	.00018	.191	.0031	.042	.285
$MnSO_4$ + S	90.80	6.35	.081	.024	.00103	.00018	.203	.0028	.040	.264
S	90.54	5.96	.084	.024	.00105	.00012	.217	.0029	.040	.260

* Ash calculated on water-free basis

(a) Pounds per acre.

Table 10, showing the mineral composition of potato vines produced under varying applications of manganese and sulphur on the Mn-S Series of the College Muck Plots.

Plot	Total applications 1934 - 1937	Ash	CaO	MgO	Fe_2O_3	Mn_3O_4	K_2O	Na_2O	S	P_2O_5
			Percentage calculated on oven dry weight basis							
1	(a) $MnSO_4$ -400	9.63	3.97	.98	.043	.00266	1.12	.246	.156	.99
2	Control	9.85	4.37	.73	.058	.00146	.96	.232	.144	1.07
3	$MnSO_4$-400 S - 1000	9.07	3.27	.85	.038	.00294	1.27	.364	.205	1.11
4	$MnSO_4$-800	8.81	3.38	.97	.035	.00319	1.02	.246	.176	1.02
5	Control	9.91	4.30	.82	.072	.00242	.91	.244	.128	1.12
6	$MnSO_4$-800 S-1000	9.47	3.61	.93	.039	.00374	1.09	.252	.150	1.17
7	S-1000	9.28	3.88	.74	.043	.00261	.74	.256	.151	1.14
8	Control	8.98	3.81	.87	.064	.00242	.93	.243	.118	1.16
9	S-3000	7.66	3.42	.72	.043	.00277	.74	.235	.120	1.11

			Average composition in per cent					
Treatment	Ash	CaO	MgO	Fe_2O_3	Mn_3O_4	K_2O	Na_2O	S	P_2O_5
Control	9.59	4.16	.73	.065	.00215	.93	.240	.130	1.12
$MnSO_4$	9.22	3.67	.97	.039	.00292	1.07	.246	.166	1.00
$MnSO_4$ + S	9.27	3.44	.89	.038	.00334	1.18	.308	.178	1.14
S	8.47	3.65	.73	.043	.00269	.74	.245	.135	1.12

(a) Pounds per acre.

Table 11, showing the mineral composition of potato tubers produced in 1937 under varying applications of manganese and sulphur on Mn-S Series of College Muck Plots.

Plot	Total applications 1934 - 1937 (a)	H_2O	Percentage calculated on field weight basis*								
			Ash	CaO	MgO	Fe_2O_3	Mn_3O_4	K_2O	Na_2O	S	P_2O_5
1	$MnSO_4$-400	84.32	7.04	.018	.047	.0016	.00012	.515	.022	.045	.392
2	Control	84.48	7.18	.018	.049	.0018	.00008	.504	.024	.058	.371
3	$MnSO_4$-400 S-1000	84.68	6.99	.017	.044	.0016	.00011	.539	.037	.055	.446
4	$MnSO_4$-800	84.09	6.96	.022	.050	.0016	.00015	.584	.044	.057	.391
5	Control	86.02	7.36	.018	.046	.0018	.00008	.471	.021	.053	.382
6	$MnSO_4$-800 s-1000	83.17	6.66	.020	.048	.0016	.00013	.593	.036	.057	.442
7	S-1000	84.16	6.89	.019	.046	.0019	.00008	.541	.041	.058	.411
8	Control	86.69	7.20	.017	.046	.0018	.00006	.443	.013	.051	.355
9	S-3000	85.30	6.66	.018	.045	.0016	.00007	.555	.029	.057	.389

Average composition in per cent

Treatment	H_2O	Ash	CaO	MgO	Fe_2O_3	Mn_3O_4	K_2O	Na_2O	S	P_2O_5
Control	85.73	7.25	.018	.047	.0018	.00007	.473	.019	.054	.369
$MnSO_4$	84.20	7.00	.020	.048	.0016	.00013	.549	.033	.051	.391
$MnSO_4$ + S	83.92	6.82	.018	.046	.0016	.00012	.566	.036	.056	.444
S	84.73	6.77	.018	.045	.0017	.00007	.548	.035	.057	.400

* Ash calculated on water-free basis

(a) Pounds per acre.

Table 12, showing the mineral composition of sugar beet roots produced in 1937 under varying applications of manganese and sulphur on Mn-S Series of College Muck Plots.

Plot	Total applications 1934- 1937 (a)	Percentage calculated on field weight basis*									
		H_2O	Ash	CaO	MgO	Fe_2O_3	Mn_3O_4	K_2O	Na_2O	S	P_2O_5
1	$MnSO_4$-400	77.8	3.79	.033	.099	.00027	.00041	.366	.043	.014	.229
2	Control	77.4	3.43	.033	.092	.00019	.00031	.372	.039	.013	.238
3	$MnSO_4$-400 S-1000	78.5	3.59	.037	.099	.00028	.00059	.397	.035	.014	.221
4	$MnSO_4$-800	77.2	3.51	.033	.094	.00027	.00068	.370	.057	.015	.235
5	Control	76.9	3.19	.034	.109	.00019	.00070	.369	.030	.014	.252
6	$MnSO_4$-800 S-1000	76.7	3.19	.043	.095	.00022	.00084	.392	.036	.013	.227
7	S - 1000	76.4	3.16	.030	.104	.00023	.00057	.362	.038	.016	.235
8	Control	77.2	4.01	.036	.102	.00019	.00036	.406	.040	.014	.232
9	S - 3000	76.5	3.16	.035	.099	.00026	.00046	.402	.036	.016	.276
10	S - 2000	77.2	3.72	.038	.103	.00022	.00037	.406	.041	.013	.244
11	Control	75.3	3.40	.038	.114	.00019	.00037	.394	.038	.015	.230
12	$CuSO_4$-200	77.9	4.28	.037	.101	.00016	.00031	.405	.046	.012	.250

Average composition

Treatment	H_2O	Ash	CaO	MgO	Fe_2O_3	Mn_3O_4	K_2O	Na_2O	S	P_2O_5
Control	76.7	3.51	.035	.104	.00019	.00043	.385	.037	.014	.238
$MnSO_4$	77.5	3.65	.033	.096	.00027	.00054	.368	.050	.014	.232
$MnSO_4$ + S	77.6	3.39	.040	.097	.00025	.00071	.394	.036	.013	.224
S	76.7	3.52	.034	.102	.00024	.00047	.390	.038	.015	.252

* Ash calculated on water-free basis

(a) Pounds per acre.

Table 13, showing the mineral composition of sugar beet tops and crown produced in 1937 under varying applications of manganese and on Mn-S Series of College Muck Plots.

Plot	Total applications 1934 - 1937 (a)	Percentage calculated on field weight basis*									
		H_2O	Ash	CaO	MgO	Fe_2O_3	Mn_3O_4	K_2O	Na_2O	S	P_2O_5
1	$MnSO_4$-400	70.5	16.88	.541	.620	.0200	.00105	.944	.242	.062	.512
2	Control	70.3	14.09	.480	.622	.0164	.00119	.715	.225	.056	.499
3	$MnSO_4$-400 S - 1000	72.8	14.29	.475	.630	.0168	.00240	.761	.242	.046	.494
4	$MnSO_4$-800	72.1	14.69	.403	.436	.0125	.00235	.915	.236	.059	.497
5	Control	73.2	13.03	.338	.439	.0107	.00140	.777	.234	.049	.464
6	$MnSO_4$-800 S - 1000	70.8	13.77	.523	.492	.0132	.00283	.716	.251	.050	.472
7	S - 1000	72.0	14.49	.429	.485	.0137	.00094	.728	.222	.057	.486
8	Control	75.2	14.96	.411	.451	.0131	.00125	.721	.230	.054	.483
9	S - 3000	75.0	14.54	.440	.441	.0151	.00149	.733	.227	.060	.455
10	S - 2000	73.1	14.56	.422	.507	.0150	.00131	.712	.234	.057	.472
11	Control	75.3	13.51	.284	.348	.0112	.00082	.711	.218	.049	.461
12	$CuSO_4$-200	74.9	14.25	.335	.432	.0135	.00096	.712	.210	.052	.454

Average composition

Treatment	H_2O	Ash	CaO	MgO	Fe_2O_3	Mn_3O_4	K_2O	Na_2O	S	P_2O_5
Control	73.5	13.90	.378	.465	.0128	.00166	.731	.227	.052	.477
$MnSO_4$	71.3	15.78	.472	.528	.0162	.00170	.929	.239	.060.	.504
$MnSO_4$ + S	71.8	14.03	.499	.561	.0150	.00261	.738	.246	.048	.483
S	73.4	14.53	.430	.478	.0146	.00091	.724	.228	.058	.471

* On water-free basis.

(a) Pounds per acre.

Table 14, showing the mineral composition of strawberry leaves collected in late fall of 1937 on Mn-S Series of College Muck Plots.

Plot	Total applications 1934 - 1937 (a)	Percentage calculated on water-free basis						
		Ash	CaO	MgO	Fe_2O_3	Mn_3O_4	S	P_2O_5
2	Control	7.68	2.33	.63	.072	.0034	.115	1.27
4	$MnSO_4$ - 800	7.81	2.41	.63	.076	.0079	.125	1.20
5	Control	7.30	2.43	.63	.057	.0049	.121	1.09
6	$MnSO_4$ - 800 S - 1000	7.18	2.46	.70	.053	.0097	.118	1.33
8	Control	8.52	3.05	.60	.068	.0027	.141	1.14
9	S - 3000	8.29	2.26	.67	.073	.0035	.139	1.27
	Average of Controls	7.83	2.60	.62	.066	.0037	.126	1.13

(a) Pounds per acre.

Table 15, showing mineral composition of raspberry leaves collected in October, 1937, from College Plots.

Plot	Total applications 1934 - 1937 (a)	Percentage calculated on water-free basis						
		Ash	CaO	MgO	Fe_2O_3	Mn_3O_4	S	P_2O_5
2	Control	7.09	1.78	.93	.089	.0057	.069	1.14
3	$MnSO_4$-400 S - 1000	7.14	1.92	1.00	.053	.0066	.069	1.09
4	$MnSO_4$-800	8.91	1.78	1.00	.057	.0074	.080	1.09
5	Control	6.98	1.77	.99	.075	.0079	.044	.99
6	$MnSO_4$-800 S - 1000	7.92	1.97	.91	.057	.0103	.059	.84
7	S - 1000	7.33	1.71	.90	.079	.0123	.063	1.10
8	Control	7.84	1.79	.88	.085	.0063	.070	1.21
9	S - 3000	7.72	1.82	.88	.082	.0086	.070	1.20
11	Control	7.95	1.75	.86	.091	.0063	.102	1.04

Average composition in per cent

Treatment	Ash	CaO	MgO	Fe_2O_3	Mn_3O_4	S	P_2O_5
Control	7.47	1.77	.93	.085	.0066	.071	1.09
$MnSO_4$	8.91	1.78	1.00	.057	.0074	.080	1.09
$MnSO_4$ + S	7.53	1.94	.95	.055	.0085	.064	.96
S	7.52	1.76	.89	.080	.0104	.066	1.15

(a) Pounds per acre.

Discussion of Data.

The data showed very few differences due to treatments; however, there were some interesting points which were considered important. As already mentioned, the plant material which was analyzed in this study was taken at the time when the crop was harvested and consequently may not show as great differences as might have been found during the growing season. The data indicated that the treatments had little or no effect upon the per cent of water, ash, calcium, magnesium, sodium, and sulphur contents of the plants. In the majority of the cases, the application of manganese sulfate increased the manganese content of the plant, especially in the case of the combination of manganese sulfate and sulphur treatment.

The most outstanding result of the analysis is that given by iron. The crops which developed the chlorotic condition had a high iron content unless they were treated with either manganese sulfate or sulphur. The non-responsive crops, sugar beets and strawberries, failed to show similar results and, in the case of sugar beets, the lowest iron content was in the controls. These data would be indicative of some influence of the manganese upon the iron in the plant.

Although these tables showed instances of apparent effect upon the phosphorous and potassium content of the crop by the soil treatment, later work failed to substantiate these results. Table 10 showed a substantial increase in potassium content with the application of manganese sulfate, but the contrary appeared to be the result with the application of sulphur. Later work has never produced similar results; however, this table reflects to some extent the data obtained by successive water extractions in graph 3.

Mineral Composition of Normal and Chlorotic Plants at Different Stages of Growth.

Due to the interesting data on iron and manganese content of the mature crops, an investigation into the mineral composition of normal and chlorotic plants at different stages of growth was made during 1938. Normal plants were produced on those plots receiving either manganese sulfate, sulphur or the combination of the two. During the spring of 1938, additional treatments were applied to the treated plots, as follows: plots 1 and 3 received a 200-pound-per-acre application of manganese sulfate, and plots 4 and 6, 400 pounds of the same salt; plots 3, 6, 7, 9, and 10 each received an additional treatment of a 500-pound-per-acre application of sulphur. In addition to these treatments, all plots received an 800-pound-per-acre application of a 0-8-24 fertilizer and 350 pounds per acre of common salt (NaCl). Analytical samples were collected at various times during the season from the responsive crops, barley, raspberries, peas, alsike clover and the non-responsive crops, sweet clover and peppermint.

Samples of peas were taken on June 23, 1938, for a study of the mineral composition of the chlorotic tissue, as compared to that of the normal tissue. The plants from plots 2, 5, and 8 were chlorotic, inasmuch as the lower leaves and stems were yellow and only a few upper leaves and stems had the normal green color. The plants on the plots 1 and 4 receiving manganese sulfate; 7 and 9, sulphur; and 3 and 6, the combination of sulphur and manganese sulfate had a normal green color throughout the vegetative growth.

The plants which were chlorotic were divided into chlorotic tissue and normal tissue. The plants from the plots producing the normal plants were divided in a manner so as to give comparable parts to those of the chlorotic plants. This material was analyzed for ash, calcium, manganese, potassium, iron and nitrogen, and the results are shown in the following table:

Table 16, showing comparison of the analyses of chlorotic and normal pea plant tissue.

				Per cent calculated on green weight of crop						
Plot (b)	Treatment-1938	 (c)	Condition	D.M.	Ash (a)	CaO	Fe_2O_3	Mn_3O_4	K_2O	N
1 upper	$MnSO_4$	200	normal	13.82	11.48	.275	.00549	.00027	.631	.678
1 lower	$MnSO_4$	200	normal	15.23	13.21	.563	.00422	.00025	.591	.503
2 upper	Control	0	normal	12.16	13.10	.206	.00419	.00011	.672	.317
2 lower	Control	0	chlorotic	15.55	15.55	.600	.01053	.00014	.692	.510
3 upper	$MnSO_4$ Sulphur	200 500	normal	13.89	11.62	.325	.00356	.00026	.586	.684
3 lower	$MnSO_4$ Sulphur	200 500	normal	15.47	13.32	.712	.00491	.00031	.538	.511
4 upper	$MnSO_4$	400	normal	14.09	10.39	.295	.00445	.00032	.572	.698
4 lower	$MnSO_4$	400	normal	14.00	12.60	.596	.00461	.00028	.535	.455
5 upper	Control	0	normal	10.73	12.63	.173	.00328	.00012	.609	.810
5 lower	Control	0	chlorotic	14.04	15.24	.567	.00772	.00013	.531	.463
6 upper	$MnSO_4$ Sulphur	400 500	normal	13.33	12.63	.220	.00330	.00047	.606	.758
6 lower	$MnSO_4$ Sulphur	400 500	normal	13.23	12.78	.617	.00452	.00077	.499	.437
7 upper	Sulphur	500	normal	14.03	11.39	.227	.00343	.00016	.620	.822
7 lower	Sulphur	500	normal	13.91	13.75	.584	.00536	.00013	.535	.473
8 upper	Control	0	normal	11.87	12.79	.172	.00406	.00012	.674	.819
8 lower	Control	0	chlorotic	13.00	16.40	.492	.00824	.00012	.608	.468
9 upper	Sulphur	500	normal	13.29	10.94	.249	.00318	.00015	.577	.718
9 lower	Sulphur	500	normal	14.00	13.32	.612	.00456	.00014	.568	.462

(a) Ash reported on basis of oven dry material.

(b) Upper and lower refer to the portion of plant taken for analysis.

(c) Pounds per acre.

Table 17, showing averages of preceding data.

Treatment		Dry Matter	Ash	CaO	Fe_2O_3	Mn_3O_4	K_2O	N
Control	upper	11.59	12.84	.184	.00384	.00011	.651	.815
	lower	14.20	15.73	.553	.00883	.00013	.610	.480
$MnSO_4$	upper	13.95	10.93	.285	.00497	.00029	.601	.688
	lower	14.61	12.90	.579	.00441	.00029	.563	.479
$MnSO_4$ + S	upper	13.61	12.12	.272	.00343	.00036	.596	.721
	lower	14.35	13.05	.664	.00471	.00054	.518	.474
S	upper	13.66	11.16	.238	.00330	.00015	.598	.770
	lower	13.95	13.53	.598	.00496	.00013	.551	.467

Peas are a crop in which the chlorotic condition develops in the lower leaves first and progressively approaches the growing tip. There is always a clear-cut boundary between the growing parts which are normal and the older tissue which is chlorotic. For this reason, the chemical data should give very clear-cut evidence as to the chemical factors that may be involved. Again the outstanding point is that of iron. Normal tissue has markedly less iron than the chlorotic tissue which has two and a half times as much as does the normal tissue. These data clearly and conclusively indicate that the effects of either the sulphur or the manganese sulfate treatments are one of activation of the iron.

The calcium content of the plant appears to increase with age of the plant tissue. The active growing portions of the plant have about half as much calcium as is found in the lower portion of the plant. The treatments do not appear to have any influence upon the calcium content. Potassium has an opposite relationship in respect to the age of tissue, with the newer tissue having the higher content. Treatments do not have any influence upon its content in the plants. No important difference appears in either the content of dry matter or nitrogen, while the ash content is higher in the case of the chlorotic tissue.

Treatments have a marked effect upon the manganese content of the plants. The lower portion of those on the sulphured plots show an increase of 20 per cent over the controls; those receiving manganese sulfate, 242 per cent increase; while those receiving the combination, show a 374 per cent increase in comparable tissue.

These data suggest a similarity to the work of Oserkowsky (58) in which he showed a higher total iron content in lime-induced chlorotic tissue as compared to normal tissue. Data of Marsh and Shive (49) revealed a similar condition existing in comparable tissue of soybeans in which chlorosis is caused by lowering the hydrogen ion concentration in nutrient solutions. A study was made as to factors which might have caused such observations. The pH of the cell sap of the chlorotic and normal tissue of canning peas reported in Table 16 was determined by a Beckman potentiometer. The chlorotic tissue in every case had a higher pH, varying from 6.40 to 6.65, while the normal tissue varied from 6.15 to 6.30. Hoffer (32) associated the higher pH with deposition of iron in nodes of corn. Allyn (2) is of the opinion that a high soluble calcium content in the soil may disturb the metabolism of iron within the plant. Since these reports indicated some kind of a break down in the metabolism of iron within the plant, it was considered advisable to establish where the iron was concentrated in the plant tissue. Using the technique of Kliman (42) for staining ferric and ferrous iron in plant tissue, some very interesting results were obtained in the tissue of canning peas and soybeans. The ferric iron present was found deposited heavily in the veins of the chlorotic leaves. The normal leaves showed no ferric iron in the veinal tissue and a mere trace in the rest of the leaf. The ferrous iron in the normal leaves was uniformly distributed throughout the leaf, while, in the chlorotic leaf, the stains indicated considerable ferrous iron but an extremely uneven distribution with a tendency to become concentrated near the veinal tissue and adjacent to the ferric iron. These results would indicate that the beneficial results of manganese and of sulphur are due to their effect on the state of oxidation of the iron in the plant.

Seasonal Trend of Mineral Content of Barley Plants Taken at Ten-day Intervals.

Barley was seeded on a portion of the manganese-sulphur series for the purpose of studying the mineral composition of the crop at different stages of its growth. It was seeded on May 5, 1938, and the first samples for analysis were taken on June 13. Thereafter it was sampled at ten-day intervals throughout the season. The final sampling was made on August 2, at which time the barley was ripe. It should be noted here that at the mature stage most of the plots were heavily infested with stem rust which was very severe in the cases of plots 2, 5, 8, 11 and 12, so much so that they failed to mature properly. Plots 3, 6, 7 and 9 matured normally.

Early in the growth of the barley, the plants on plots 2, 5, 8, 11 and 12 became chlorotic, the chlorosis closely resembling that produced by nitrogen starvation. As growth progressed, the yellowing gradually became more pronounced up to the hot weather of July, at which time a few plants turned dark green on the control plots and especially on plot 5 on which dark green plants became very numerous. At heading time, July 20, all plants on all of these plots, with the exception of 12, were dark green and on this plot a week later they also became green.

In the first five samplings, the area taken for a sample was determined by the size of the sample needed for analysis. The August second sampling was taken with the idea of obtaining a comparative yield on each plot. An area six feet long and two feet in width was taken. Calcium, manganese, and iron were determined in these samples by methods previously described.

The highest grain yield, (Table 18), was obtained on plots receiving only sulphur. The three plots showed a 600 per cent increase in yield over the controls. The manganese sulfate and sulphur combinations showed a 568 per cent increase and the plots receiving only manganese sulfate showed a 386 per cent increase. The yields of straw have approximately the same relationship.

The date given in Table 19 on the dry matter content indicate that on all dates before maturity, the content of the several constituents is higher on all treated plots. At maturity none of the treatments varied from the controls to any significant degree in percentage of dry matter.

Table 18, showing yields of barley in 1937 on Mn-S Series of College Muck Plots taken from from one row comprising an area of 12 square feet.

Plot	Treatment-1938 (a)	Number of heads	Wt. of heads — grams	Yield of straw per area — grams
1	$MnSO_4$ - 200	127	172	174
2	Control	26	19	68
3	$MnSO_4$ - 200 S - 1500	196	259	246
4	$MnSO_4$ - 400	151	211	225
5	Control	73	65	91
6	$MnSO_4$ - 400 S - 1500	207	307	276
7	S - 1500	249	356	317
8	Control	57	61	96
9	S - 3500	229	301	293
10	S - 2500	208	229	238
11	Control	74	58	108
12	$CuSO_4$ - 50	2	1	13

(a) Pounds per acre.

Table 19, showing the effects of manganese and sulphur, applied on an alkaline muck on the seasonal trend of dry matter content of barley plants grown thereon in 1938.

Plot	Treatment	Percentage calculated on oven dry basis					
		June 13	June 23	July 3	July 13	July 23	Aug. 2
1	$MnSO_4$ - 200 (a)	11.99	12.35	16.83	23.89	26.60	89.84
2	Control	11.04	10.75	11.95	17.01	21.71	90.48
3	$MnSO_4$ - 200 S - 1500	12.46	13.52	14.36	25.06	27.78	90.12
4	$MnSO_4$ - 400	12.29	12.12	14.16	23.60	25.22	89.70
5	Control	10.86	9.95	10.89	19.55	23.15	89.55
6	$MnSO_4$ - 400 S - 1500	11.67	11.72	12.52	25.63	26.73	90.20
7	S - 1500	11.89	10.72	13.60	23.55	25.96	89.75
8	Control	10.57	9.80	10.36	17.39	19.89	89.80
9	S - 3500	11.24	12.12	14.31	25.44	24.45	89.88

Averages

	June 13	June 23	July 3	July 13	July 23	Aug 2
Control	10.82	10.17	11.07	17.98	21.58	89.94
$MnSO_4$	12.14	12.23	15.49	23.74	25.91	89.77
$MnSO_4$ + S	12.06	12.62	13.44	25.34	27.25	90.16
S	11.56	11.42	13.95	24.49	25.20	89.81

(a) Pounds per acre.

The chemical data given in Tables 20, 21 and 22, show several interesting points. There appears to be no real differences between the calcium contents of the barley resulting from the various treatments. In the case of the manganese, all the treatments produced higher percentages than did the controls. One of the highest values for manganese in crops grown on the sulphur plots was obtained in the barley. These results gave the strongest evidence offered by all crops analyzed in support of the theory that the soil acidity increased the solubility of the manganese. The unusual data that appeared in the August 2 analysis is hard to explain. The control plots gave the highest manganese content of the crop at that date for which no explanation can be given. The iron data are in keeping with those that had been obtained in other crops. From June 13 to July 13 the iron content is in inverse order to the yields, with the chlorotic plants from control plots having the highest values. On July 20, when the plots had become green, no difference existed between the iron content of these plants and of those on the treated plots. This relationship definitely establishes the iron factor as the determining one in chlorosis on alkaline soil.

Table 20, showing the effects of manganese and sulphur, applied on an alkaline muck, on the seasonal trend of the CaO content of barley plants

Plot	Treatment Lbs. per acre	June 13 %	June 23 %	July 3 %	July 13 %	Straw		Heads	
						July 23 %	Aug. 2 %	July 23 %	Aug. 2 %
Calculated on over-dry basis									
1	$McSO_4$-200	1.112	1.295	1.118	.945	1.162	1.186	.371	.189
2	Control	1.041	1.309	1.116	1.414	1.302	1.086	.295	.315
3	$MnSO_4$-200 S - 1500	1.119	1.176	1.044	.903	1.029	.997	.252	.210
4	$MnSO_4$-400	1.099	1.155	1.160	.938	1.190	1.044	.329	.189
5	Control	1.067	1.491	1.303	1.085	1.141	1.052	.315	.283
6	$MnSO_4$-400 S - 1500	1.177	1.253	1.022	.707	1.064	1.013	.224	.194
7	S - 1500	1.132	1.281	1.141	.833	1.022	1.034	.273	.200
8	Control	1.161	1.491	1.406	1.309	1.330	.934	.301	.283
9	S - 3500	1.293	1.260	1.047	.840	1.029	1.002	.238	.194
Averages on over-dry basis									
2,5,8	Control	1.090	1.430	1.275	1.260	1.258	1.024	.304	.294
1,4	Mn	1.105	1.225	1.139	.941	1.176	1.115	.349	.189
3,6	Mn + S	1.148	1.214	1.033	.805	1.046	1.005	.238	.202
7,9	Sulphur	1.212	1.270	1.094	.836	1.025	1.018	.255	.197
Averages calculated on field basis									
2,5,8	Control	.118	.145	.140	.223	.269	.925	.088	.270
1,4	Mn	.134	.163	.175	.219	.305	1.004	.123	.172
3,6	Mn + S	.138	.153	.158	.199	.285	.905	.091	.184
7,9	Sulphur	.140	.144	.152	.201	.258	.917	.091	.179

Table 21, showing the effects of manganese and sulphur on the seasonal trend of the Mn_3O_4 content of barley plants.

Plot	Treatment	June 13 %	June 23 %	July 3 %	July 13 %	July 23 %	Straw Aug. 2 %	July 23 %	Heads Aug. 2 %
				Calculated on oven-dry basis					
1	$MnSO_4$-200	.00217	.00308	.00277	.00198	.00298	.00246	.00187	.00305
2	Control	.00139	.00231	.00186	.00150	.00206	.01083	.00065	.01332
3	$MnSO_4$-200 S - 1500	.00356	.00385	.00462	.00347	.00409	.00302	.00305	.00434
4	$MnSO_4$-400	.00384	.00434	.00421	.00302	.00434	.00302	.00239	.00354
5	Control	.00126	.00171	.00257	.00168	.00255	.00267	.00123	.01194
6	$MnSO_4$-400 S - 1500	.00615	.00661	.00661	.00555	.00534	.00555	.00288	.00579
7	S - 1500	.00231	.00257	.00289	.00212	.00264	.00246	.00264	.00385
8	Control	.00126	.00128	.00169	.00121	.00271	.00296	.00065	.01332
9	S - 3500	.00264	.00319	.00384	.00222	.00347	.00215	.00239	.00434
				Averages on oven-dry basis					
	Control	.00130	.00177	.00204	.00146	.00244	.00549	.00084	.01286
	Mn	.00300	.00371	.00349	.00250	.00366	.00274	.00213	.00329
	Mn + S	.00485	.00523	.00561	.00451	.00471	.00428	.00296	.00506
	Sulphur	.00247	.00288	.00336	.00217	.00305	.00230	.00251	.00409
				Averages on field basis					
	Control	.00014	.00018	.00023	.00026	.00052	.00497	.00025	
	Mn	.00037	.00048	.00053	.00058	.00094	.00247	.00075	
	Mn + S	.00058	.00065	.00083	.00112	.00128	.00387	.00116	
	Sulphur	.00028	.00033	.00047	.00052	.00077	.00208	.00091	

Table 22, showing the effects of manganese and sulphur, applied on an alkaline muck, on the seasonal trend of the Fe_2O_3 content of barley plants grown thereon in 1938.

Plot	Treatment	June 13 %	June 23 %	July 3 %	July 13 %	Straw		Heads	
						July 23 %	Aug. 2 %	July 23 %	Aug. 2 %
	Calculated on oven-dry basis								
1	$MnSO_4$-200	.0232	.0204	.0177	.0148	.0135	.0194	.0101	.0117
2	Control	.0374	.0383	.0268	.0242	.0148	.0226	.0114	.0058
3	$MnSO_4$-200 S - 1500	.0236	.0204	.0191	.0124	.0128	.0141	.0117	.0094
4	$MnSO_4$-400	.0225	.0255	.0210	.0114	.0160	.0166	.0088	.0091
5	Control	.0288	.0396	.0288	.0177	.0167	.0217	.0068	.0094
6	$MnSO_4$-400	.0227	.0204	.0191	.0106	.0153	.0148	.0127	.0117
7	S - 1500	.0232	.0223	.0171	.0106	.0114	.0180	.0117	.0127
8	Control	.0337	.0333	.0294	.0191	.0160	.0180	.0076	.0127
9	S - 2500	.0236	.0210	.0160	.0114	.0153	.0173	.0104	.0105
	Averages on oven-dry basis								
	Control	.0333	.0371	.0283	.0203	.0158	.0208	.0086	.0093
	Mn	.0228	.0229	.0193	.0131	.0147	.0180	.0094	.0104
	Mn + S	.0231	.0204	.0191	.0115	.0140	.0144	.0122	.0105
	Sulphur	.0234	.0216	.0165	.0110	.0133	.0176	.0110	.0116
	Averages on field basis								
	Control	.00360	.00357	.00314	.00358	.00341	.00187	.00251	
	Mn	.00277	.00301	.00297	.00305	.00381	.00162	.00330	
	Mn + S	.00279	.00257	.00278	.00285	.00381	.00131	.00471	
	Sulphur	.00270	.00248	.00232	.00265	.00334	.00158	.00390	

Seasonal Trend of Mineral Composition of Legumes Sampled at 15-day Intervals.

The following legumes, alfalfa, alsike clover, and sweet clover, were seeded in July, 1938, on the Manganese-Sulphur Series of the College Muck Plots. These three legumes represent three degrees of responsiveness to Manganese fertilizers. Alfalfa is a very responsive crop; alsike clover, mildly responsive; and sweet clover, non-responsive. The first samples were taken August 27, 1938, and samples at approximately fifteen-day intervals thereafter. In the case of alfalfa, very marked differences due to treatment could be observed. CaO, MgO, Mn_3O_4 and Fe_2O_3 were determined in the samples collected.

In Tables 23, 24 and 25, is shown the dry matter content of these three legumes at each date of sampling. For the most part, no differences could be recognized as resulting from treatments. In most cases, the plants from the controls were slightly higher in dry matter than were those from the treated plots.

Table 23, Mn - S series legume experiment showing per cent of dry matter content of alfalfa sampled at approximately 15-day periods during 1938.

Plot	Treatment lbs. per acre	Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
1	$MnSO_4$-200	23.99	22.66	30.01	39.36	38.98
2	Control	24.40	23.67	30.23	42.03	38.59
3	$MnSO_4$-200 S - 1500	25.33	22.68	28.30	31.72	36.29
4	$MnSO_4$-400	22.19	21.57	30.93	29.32	37.74
5	Control	24.12	22.52	28.39	32.18	35.38
6	$MnSO_4$-400 S - 1500	22.98	22.12	27.78	35.64	38.13
7	S - 1500	25.24	22.41	27.78	33.87	38.30
8	Control	24.68	22.85	28.99	28.04	34.31
9	S - 3500	25.55	22.91	28.86	32.63	34.79
10	S - 2500	24.93	21.33	27.91	32.86	35.94
11	Control	25.92	22.42	30.14	34.81	36.88
12	$CuSO_4$-50	24.59	21.09	27.13	29.88	37.19
	Averages					
	Control	24.78	22.86	29.44	37.15	36.29
	Mn	23.09	22.12	30.47	34.34	38.36
	Mn + S	24.15	22.40	28.04	33.68	37.21
	Sulphur	25.24	22.22	28.18	33.12	36.34
	Ratios to control					
	$\frac{\text{Mn}}{\text{Control}}$	.93	.97	1.03	.92	1.06
	$\frac{\text{Mn - S}}{\text{Control}}$	.97	.98	.95	.91	1.03
	$\frac{\text{Sulphur}}{\text{Control}}$	1.02	.97	.96	.90	1.00

Table 24, Mn-S series legume experiment showing per cent of dry matter content of alsike clover sampled at approximately 15-day periods during 1938.

Plot	Treatment lbs. per acre	Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
1	$MnSO_4$-200	20.35	19.05	22.28	26.91	35.80
2	Control	20.67	18.02	22.33	27.56	36.23
3	$MnSO_4$-200 S - 1500	22.11	18.32	23.88	25.63	36.14
4	$MnSO_4$-400	22.67	17.57	23.25	26.98	39.52
5	Control	24.42	18.35	24.26	28.83	38.68
6	$MnSO_4$-400 S - 1500	21.02	19.42	24.93	27.36	40.03
7	S - 1500	24.76	19.23	23.68	26.26	38.07
8	Control	23.90	17.76	23.94	25.82	32.97
9	S - 2500	21.82	18.12	23.59	28.95	38.00
10	S - 2500	22.08	18.76	21.12	26.79	34.71
11	Control	23.58	18.28	22.34	27.28	34.94
12	$CuSO_4$-50	23.52	17.89	20.86	24.08	36.59
	Averages					
	Control	23.14	18.10	23.22	27.37	35.70
	Mn	21.51	18.31	22.76	26.94	37.66
	Sulphur	22.89	18.70	22.80	27.33	36.93
	Mn + S	21.58	18.87	24.40	26.49	38.08
	Ratios to control					
	$\frac{\text{Mn}}{\text{Control}}$	.93	1.01	.98	.98	1.05
	$\frac{\text{Mn + S}}{\text{Control}}$	.93	1.04	1.05	.97	1.07
	$\frac{\text{Sulphur}}{\text{Control}}$	.99	1.03	.98	1.00	1.03

Table 25, Mn-S series legume experiment showing per cent of dry matter content of sweet clover taken at about 15-day intervals.

Plot	Treatment lbs. per acre	Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
1	$MnSO_4$-200	17.55	16.25	21.91	24.69	27.63
2	Control	17.05	17.24	23.03	25.26	28.98
3	S - 1500 $MnSO_4$-200	18.79	15.91	21.03	24.03	29.32
4	$MnSO_4$-400	18.82	16.34	19.94	24.41	27.86
5	Control	17.43	16.70	21.85	25.04	29.66
6	$MnSO_4$-400 S - 1500	18.21	16.20	22.34	24.68	27.35
7	S - 1500	18.53	16.50	22.78	25.86	28.71
8	Control	17.11	16.09	22.69	24.87	27.42
9	S - 3500	18.63	16.62	21.79	25.22	29.68
10	S - 2500	18.56	16.81	21.86	23.80	29.09
11	Control	17.19	16.05	21.96	25.28	28.33
12	$CuSO_4$-500	16.69	16.34	19.04	24.87	30.70
			Averages			
	Control	17.19	16.52	22.38	25.11	28.60
	Mn	18.18	16.29	20.92	24.55	27.74
	Mn + S	18.50	16.05	21.68	24.35	28.33
	Sulphur	18.57	16.64	22.14	24.96	29.16
			Ratios to control			
	$\frac{\text{Mn}}{\text{Control}}$	1.06	.99	.93	.98	.97
	$\frac{\text{Mn + S}}{\text{Control}}$	1.08	.97	.97	.97	.99
	$\frac{\text{S}}{\text{Control}}$	1.08	1.01	.99	.99	1.02

Perhaps one of the best indications of the part played by calcium in this problem will be seen in Tables 26 and 27, and graphs 7 and 8, which show the effect of soil moisture and temperature upon the CaO content of the responsive crop, alfalfa, as compared to the non-responsive crop, sweet clover. It is to be remembered that the greatest response of manganese fertilizers occurred when soils were cold and wet. On September 13, 1938, these plots received a heavy rain and it is interesting to note that the calcium content in the samples gathered on September 15, made a very great increase in the alfalfa; samples of sweet clover from the same date gave no increase in calcium content. In the samples taken October 1, the calcium content of the alfalfa was lower than in those taken September 15, while the sweet clover showed practically no fluctuation. Many investigators have reported the effect of lime as a cause of chlorosis, yet, as far as one has been able to observe in the data of this investigation, this was the only instance in which calcium played a part. It is possible that its intake may be associated with certain climatic factors. Jones (40) reported that when the soil temperature fell below a certain minimum, 18°C., gardenias became chlorotic. At temperatures above 20°C. they were normal. A higher calcium content of the chlorotic gardenias is shown in his analyses. A low soil temperature would be conducive to a higher soluble calcium level in the soil, with attendant general lowering in concentration of all other elements. Under conditions of this nature, a plant, in order to maintain its cell sap concentration, may take in abnormal quantities of calcium, which would result in a higher pH of the sap. This in turn would decrease the availability of the iron in the plant. A higher pH of the cell sap has been associated with a lower available iron content. A high assimilation of calcium will result in the precipitation of iron in the cells of plants according to McGeorge (54). If a high temperature should prevail again, the calcium content of the soil solution would decrease, as would also the calcium content of the plant in respect to the other elements. The result would be the disappearance of the chlorotic condition.

The manganese contents of these crops were similar to those obtained in other crops. The manganese sulfate and sulphur treatments gave the highest manganese level for all sampling dates as shown in Tables 29 and 31 and graphs 9 and 10. Manganese sulfate treatment resulted in considerable increase in manganese content of crops over that of plants from control in the case of alfalfa and alsike clover and slightly higher in the case of sweet clover. The controls and sulphur treatments were very similar and in some cases the plants on the control plot were higher in manganese than those from the sulphured plots.

The difference in manganese content in plants harvested at different dates reveals that it dropped to some extent after rains, with those on the plants receiving manganese sulfate showing the greatest drop. However, plants on the sulphur-treated plots and controls showed very little difference between dates, irrespective of rainfall.

Unquestionably the information given by the data in Tables 32 and 34 and graphs 11 and 12, on the seasonal trend of iron content is in line with the previous data. The uniformity of the iron content in sweet clover, a non-responsive crop indicates that no disturbance has taken place in its metabolism of iron. In the case of alfalfa, the high iron content occurred in those plants taken from plots having the chlorotic plants, the control plots.

The seasonal trend of Fe_2O_3 was not very variable in the case of sweet clover; mildly variable in the case of alsike; and in alfalfa, it appeared to be affected by prevailing climatic conditions. On September 15, after the rain, the alfalfa plants showed very small differences in percentage of iron and also contained the lowest quantity in the seasonal trend at this point. At this date, the ratio of Ca to Fe was the greatest. The abruptness in the rise of the iron in the control plants after that date establishes clear evidence as to the accumulation of iron, due to its desposition in an unavailable form.

The most unusual and unexpected data of this study came in the magnesium content of these plants. It was unexpected inasmuch as the data so far had given no indication that magnesium might be a factor, and unusual in that there appeared to be almost a complete opposite relationship between the non-responsive plants and the responsive plants.

The magnesium content correlates very well with the iron content in that the chlorotic plants contain the larger quantities. There was very little difference between the effects of the sulphur, manganese sulfate, and the combination treatments in the case of the alfalfa. A comparison of the data for alfalfa and sweet clover shows that the plants from the control plots of the sweet clover had the least magnesium and the order of the magnesium contents of the sweet clover from the various treatments were in reverse order to that of alfalfa. Haas (28), working with walnut yellows, found that the diseased leaves were higher in ash, magnesium, and iron which would be very comparable to the results obtained in these analyses. Analysis of peppermint plants gave data very similar in all respects to that for the non-responsive crop, sweet clover, while data obtained from raspberry leaves were comparable to those for barley and alfalfa.

Table 26, Showing content of CaO in alfalfa plants sampled at 15-day intervals on Mn-S series

Plot	Treatment lbs. per acre	Percentage calculated on oven-dry basis				
		Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
1	$MnSO_4$-200	1.983	2.829	1.856	2.163	2.184
2	Control	2.184	3.089	1.979	2.496	2.357
3	$MnSO_4$-200 S - 1500	2.170	3.193	2.079	2.371	2.420
4	$MnSO_4$-400	2.184	3.193	2.063	2.444	2.503
5	Control	2.836	3.619	2.244	2.288	2.531
6	$MnSO_4$-400 S - 1500	2.482	3.328	1.959	2.496	2.482
7	S - 1500	2.252	3.203	1.875	2.309	2.580
8	Control	2.433	3.380	2.153	2.683	2.441
9	S - 3500	2.219	3.546	1.921	2.236	2.399
10	S - 2500	2.087	3.401	1.908	2.288	--
11	Control	2.177	3.078	1.862	2.496	2.503
12	$CuSO_4$-50	2.198	3.037	2.024	2.517	2.371
	Averages					
	Control	2.407	3.291	2.059	2.491	2.458
	Mn	2.084	3.011	1.959	2.303	2.343
	Mn + S	2.326	3.260	2.019	2.433	2.451
	Sulphur	2.186	3.383	1.901	2.278	2.630
	Ratio to control					
	$\frac{\text{Mn}}{\text{Control}}$	.87	.91	.95	.92	.95
	$\frac{\text{Mn + S}}{\text{Control}}$	.97	.99	.98	.98	1.00
	$\frac{\text{S}}{\text{Control}}$	.91	1.03	.92	.91	1.07

Table 27, showing the content of CaO in sweet clover plants sampled at 15-day intervals on Mn-S series

Plot	Treatment lbs. per acre	Percentage calculated on oven-dry basis				
		Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
1	$MnSO_4$-200	2.614	2.967	2.561	3.349	3.196
2	Control	2.905	2.580	2.024	3.085	2.877
3	$MnSO_4$-200 S - 1500	2.801	2.932	2.548	3.446	3.203
4	$MnSO_4$-400	2.704	2.981	2.548	2.947	3.065
5	Control	2.759	2.711	2.393	3.189	3.397
6	$MnSO_4$-400 S - 1500	3.030	2.912	2.502	3.155	3.161
7	S - 1500	2.947	2.773	2.224	3.189	3.231
8	Control	2.912	2.553	2.102	3.245	2.995
9	S - 3500	2.947	3.009	2.587	3.196	3.328
10	S - 2500	2.739	2.773	2.360	3.052	2.975
11	Control	2.967	2.537	2.296	3.120	3.265
12	$CuSO_4$-50	2.857	2.537	2.276	2.947	2.794
			Averages			
	Control	2.886	2.595	2.204	3.160	3.133
	Mn	2.659	2.974	2.554	3.148	3.130
	Mn + S	2.915	2.922	2.525	3.300	3.182
	Sulphur	2.878	2.852	2.390	3.146	3.178
			Ratio to control			
	$\frac{\text{Mn}}{\text{Control}}$	.92	1.15	1.16	1.00	1.00
	$\frac{\text{Mn + S}}{\text{Control}}$	1.01	1.13	1.15	1.04	1.02
	$\frac{\text{S}}{\text{Control}}$	1.00	1.10	1.08	1.00	1.01

Table 28, showing the content of CaO in alsike clover plants sampled at 15-day intervals.

Plot	Treatment lbs. per acre	Percentage calculated on oven-dry basis				
		Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
1	$MnSO_4$-200	2.392	2.400	1.820	2.288	3.120
2	Control	2.427	2.400	1.733	2.319	2.790
3	$MnSO_4$-200 S - 1500	2.794	2.554	2.635	2.850	3.654
4	$MnSO_4$-400	2.413	2.290	2.273	2.798	2.919
5	Control	2.974	2.684	2.118	2.725	3.231
6	$MnSO_4$-400 S - 1500	2.357	2.360	1.869	2.496	2.863
7	S - 1500	2.808	2.787	1.791	2.267	2.628
8	Control	2.884	2.231	1.953	2.278	2.628
9	S-3500	2.670	2.341	1.733	2.586	2.836
10	S - 2500	2.336	2.561	1.830	2.150	2.531
11	Control	2.850	2.057	2.031	2.184	2.461
12	$CuSO_4$-50	2.926	2.141	1.891	2.392	2.961
	Averages					
	Control	2.784	2.293	1.959	2.376	2.777
	Mn	2.402	2.345	2.041	2.543	3.019
	Mn + S	2.575	2.457	2.052	2.673	3.258
	Sulphur	2.605	2.563	1.785	2.334	2.665
	Ratio to control					
	$\frac{\text{Mn}}{\text{Control}}$	.86	1.02	1.04	1.07	1.09
	$\frac{\text{Mn + S}}{\text{Control}}$	.92	1.07	1.05	1.13	1.17
	$\frac{\text{S}}{\text{Control}}$	.94	1.12	.91	.98	.96

Figure 7, showing CaO content in sweet clover sampled at 15-day intervals.

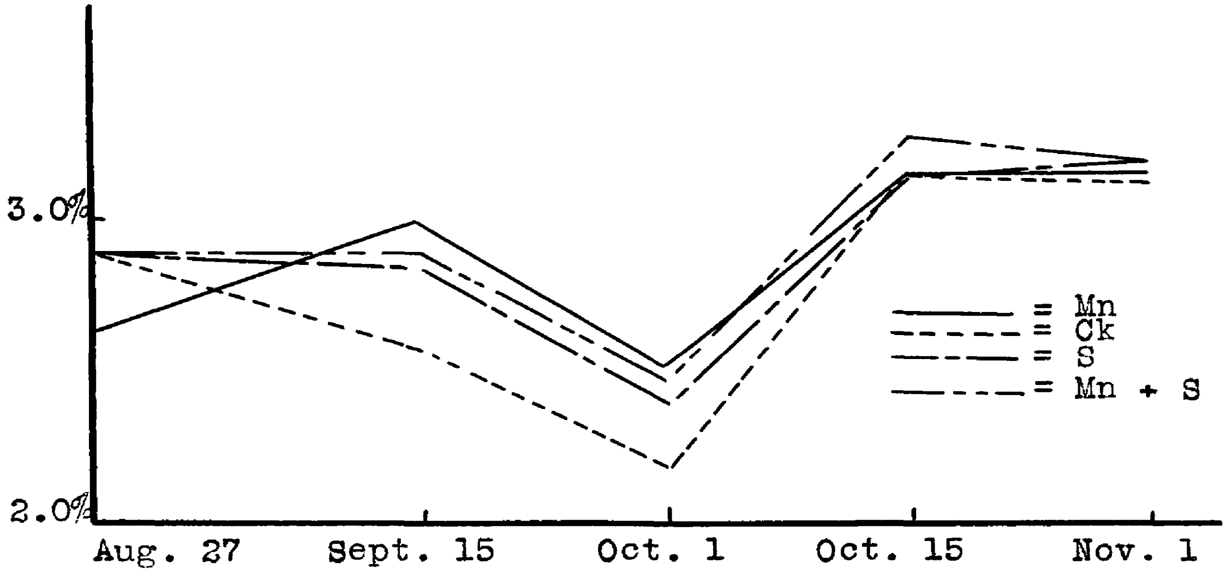


Figure 8, showing CaO content in alfalfa sampled at 15-day intervals.

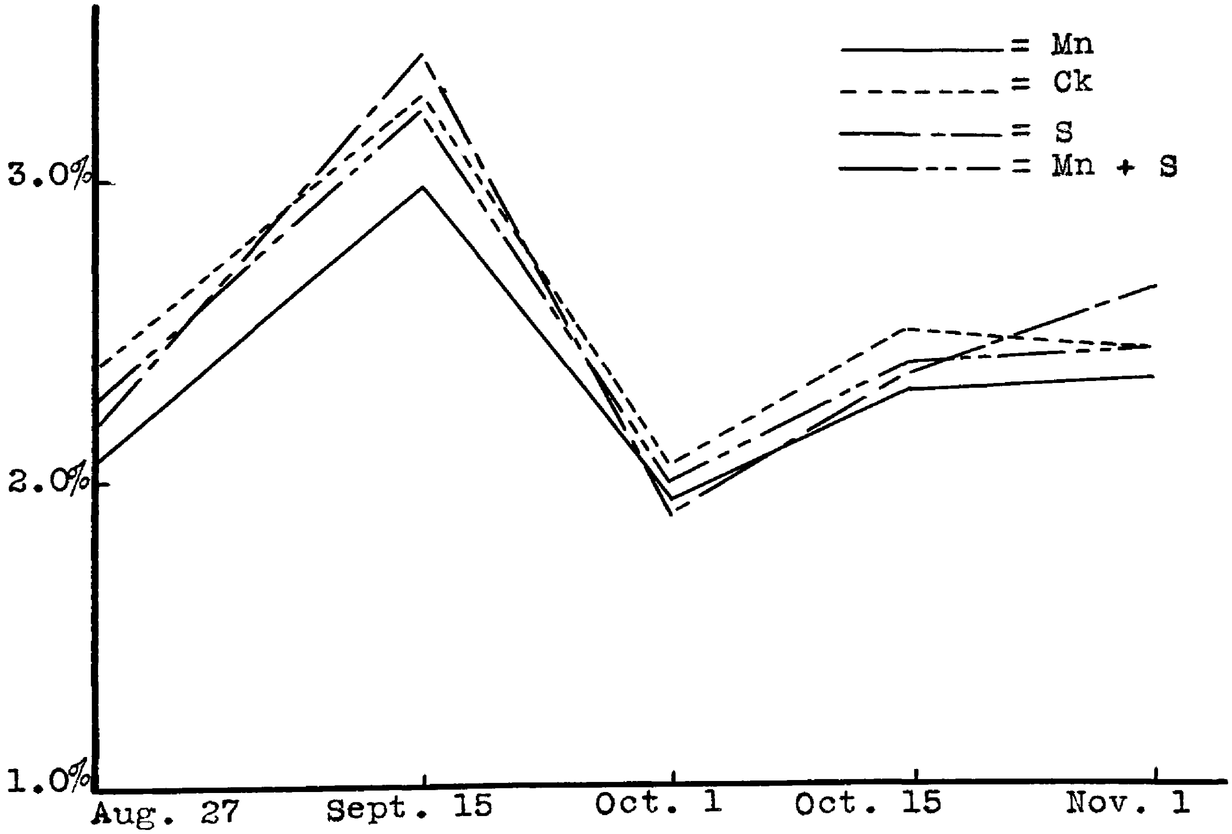


Table 29, showing the content of Mn_3O_4 in alfalfa plants sampled at 15-day intervals on Mn-S series

Plot	Treatment lbs. per acre	Percentage calculated on oven-drybasis				
		Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
1	$MnSO_4$-200	.00410	.00307	.00433	.00334	.00381
2	Control	.00222	.00242	.00173	.00247	.00216
3	$MnSO_4$-200 S - 1500	.00632	.00455	.00347	.00412	.00569
4	$MnSO_4$-400	.00693	.00541	.00364	.00511	.00541
5	Control	.00288	.00307	.00277	.00394	.00465
6	$MnSO_4$-400 S - 1500	.01304	.00724	.00992	.00140	.01082
7	S - 1500	.00258	.00277	.00364	.00284	.00374
8	Control	.00199	.00273	.00277	.00299	.00347
9	S - 3500	.00305	.00242	.00301	.00288	.00260
10	S - 2500	.00277	.00260	.00204	.00256	.00260
11	Control	.00199	.00260	.00173	.00216	.00260
12	$CuSO_4$-50	.00231	.00216	.00192	.00225	.00211
	Averages					
	Control	.00228	.00271	.00225	.00289	.00322
	Mn	.00502	.00425	.00399	.00423	.00461
	Mn + S	.00968	.00590	.00670	.00726	.00826
	Sulphur	.00280	.00260	.00290	.00276	.00296
	Ratio to control					
	$\frac{\text{Mn}}{\text{Control}}$	2.43	1.57	1.77	1.46	1.43
	$\frac{\text{Mn + S}}{\text{Control}}$	4.25	2.18	2.97	2.51	2.56
	$\frac{\text{S}}{\text{Control}}$	1.23	.96	1.29	.95	.93

Table 30, showing Mn$_3$04 content of alsike clover plants sampled at 15-day intervals on Mn-S series

Plot	Treatment lbs. per acre	Percentage calculated on oven-dry basis				
		Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
1	MnSO$_4$-200	.00485	.00385	.00420	.00478	.00513
2	Control	.00183	.00182	.00231	.00277	.00279
3	MnSO$_4$-200 S - 1500	.00528	.00496	.00577	.00659	.00728
4	MnSO$_4$-400	.00624	.00461	.00527	.00572	.00680
5	Control	.00225	.00211	.00347	.00354	.00347
6	MnSO$_4$-400 S - 1500	.00747	.00862	.00739	.00818	.00902
7	S - 1500	.00253	.00298	.00231	.00329	.00388
8	Control	.00218	.00201	.00347	.00395	.00513
9	S - 3500	.00277	.00241	.00235	.00329	.00312
10	S - 2500	.00260	.00274	.00208	.00347	.00378
11	Control	.00194	.00248	.00208	.00364	.00378
12	CuSO$_4$-50	.00218	.00216	.00231	.00277	.00347

Averages

Control	.00205	.00210	. 00283	.00347	.00379
Mn	.00555	.00423	.00473	.00525	.00596
Mn + S	.00637	.00679	.00658	.00739	.00815
Sulphur	.00263	.00271	.00225	.00335	.00359

Ratio to control

$\frac{\text{Mn}}{\text{Control}}$	2.70	2.01	1.67	1.51	1.57
$\frac{\text{Mn + S}}{\text{Control}}$	2.10	3.22	2.32	2.12	2.15
$\frac{\text{S}}{\text{Control}}$	1.28	1.29	.79	.96	.95

Table 31, showing Mn_3O_4 content of sweet clover plants taken at 15-day intervals on Mn-S series

Plot	Treatment lbs. per acre	Percentage calculated on oven-dry basis				
		Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
1	$MnSO_4$-200	.00216	.00230	.00182	.00242	.00301
2	Control	.00199	.00177	.00173	.00190	.00232
3	$MnSO_4$-200 S - 1500	.00427	.00223	.00236	.00277	.00315
4	$MnSO_4$-400	.00333	.00248	.00260	.00270	.00347
5	Control	.00249	.00231	.00216	.00336	.00322
6	$MnSO_4$-400 S - 1500	.00604	.00461	.00485	.00534	.00694
7	S - 1500	.00260	.00208	.00164	.00246	.00236
8	Control	.00160	.00187	.00156	.00211	.00253
9	S - 3500	.00199	.00177	.00173	.00208	.00232
10	S - 2500	.00205	.00209	.00173	.00232	.00232
11	Control	.00177	.00209	.00157	.00190	.00260
12	$CuSO_4$-50	.00194	.00210	.00189	.00197	.00246

Average

	Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
Control	.00197	.00201	.00176	.00232	.00267
Mn	.00274	.00239	.00221	.00256	.00324
Mn + S	.00516	.00342	.00361	.00406	.00504
Sulphur	.00222	.00198	.00170	.00229	.00233

Ratio to control

	Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
$\frac{\text{Mn}}{\text{Control}}$	1.39	1.19	1.26	1.10	1.21
$\frac{\text{Mn + S}}{\text{Control}}$	2.62	1.70	2.05	1.75	1.89
$\frac{\text{S}}{\text{Control}}$	1.13	.99	.97	.98	.87

Figure 9, showing M_3O_4 content in alfalfa sampled at 15-day intervals.

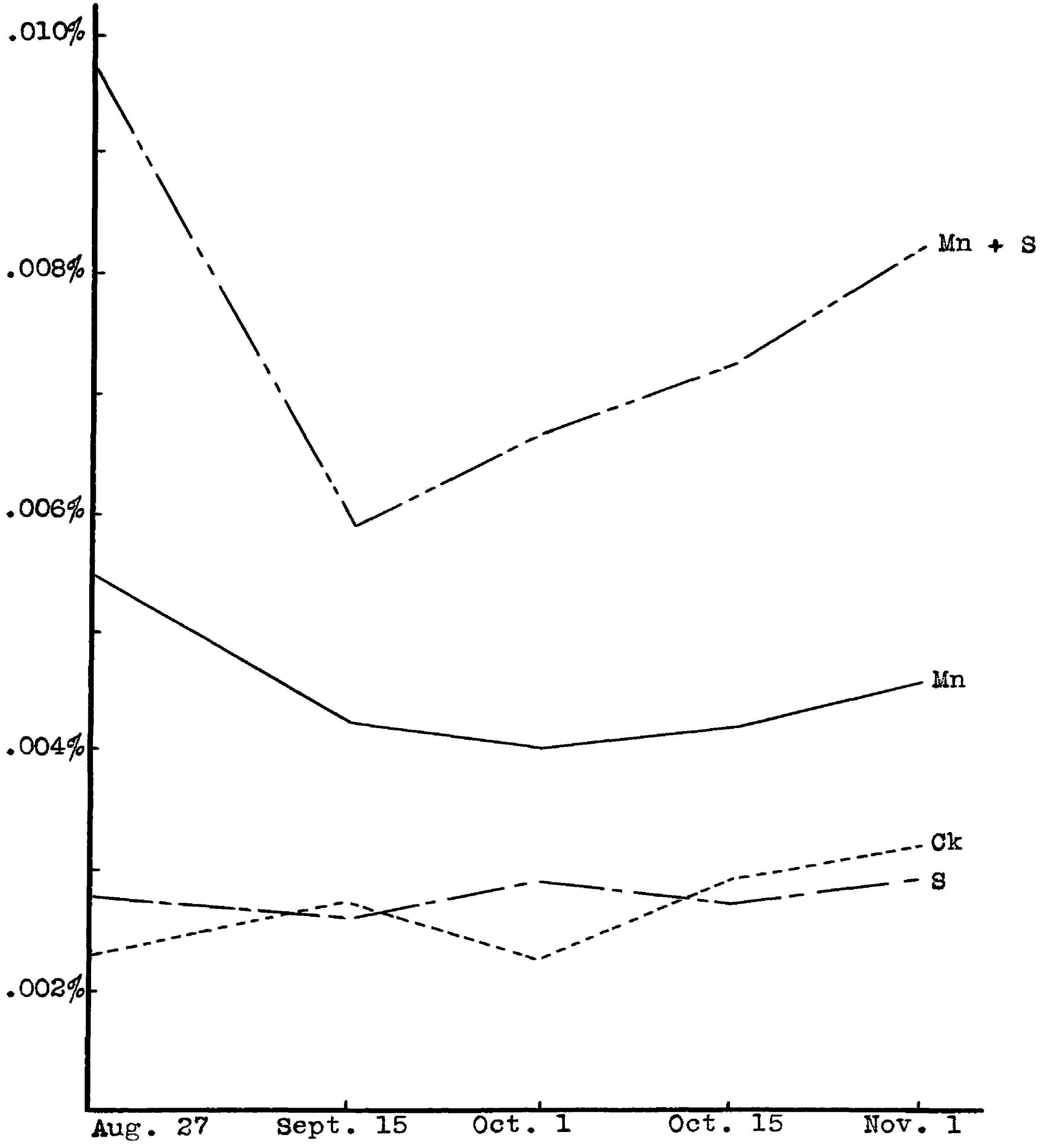


Figure 10, showing Mn_3O_4 content in sweet clover sampled at 15-day intervals.

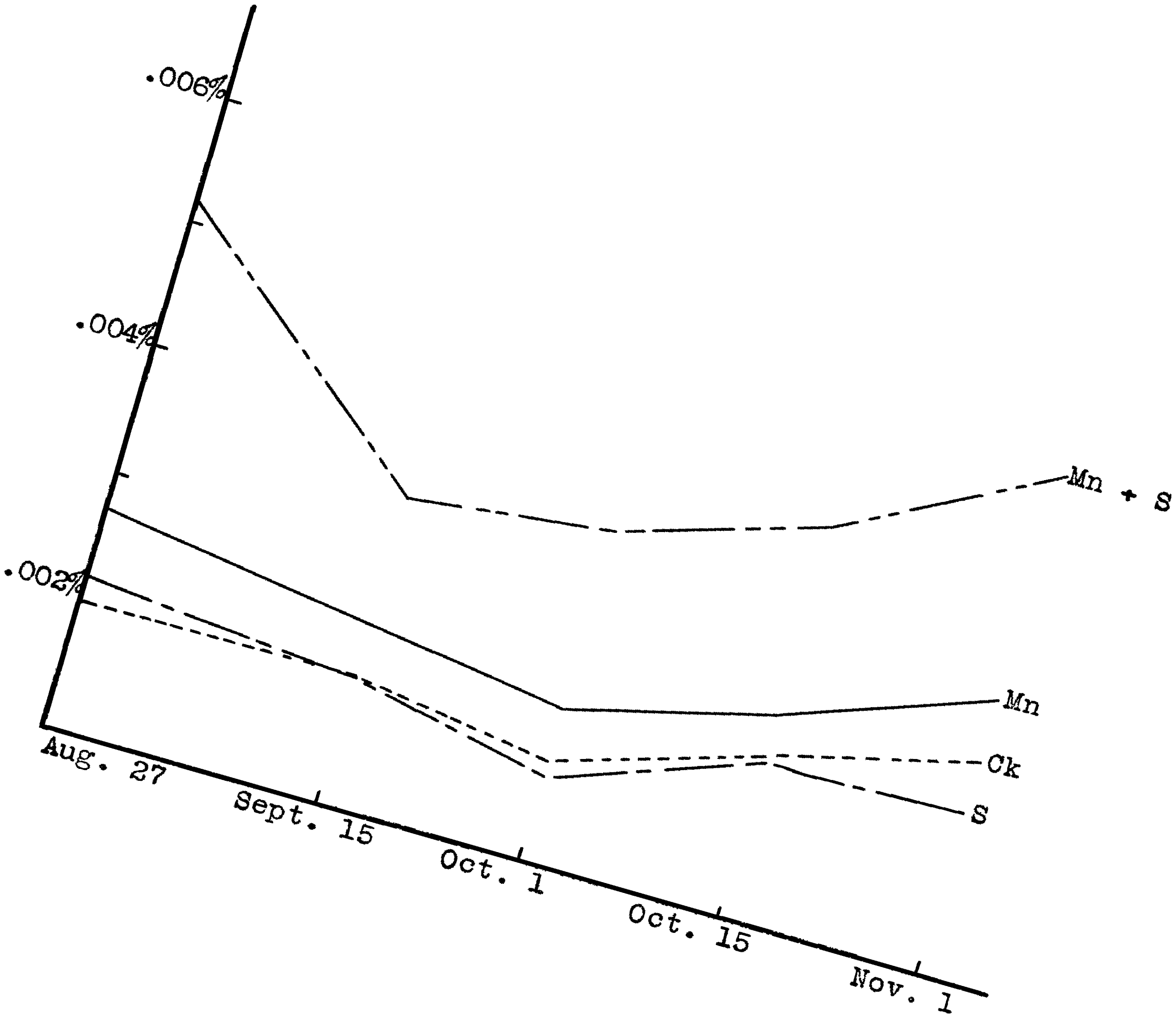


Table 32, showing the content of Fe_2O_3 in alfalfa plants sampled at 15-day intervals on Mn-S series

Plot	Treatment lbs. per acre	Percentage calculated on oven-dry basis				
		Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
1	$MnSO_4$-200	.04730	.03562	.03788	.04812	.04738
2	Control	.8188	.04037	.04018	.06935	.07844
3	$MnSO_4$-200 S - 1500	.06443	.03622	.03458	.03985	.04537
4	$MnSO_4$-400	.05321	.03725	.02899	.04450	.05628
5	Control	.08633	.04242	.05066	.04865	.05924
6	$MnSO_4$-400 S - 1500	.06503	.03002	.02282	.04140	.05332
7	S - 1500	.07390	.04037	.03071	.04140	.05430
8	Control	.09164	.03520	.04018	.06210	. 04936
9	S - 3500	.07330	.03622	.03114	.04760	.04787
10	S - 2500	.06739	.03105	.03229	.04450	.04887
11	Control	.08558	.04037	.04018	.05590	.05574
12	$CuSO_4$ - 50	.08070	.03777	.04348	.05072	.05034

Averages

	Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
Control	.08636	.03959	.04280	.05900	.06159
Mn	.05025	.03543	.03343	.04631	.05138
Mn + S	.06473	.03312	.02870	.04062	.04934
Sulphur	.07153	.03588	.03138	.04450	.05035

Ratio to control

	Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
$\frac{\text{Mn}}{\text{Control}}$	.58	.89	.78	.78	.83
$\frac{\text{Mn + S}}{\text{Control}}$	.75	.84	.67	.69	.78
$\frac{\text{S}}{\text{Control}}$	.83	.91	.73	.75	.82

Table 33, showing the content of Fe_2O_3 in alsike clover plants sampled at 15-day intervals on Mn-S series

Plot	Treatment lbs. per acre	Percentage calculated on oven-dry basis				
		Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
1	$MnSO_4$-200	.03547	.01837	.02023	.02608	.03603
2	Control	.03793	.01952	.02511	.02980	.03751
3	$MnSO_4$-200 S - 1500	.03448	.01569	.01937	.02442	.03702
4	$MnSO_4$ -400	.03253	.02009	.02023	.02608	.03751
5	Control	.03942	.02009	.02224	.03146	.04147
6	$MnSO_4$ -400 S - 1500	.03448	.01837	.01937	.02608	.03949
7	S - 1500	.03448	.01837	.02023	.03064	.03949
8	Control	.04779	.02124	.02152	.03230	.03553
9	S - 3500	.03547	.01952	.01995	.03064	.03949
10	S - 2500	.03841	.01908	.01995	.02732	.03751
11	Control	.03547	.02009	.02267	.02816	.03849
12	$CuSO_4$ - 50	.04926	.02124	.02267	.02650	.05034

Averages

		Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
	Control	.04015	.02023	.02288	.03057	.03825
	Mn	.03400	.01923	.02023	.02608	.03677
	Mn + S	.03448	.01700	.01937	.02525	.03825
	Sulphur	.03621	.01899	.02004	.02953	.03883

Ratio to control

		Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
	$\frac{\text{Mn}}{\text{Control}}$	.85	.95	.88	.85	.96
	$\frac{\text{Mn + S}}{\text{Control}}$	.86	.84	.85	.83	1.00
	$\frac{\text{S}}{\text{Control}}$	.90	.94	.88	.97	1.02

Table 34, showing the content of Fe_2O_3 in sweet clover plants sampled at 15-day intervals on Mn-S series.

	Treatment	Percentage calculated on oven-dry basis				
Plot	lbs. per acre	Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
1	$MnSO_4$-200	.02267	.01738	.01822	.02070	.02173
2	Control	.01970	.01656	.01822	.01864	.02270
3	$MnSO_4$-200 S - 1500	.02167	.01738	.01607	.02070	.02371
4	$MnSO_4$-400	.02464	.01822	.01822	.01946	.02371
5	Control	.02464	.01698	.01736	.02070	.02371
6	$MnSO_4$-400 S - 1500	.02267	.01988	.01550	.01946	.02371
7	S - 1500	.02167	.01822	.01478	.02028	.02614
8	Control	.02167	.01576	.01894	.02070	.02371
9	S - 3500	.02167	.01780	.01736	.01864	.02371
10	S - 2500	.02267	.01656	.01650	.01904	.02371
11	Control	.02267	.01490	.01578	.02070	.02371
12	$CuSO_4$-50	.02363	.01614	.01794	.01988	.02468

Averages

	Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
Control	.02217	.01605	.01757	.02018	.02346
Mn	.02365	.01780	.01822	.02008	.02272
Mn + S	.02217	.01863	.01579	.02008	.02371
Sulphur	.02201	.01753	.01621	.01932	.02452

Ratio to control

	Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
$\frac{\text{Mn}}{\text{Control}}$	1.07	1.11	1.04	1.00	.97
$\frac{\text{Mn + S}}{\text{Control}}$	1.00	1.16	.90	1.00	1.01
$\frac{\text{S}}{\text{Control}}$	.99	1.09	.92	.96	1.05

Figure 11, showing Fe_2O_3 content in sweet clover sampled at 15-day intervals.

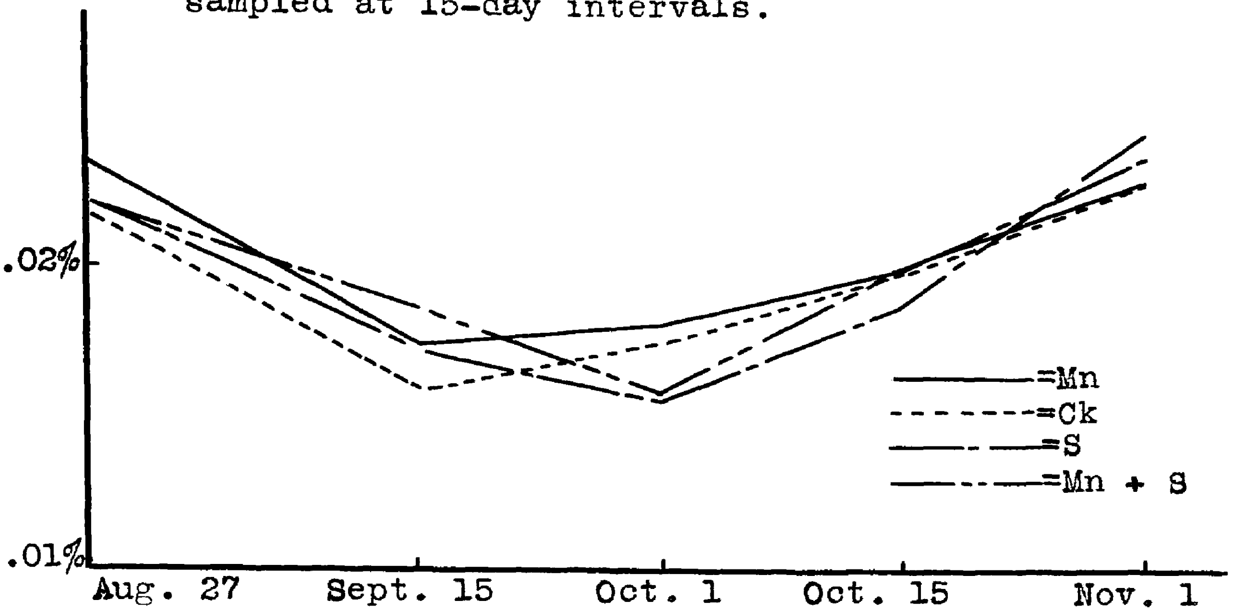


Figure 12, showing Fe_2O_3 content in alfalfa sampled at 15-day intervals.

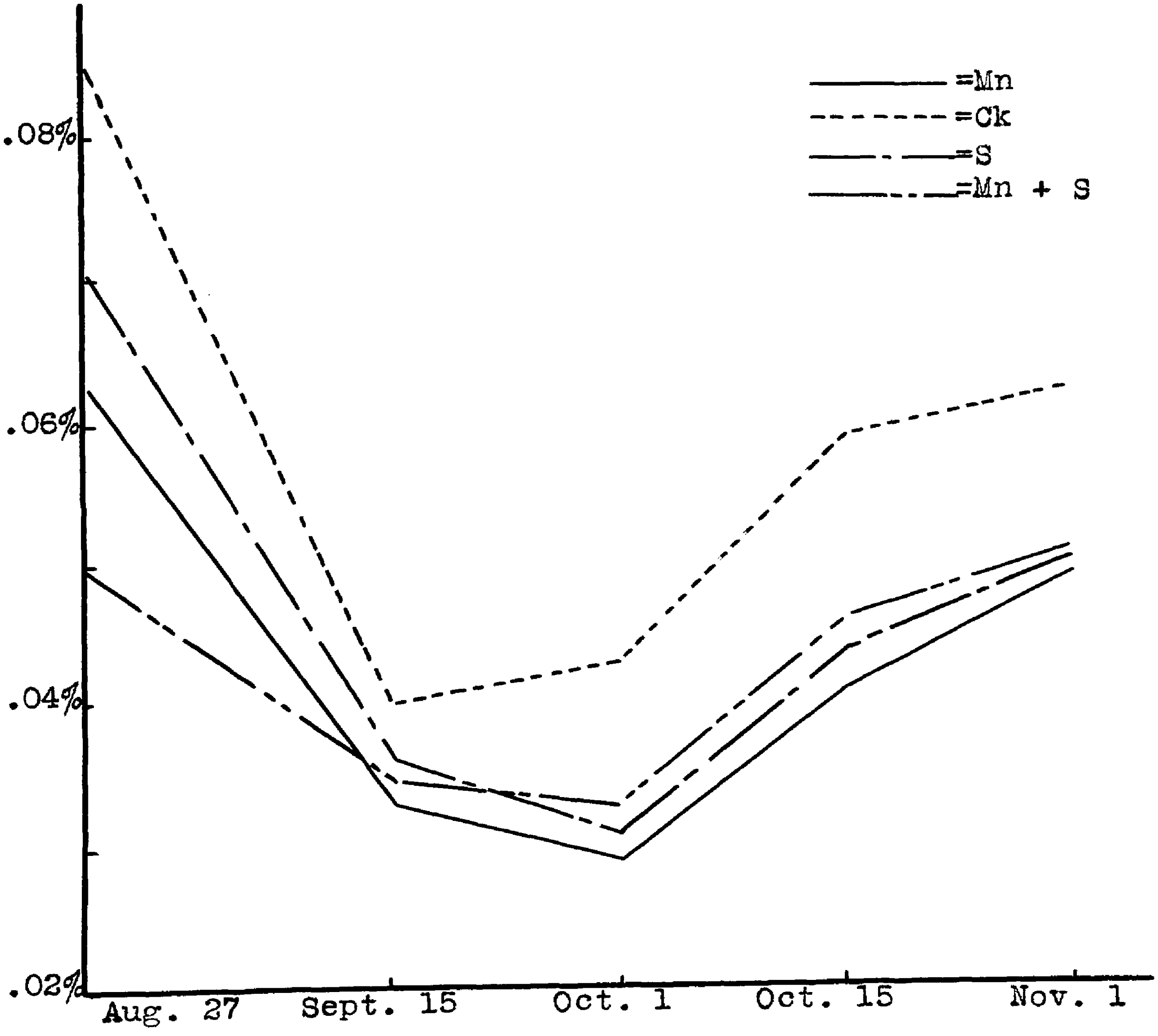


Table 35, showing the content of MgO in alfalfa plants sampled at 15-day intervals on Mn-S series

Plot	Treatment lbs. per acre	Percentage calculated on oven-dry basis				
		Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
1	$MnSO_4$-200	.463	.402	.437	.398	.442
2	Control	.485	.478	.480	.472	.466
3	$MnSO_4$-200 S - 1500	.461	.407	.405	.399	.391
4	$MnSO_4$-400	.483	.411	.391	.418	.442
5	Control	.579	.432	.517	.441	.418
6	$MnSO_4$-400 S - 1500	.519	.407	.449	.394	.432
7	S - 1500	.483	.412	.449	.390	.377
8	Control	.502	.468	.497	.498	.452
9	S - 3500	.531	.420	.435	.420	.432
10	S - 2500	.483	.438	.406	.420	.458
11	Control	.563	.458	.471	.462	.437
12	$CuSO_4$-50	.483	.478	.483	.478	.403
	Averages					
	Control	.532	.459	.491	.468	.443
	Mn	.473	.406	.414	.408	.442
	Mn + S	.490	.407	.427	.396	.411
	Sulphur	.499	.423	.430	.410	.422
	Ratio to control					
	$\frac{\text{Mn}}{\text{Control}}$	.89	.88	.84	.87	1.00
	$\frac{\text{Mn + S}}{\text{Control}}$	.92	.89	.87	.84	.93
	$\frac{\text{S}}{\text{Control}}$	.94	.92	.87	.88	.95

Table 36, showing the content of MgO in alsike clover plants sampled at 15-day intervals on Mn-S series

Plot	Treatment lbs. per acre	Percentage calculated on oven-dry basis				
		Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
1	$MnSO_4$-200	.480	.414	.454	.481	.543
2	Control	.572	.432	.414	.482	.538
3	$MnSO_4$-200 S - 1500	.567	.414	.449	.512	.603
4	$MnSO_4$-400	.514	.414	.444	.485	.531
5	Control	.637	.432	.436	.482	.531
6	$MnSO_4$-400 S - 1500	.611	.401	.470	.474	.531
7	S - 1500	.555	.421	.450	.484	.531
8	Control	.563	.432	.422	.444	.468
9	S - 3500	.570	.406	.374	.444	.495
10	S - 2500	.563	.406	.403	.466	.502
11	Control	.640	.414	.442	.460	.502
12	$CuSO_4$- 50	.534	.428	.410	.478	.531

Averages

	Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
Control	.603	.427	.428	.467	.510
Mn	.497	.414	.448	.483	.537
Mn + S	.589	.407	.459	.493	.567
Sulphur	.563	.411	.409	.465	.509

Ratio to control

	Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
$\frac{\text{Mn}}{\text{Control}}$	.82	.97	1.05	1.04	1.05
$\frac{\text{Mn + S}}{\text{Control}}$	.98	.95	1.07	1.06	1.11
$\frac{\text{S}}{\text{Control}}$	.93	.96	.96	1.00	1.00

Table 37, showing the MgO content of sweet clover plants sampled at 15-day periods on Mn-S series

Plot	Treatment lbs. per acre	Percentage calculated on oven-dry basis				
		Aug. 27	Sept. 15	Oct. 1	Oct. 15	Nov. 1
1	$MnSO_4$-200	.707	.536	.521	.471	.471
2	Control	.635	.501	.478	.495	.471
3	$MnSO_4$-200 S - 1500	.736	.540	.534	.483	.483
4	$MnSO_4$-400	.693	.548	.553	.507	.471
5	Control	.659	.499	.505	.495	.471
6	$MnSO_4$-400 S - 1500	.719	.555	.507	.507	.538
7	S - 1500	.712	.541	.461	.471	.483
8	Control	.536	.476	.447	.464	.434
9	S - 3500	.679	.522	.492	.464	.492
10	S - 2500	.621	.518	.453	.519	.524
11	Control	.579	.462	.437	.422	.444
12	$CuSO_4$-50	.591	.476	.488	.434	.471
	Averages					
	Control	.602	.484	.467	.469	.455
	Mn	.700	.542	.537	.489	.471
	Mn + S	.727	.547	.520	.495	.510
	Sulphur	.671	.527	.469	.485	.500
	Ratio to control					
	$\frac{\text{Mn}}{\text{Control}}$	1.16	1.12	1.15	1.04	1.03
	$\frac{\text{Mn + S}}{\text{Control}}$	1.21	1.13	1.11	1.05	1.12
	$\frac{\text{S}}{\text{Control}}$	1.11	1.09	1.00	1.04	1.10

Figure 13, showing MgO content in alfalfa sampled at 15-day intervals.

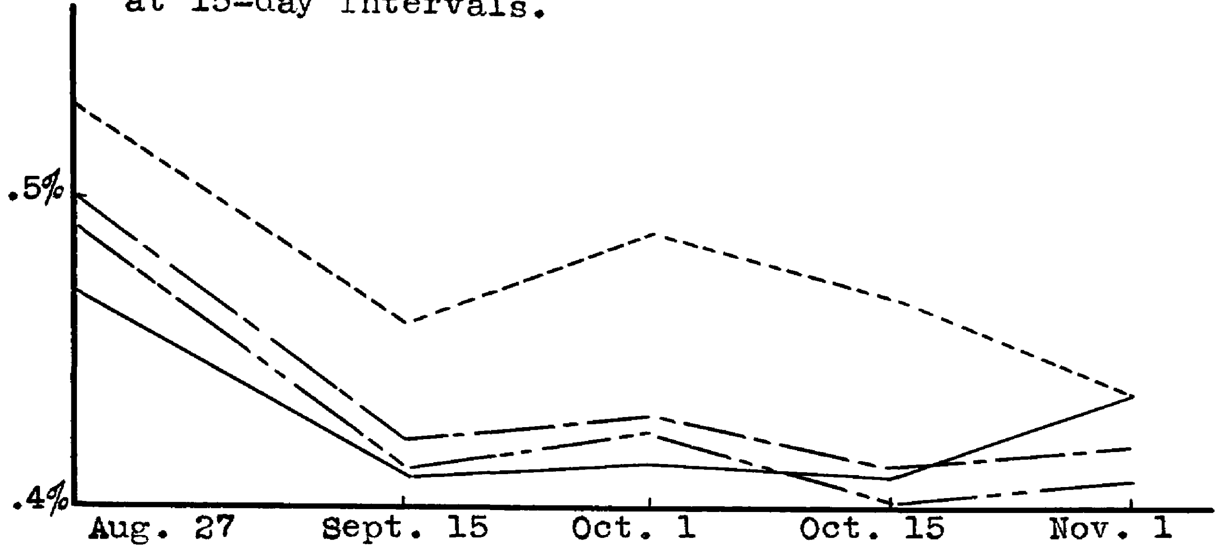
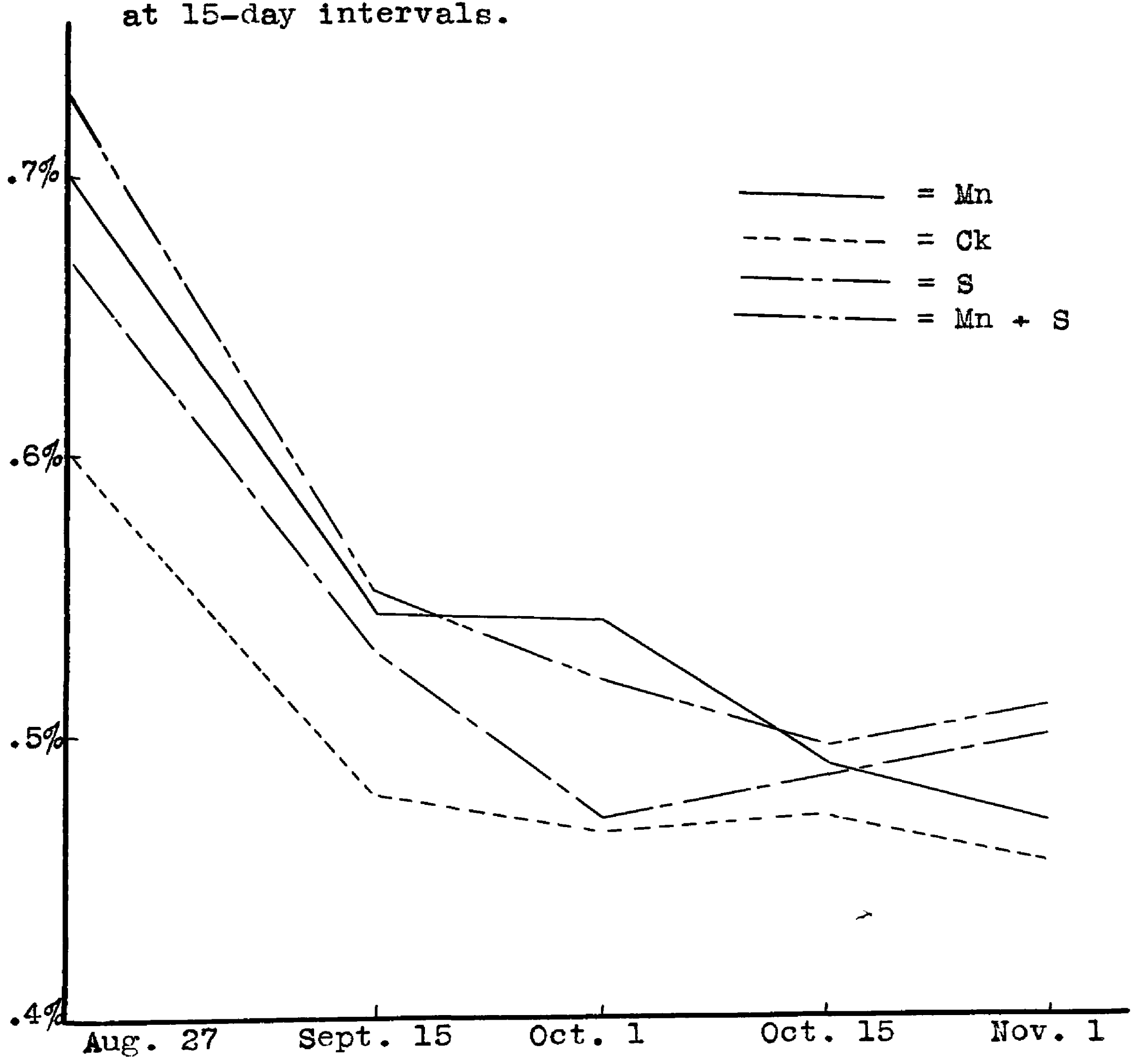


Figure 14, showing MgO content in sweet clover sampled at 15-day intervals.



FALLACY OF PRESENT THEORIES

The data thus far presented do not appear to support the theory that manganese is an essential nutrient. Perhaps one would be safer to say that, if it is essential, it also plays an accessory function in plants, which function may be of far more importance than the part it plays as a nutrient. Analysis of plants show that there is a great variability in the manganese content in plants. The variation between the quantities of manganese present in two samples of a plant species obtained from different sources may easily be tenfold. This situation may occur under one set of conditions with no effect upon the metabolic processes of the plant yet, under another, serious nutritional disturbance may result.

It is the contention here that the data do not support the nutrient theory of manganese and that it functions more in an accessory capăcity than as a required nutrient. There are several factors which form the basis for this contention, one of which is the relationship between crop yield and manganese content of the crop. The data concerning the manganese content of crops presented in this thesis show that the treatments combining manganese sulfate and sulphur give the highest values. In the case of manganese sulfate alone, it is high as compared to the controls. Little difference exists between the controls and the sulphur treatments; in some crops the sulphur treatment is slightly higher and in others, it is slightly lower. Crop yields do not follow the manganese content of the crop since the sulphur and the combination of sulphur and manganese sulfate give consistently the highest yields and also superior quality. Even the 400-pound application of manganese sulfate appears to be on the average only two-thirds as efficient in its effect on yields as are the above treatments. If manganese were the only limiting factor, there would be no reason for this inconsistency and, for this reason, the function of either manganese or sulphur must be an influence upon the other factors of plant nutrition.

Further support of this theory is given in some of Harmer's unpublished data (31) which showed increasing yields with increasing amounts of manganese sulfate applied up to 3,000 pounds per acre. His applications were 100, 250, 500, 1,000, 2,000 and 3,000 pounds per acre and onions were grown as a test crop. The crops were grown during the spring and early summer season. From the other analytical work it is known that, in all onions receiving more than a 250-pound-per-acre application, the manganese content would be high and therefore the increase in yield resulting from application above this quantity would be suggestive of the possibility of an intensity factor.

Peculiar responses of several crops were observed in the plant growth during the growing season, such as chlorotic plants becoming normal during period of hot weather and dry soil conditions. When the jars of soil, used in the greenhouse experiment reported above were sown to spinach in August, they produced a crop in which the controls outyielded the treated jars by three-fold. Soybeans grown in the greenhouse during August on the same manganese-deficient muck were chlorotic on those jars receiving manganese salts, while such treatments as copper sulfate and ferric sulfate produced normal plants. Later in the fall very remarkable growth was produced by spinach treated with copper and zinc separatively. *These occurred on a soil which gave very marked response to manganese in the greenhouse during the spring.* This situation is substantiated by results secured by Harmer who found in past years that the results he secured with manganese and sulphur applied in greenhouse experiments on other alkaline mucks in early winter was no indication as to what results would be secured on the same soils in the spring. Strongly acid organic soils have been found which have only a mere trace of manganese upon total analysis; in many cases, as mentioned before, they were often lower than the critical amounts given by some investigators. It may be concluded from this that the essentiality of manganese under these conditions would not be as great as under neutral or alkaline conditions. Sometimes, indeed, one might expect that there is no need for it under these conditions, a thought which is suggested by Martin's work with sugar cane shoots (50).

It has been shown by several workers, that there is a disturbance in the distribution of iron in the chlorotic plants. Normal plants appeared always to have a lower iron content than did the chlorotic plants, regardless of whether the material is reported on oven-dry basis or on its field condition. Kliman (42) found that iron in plant tissue existed as the ferrous and anionic forms. In the work reported in this paper it has been shown that ferric iron is concentrated in veinal tissue of chlorotic leaves, a disturbance in metabolism of iron is evident. Oserkowsky (58) has divided iron in the plant into two classes, available and unavailable. The iron which he considered available was that portion which was soluble in half-normal hydrochloric acid. Using this test, he found that chlorotic tissue contained less available iron than normal tissue. Ingalls and Shive (35) made a study of the relationship between the distribution of iron as related to hydrogen ion concentrations of tissue fluids. They reported that (1) Hydrogen ions concentration of tissue fluid corresponded to light intensity; (2) all plants showed differences in hydrogen ion concentration between leaves and stem; and (3) soluble and total iron content of tissue sap was greatly influenced by its hydrogen ion concentration. In the metabolism of iron an equilibrium between ferrous and ferric iron is set up, the direction of which in normal plants is strongly towards the ferrous form. The intensity of the directional trend can be shifted by internal and external factors which factors, if in dominance, may cause physiological disturbances within the plant. If sufficiently strong, these will cause a complete break down of the plant tissue.

Much of the recent work has shown support of the theory that the function of manganese is that of an activation of iron. Hopkins (34) has stated that the effect of manganese lies in the activation of iron and he believes that the iron is reduced in photosynthesis and that manganese reoxidizes it. The data presented in this paper strongly supports this theory. The iron apparently has entered the plant and has failed to function properly in the metabolic processes of plant growth due possibly to (1) an unfavorable mineral balance; (2) an unfavorable hydrogen ion concentration or (3) an unfavorable oxidation-reduction system of such intensity as to disrupt the functioning of iron, or perhaps a combination of any two or of all three. This paper is in full agreement with Willis and Piland's idea that copper and manganese will produce opposite effects and therefore both could function as activators of iron.

Since it is firmly believed that at least some of the minor elements play a part in the functioning of iron, and since some workers have suggested that climate may be a factor (30), it would be interesting to study the geographical location of the reported deficiencies of manganese, copper, iron, boron, zinc and cobalt. After mapping the distributions of these locations, one immediately notes that (1) the concentration of these deficiencies are in rather definite areas such as the humid climate regions which have a considerable precipitation during the cool part of the season, as western Europe, England, Australia, Hawaii, South Africa, Southern

France, and Northern Italy, the Baltic countries, Japan, Eastern Coast of United States and Canada, country adjacent to the Great Lakes, Florida, Gulf Coast of Texas, California, Oregon, Washington and several other western states; (2) in most regions, two or more minor element deficiencies occur; (3) many of these regions are regions in which nutritional troubles occur in animals, such as bush sickness and anemia.

Since the indications are that, in a lime-induced chlorosis, the metabolism of iron in the physiological processes breaks down, and the plants actually suffer from the lack of iron at the points where it is vitally necessary, it is quite apparent that such a break down necessarily involves a state of oxidation of the iron. For that reason, any factor which would affect this oxidation-reduction system or direction of the intensity of this system, would probably upset this equilibrium to the extent that a physiological disturbance would result. Several factors that may affect these intensities may act as follows: (1) Abnormal mineral composition may upset this equilibrium and produce a chlorosis. Several of these are known; manganese-induced chlorosis; lime-induced chlorosis and recently Curini-Galletti (14) describes a copper-induced chlorosis. Camp and Ruether report a boron-induced chlorosis in Florida (12); Nemec and Babecka (57) describe a chlorosis induced by excessive cobalt; chromium-induced chlorosis occurs in South Africa according to Van der Merve and Anderson (74). Greenhouse work shows that excessive amounts of zinc and titanium causes the chlorotic condition to become more severe, a result to be expected since they are capable of taking part in oxidation-reduction reactions. (2) External factors such as climate and perhaps quality of light may play a very important part in the direction of the oxidation-reduction intensities in metabolic processes of plants. Little is known of the nature of these factors and their effects. Haas and Quayle (30) suggest the climatic factor; the work of Jones (40) tends to show that soil temperature is extremely important in that, when soil temperature is below 18°C., gardenias become chlorotic. Recent work shows that light intensity may be a very important factor, since it produces changes in hydrogen ion concentration of the plant sap. Hydrogen ion concentration influences the oxidation-reduction intensities. These points are very important since, first, it influences the physiological processes of the plant which is important since the break down is within the plant. Second, the chemical elements under consideration are capable of resisting changes in oxidation-reduction intensities due to changes in hydrogen ion concentration. The recent work of Cooper, Paden and Smith (13) shows that the quality of radiant energy and some of the oxidation-reduction systems materially affect the intake of ions by plants. They hold further that those reactions, which occur at approximately the same energy level and which may be effective in bringing about certain oxidation-reduction reactions necessary in physiological processes, can be arranged into groups. They state,

> "It has been observed that, on certain soils relatively high in nitrates, there has been a marked response to applications of copper and in some instances to manganese. These responses are probably related to their effect upon oxidation-reduction reactions. The greatest growth response observed from additions of boron to nutrient media seems to be in complexes where iron may not be readily available. There is a relation between magnesium chloride and the reduction of the carbonate ion, which is a very important reaction in photosynthesis. Some of the important reactions such as the reduction of nitrates to nitrites, formation of hydrogen peroxide, the free energy decrease in the formation of cupric chloride and the change of valence of manganese from Mn^{++} to Mn^{+++} are on approximately the same energy level ....".

A third theory is the one Willis proposes (81) in which microbial activity in the soil influences the oxidation-reduction potential of the soil by depletion of the available oxygen. The work of Turk (72) gives very little support to this theory since he shows no effect by minor elements on the carbon dioxide or ammonia production in well decomposed organic soils. Bacterial activity should accelerate either one or both of these processes. Fourth, the effects of water logging may bring about chlorotic plants in that it causes highly reducing conditions which release bivalent ions, and also that it depletes the oxygen supply in the soil. A lack of available oxygen can be a possible fundamental factor, however, it is not in harmony with the fact that the deposition of iron is in the ferric form in the plant tissue.

After careful consideration, it was considered that, fundamentally, only two of the above factors were significant in this problem, namely, abnormal balance of the multivalent group of minor elements and second, the climatic factors. The combined effect of these two under natural soil conditions, is not only possible but highly probable. The problem now resolves itself into the following questions.

1. <u>Do minor elements, either alone or under specific climatic factors, affect the oxidation-reduction reactions of plants in such a manner as to disturb the state of oxidation of iron in the plant</u>?

2. <u>Do the minor elements alone or in combination affect some of the oxidation-reduction systems in the plant</u>?

To establish the answer to these questions, various minor elements in systems which would produce various oxidation intensities were used and a study was made as to their effect upon the plants, paying special attention to features of climate or light factors. The state of oxidation of the iron was carefully observed. To evaluate the effect upon oxidation-reduction systems in the plant, reduced and oxidized ascorbic acid, and reduced and oxidized glutathione were determined in chlorotic and normal tissue.

<u>Experimental</u>

This experiment was designed to determine the possibility of manganese acting in the oxidation-reduction processes of plant nutrition. It is assumed that, if it does, another ion capable of oxidation and reduction reactions would be capable of functioning in its place. Furthermore, if a reducing agent were added to the so-called manganese-deficient organic soils the chlorotic condition of the plants would become more pronounced. The application of manganese sulfate was taken as the basis of the oxidizing condition. It was assumed that the system $Mn^{++} \;\underline{\hspace{3em}}\; Mn^{+++}$ was set up by this application and that it would give a potential of -1.5. The system of $Fe^{++} \;\underline{\hspace{3em}}\; Fe^{+++}$ has a potential of -.74, therefore it should take twice as much ferrous sulfate to produce the same oxidation-reduction system as is produced by manganese sulfate. Titanous chloride, potassium chromate, chromic sulfate and ferrous sulfate were applied in this manner. The reducing agents were not added in quantities that would produce identical reducing conditions. The systems used are as follows:

Oxidation-Reduction Systems.

$$\begin{array}{lcl}
Mn^{++} & \longrightarrow & Mn^{++++} \\
Cr^{+++} & \longrightarrow & Cr^{++++} \\
CrO_4^{--} & \longrightarrow & CrO_4^{-} \\
Fe^{++} & \longrightarrow & Fe^{+++} \\
Ti^{+++} & \longrightarrow & Ti^{++++} \\
Cu^{++} & \longrightarrow & Cu^{+} \\
Fe^{+++} & \longrightarrow & Fe^{++} \\
Zn^{++} & \longrightarrow & Zn^{+} \\
 & \longrightarrow &
\end{array}$$

An alkaline muck was selected for the experiment, having a pH of about 7.8. All pots were given 1,000 pounds per acre of an 0-8-24 fertilizer made up of chemically pure chemicals. Each treatment was replicated four times so as to make a statistical analysis of yield data possible.

Eight spinach plants were planted in each jar. Titanous chloride was found to be mildly toxic to the plants in the early stages of their growth and it also affected the physical condition of the soil by causing it to become granular. The spinach was harvested April 7, 1939, and the following data was obtained and is shown in Table 38.

In considering these data, one is immediately impressed by the high yield produced by manganese sulfate which is not only significantly greater than that of the control treatment but also than of all other treatments, including manganese acetate, on the five per cent basis. Significant increases over the control were obtained in the two treatments with manganese, and two with chromium while the one with ferrous sulfate was on the border line of five per cent significance. The first four were also significant on the one per cent basis. The zinc acetate treatment produced yields that were significantly lower than these produced on the control and, since this treatment has the highest reducing possibilities, this is to be expected if the oxidation-reduction theory holds good.

Since color of the plants indicates the cure of the chlorotic condition, it must be considered as a part of the data. For this reason the ferrous sulfate treatment should be considered as giving positive results, especially since

Table 38, yields of spinach on Oxidation-Reduction Experiment Green Weight in grams.

Treatment	Block A	Block B	Block C	Block D	Ave.	Color of plants	Significance
Check	70.2	85.8	74.7	62.7	73.3	Greenish - yellow	
375# $MnSO_4$	142.2	122.2	130.7	125.1^x	130.1	Dark green	**
350# Mn Acetate	126.8	102.7	107.6^x	106.3	110.9	Dark green	**
450# Zn Acetate	49.2	69.7	49.6	32.4	50.2	Yellow	**
1200# $Cr_2(SO_4)_3$	102.7	110.4	86.0	90.6	97.4	Green	**
1125# $FeSO_4$	82.2	97.4	91.2	87.7	89.6	Green	
750# $TiCl_3$	62.7	55.2	77.8	65.2	65.2	Green	
320# $CuSO_4$	88.4	88.0	56.0	52.8	71.3	Greenish - yellow	
250# $Fe_2(SO_4)_3$	66.8	58.4	70.0	73.0	67.0	Yellow	
690# K_2CrO_4	111.3	103.4	81.0	112.0	101.9	3 green 1 greenish-yellow	**

x Data supplied by missing value formula by W. D. Baten.

Analysis of Variance

Source	D.F.	S S	Var.	Exp. Error
Total	39	25824.9	662.2	
Replication	3	685.1	228.4	
Treatment	9	21603.9	2400.4**	
Error	27	3535.9	130.9	11.44

F = 18.3

Difference for significance between means 1% = 22.4
5% = 16.6

those plants treated with ferric sulfate were yellow. The titanous chloride treatments gave plants which are healthy in all respects except that the injury in their early growth stunted their final development.

In Table 39 is shown yields of Mandarin soybeans on the same jars as reported in Table 38. The treatments were altered and, in some cases, the additions brought the total application of the two to double the original. Application of 500 pounds per acre of a chemically pure 0-8-24 fertilizer was applied before planting the seed. The data obtained in the experiment gave very conclusive evidence for the manganese salts which were the only treatment which resulted in normal plants.

In Table 40 is shown the yields of Cayuga soybeans on the same jars used in the preceding table. The jars again received 500 pound-per-acre applications of a chemically pure 0-8-24 fertilizer before seeding. Very unusual responses were obtained in these jars. The jars receiving manganese salts were chlorotic during most of the growing season of this crop. Potassium chromate grew the largest plants of all treatments with copper sulfate, chromic sulfate and the iron salts following in the order named. However, it must be mentioned that the plants receiving many of these treatments were chlorotic at certain stages of growth; those receiving the chromium salts became partially chlorotic in the later stages of their growth. Those

Table 39, yields of Mandarin soybeans on Oxidation-Reduction Experiment. Seed sown April 10, 1939. Harvested June 5, 1939. Green weight in grams.

Treatment	Block A	Block B	Block C	Block D	Ave.	Significance
Control	41.2	38.1	35.7	38.8	38.4	
750# $MnSO_4$	46.2	51.5	50.1	78.0	56.4	**
700# Mn Acetate	50.5	65.0	81.3	56.2	63.2	**
450# Zn Acetate	35.6	34.0	42.5	40.7	38.2	
2380# $Cr_2(SO_4)_3$	40.2	38.5	36.8	35.8	37.8	
2250# $FeSO_4$	36.5	33.4	37.2	33.8	34.7	
1500# $TiCl_3$	37.9	40.8	31.1	40.2	37.5	
640# $CuSO_4$	35.9	35.8	37.3	40.2	37.3	
340# $Fe_2(SO_4)_3$	31.8	43.1	34.0	36.9	36.4	
1380# K_2CrO_4	33.4	30.0	37.5	41.8	33.2	

Analysis of Variance

Source	D.F.	S S	Var.	S.D.
Total	39	5114.6		
Replications	3	143.6		
Treatment	9	3591.7	399.08	
Error	27	1379.3	51.09	7.15

F = 7.81

Difference for significance between means 1% = 14.00
5% = 10.37

Table 40, yields of Cayuga soybeans (grain) on Oxidation-Reduction Experiment. Sown June 10, 1939, harvested Aug. 20, 1939. Weight in grams.

Treatment	Block A	Block B	Block C	Block D	Average	Significance
Control	12.0	12.7	14.0	13.3	13.00	
750# Mn SO_4	12.4	12.2	13.6	10.0	12.04	
700# Mn Acetate	10.6	11.8	13.6	10.9	11.72	
450# Zn Acetate	10.8	11.0	11.8	10.4	11.00	*
2380# $Cr_2(SO_4)_3$	16.8	13.0	15.4	13.6	14.70	
2250# $FeSO_4$	16.4	12.0	14.3	15.5	14.55	
1500# $TiCl_3$	4.8	4.9	6.3	4.2	4.40	**
640# $CuSO_4$	17.2	15.5	14.2	14.7	15.40	**
340# $Fe_2(SO_3)_4$	14.4	14.4	15.1	14.8	14.67	
1380# K_2CrO_4	17.7	17.7	20.5	19.5	18.85	**

Analysis of Variance

Source	D. F.	S. S.	Var.	S. D.
Total	39	519.9		
Replication	3	11.6		
Treatment	9	470.8	52.31	
Error	27	37.5	1.39	1.18

F = 37.26

Difference for significance between means 1% = 2.31
5% = 1.71

receiving copper sulfate and ferric sulfate were very chlorotic during their early growth but became normal in appearance during late growth. Those receiving ferrous sulfate were very irregular. The titanium treatment produced very injurious effects.

In Table 41 is shown data received on these jars when sown to spinach in the fall. On two blocks of this experiment, red spider destroyed the spinach to the extent that they were of little value as data. The remaining blocks gave very interesting results but some of them were entirely observational and are not expressed in the final data. Zinc acetate treatments stimulated the growth of the spinach in the early growth. This stimulation did not last long, as these plants became very chlorotic later. Marked response was obtained on those jars receiving ferrous sulfate and copper sulfate. The manganese salts were beginning to show beneficial effects late in their growth. Ascorbic acid and glutathione determinations were made on these plants and they are shown later in Tables 49 and 50, respectively.

Wolverine oats were seeded in these jars January 5, 1940, and the following data secured as given in Table 42. A very marked response was obtained from the manganese salts which was significant over that from all other treatments. The results from the zinc and titanium salts were significantly lower. The oats receiving the manganese sulfate had a healthy green color, while with all other treatments, the leaves showed infestation of grey speck disease.

Table 41, showing yields of spinach on Oxidation-Reduction Experiment. Sown Sept. 1, 1939, harvested Nov. 27 to Dec. 1, 1939. Green weight in grams.

Treatment	Block A	Block B	Average
Check	59.7	50.4	55.0
750# $MnSO_4$	57.1	45.6	51.3
700# Mn Acetate	28.0	72.7	50.3
450# Zn Acetate	54.0	44.0	49.0
2380# $Cr_2\ (SO_4)_3$	51.6	46.8	49.2
2250# $FeSO_4$	60.8	76.7	68.7
1500# $TiCl_3$	55.0	31.4	43.2
640# $CuSO_4$	62.5	74.2	68.3
340# $Fe\ (SO_4)_3$	40.6	45.5	43.0
1380# K_2CrO_4	43.2	51.0	47.1

Table 42, showing yields of Wolverine oats on Oxidation-Reduction Experiments in Greenhouse. Sown Jan. 5, and harvested Febr. 26, 1940.

Treatment	Block A	Block B	Block C	Block D	Average	Significance
Control	17.3	24.7	24.5	20.4	21.7	
750# $MnSO_4$	54.6	58.9	50.1	56.4	55.0	**
700# Mn Acetate	39.6	61.6	54.0	61.1	54.1	**
450# Zn Acetate	13.2	15.7	15.5	18.5	15.7	*
2350# $Cr_2(SO_4)_3$	23.7	22.0	26.5	16.8	22.3	
2250# $FeSO_4$	16.0	25.5	23.8	18.8	21.0	
1500# $TiCl_3$	11.7	15.6	16.7	12.0	14.0	*
640# $CuSO_4$	18.1	18.2	22.2	22.5	20.3	
500# $Fe_2(SO_4)_3$	26.5	27.6	27.0	27.4	27.1	
1380 K_2CrO_4	19.6	20.7	27.9	21.0	22.3	

Analysis of Variance

Source	D. F.	S. S.	Var.	S. D.
Total	39	8460.1		
Replications	3	160.9		
Treatment	9	7862.9	873.7	4.02
Error	27	436.2	16.16	

Difference for significance between means 1% = 7.87
5% = 5.83

Grey speck disease made its first appearance on the cultures receiving zinc and titanium salts. Those receiving manganese sulfate treatment showed the greatest resistance to the disease, with manganese acetate nearly as efficient. Table 43 gives some idea as to effect of treatment upon rate of infection.

Table 43, showing per cent of plants exhibiting grey speck disease at different stages of growth. Sown Jan. 5, 1940.

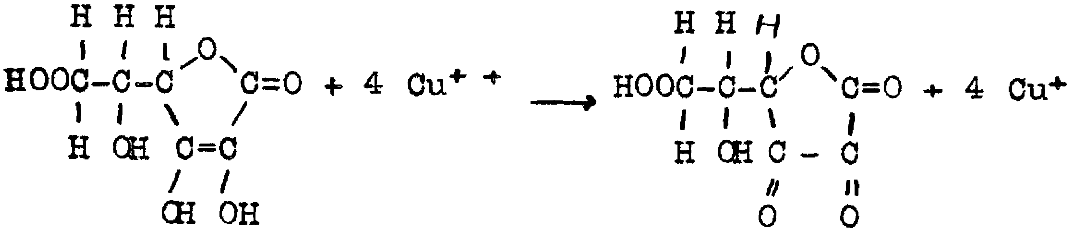
Treatment	Date of Counts	
	Feb. 17	Feb. 24
Control	34	98
Manganese sulfate	1	1
Manganese acetate	3	3
Zinc acetate	99	100
Chromic sulfate	22	91
Ferrous sulfate	14	79
Titanous chloride	91	100
Copper sulfate	24	98
Ferric sulfate	25	100
Potassium chromate	15	76

Ascorbic acid and glutathione were determined in samples of this oat and the data is reported in Tables 51 and 52.

The Effect of Minor Element Fertilization on Oxidation-Reduction Systems Within Plant.

The recent findings of various workers indicates that the application of various minor elements to nutrient solutions may affect the oxidation-reduction systems of ascorbic acid and glutathione. Euler, Myrböck and Larrson (18) have shown that the application of copper, manganese and nickel ions increased the oxidation of ascorbic acid; also that cobalt inhibited this oxidation. These results are substantiated by Green, McCarthy and King (24) who found that the aerobic oxidation of ascorbic acid is very sensitive to catalysis by copper protein complexes in a great variety of expressed plant press juices; also they found that both copper and ascorbic acid are found in relatively high concentrations in green leaves or other tissue capable of photosynthesis. Further, they believe that although the evidence is far from complete, it is not unreasonable to believe that copper and ascorbic acid may be concerned directly or indirectly with both respiration and photosynthesis in green plants. In another report (52) the same authors describe various systems in which a rapid oxidation of ascorbic acid is due to the catalytic effect of copper combined with proteins. They enumerated other plant and animal oxidative catalysts as those that lead to the quinone formation, the cytochrome systems and the hemochromogens, all of which can obviously act as catalysts for the oxidation of ascorbic acid. They conclude that the dominant active agent in many plants appears to be copper. Guzman Barron, Demeio and Klemperer (26), from their studies of the oxidation of ascorbic acid by copper and hemochromogens, propose the following equations:

(1)



$$(2)\quad 4\ Cu^{+} + 4H^{+} + 2O_2 \longrightarrow 4\ Cu^{++} + H_2O_2$$

$$(3)\quad 2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$$

Euler indicated that he obtained similar results with the use of manganese in his experiments. Rudra (60) reports that nutrient solutions containing manganese produced more ascorbic acid in the plants. In reviewing the work of these investigators, it will be found that those reporting benefit from manganese worked at a pH above 6.0. The significance of this will be brought out in later discussion.

Glutathione exists in plants and animals in both the reduced and oxidized form and, for this reason, should reflect the oxidation-reduction intensities that exist. This system might be expressed by the following equations: -

$$2\ HOOC.CHNH_2.CH_2SH \overset{Ox}{\underset{Red}{\rightleftarrows}} HOOC.CHNH_2.CH_2S\text{-}SH_2C.CHNH_2.COOH$$

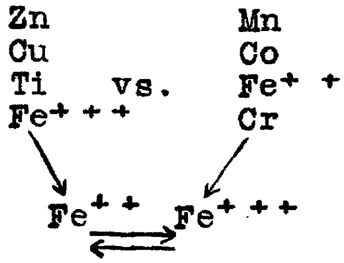
or G-S-S-G the oxidized form and G-Sh the reduced form. This system has been studied considerably in the blood of animals. Lyman and Guzman Barron (47) studied the oxidation-reduction systems in living cells. They state, "Glutathione and ascorbic acid seem to possess closely related properties in the chemical activities of biological systems, maintaining graded levels of reduction intensity necessary for the performance of certain biochemical processes." If glutathione is similar to ascorbic acid in properties, it will not be surprising to find that it enters into oxidation-reduction reactions with copper and manganese. They also bring out this reaction of glutathione and iron: -

$$(1)\ 2\ Fe^{+++} + 2GSH \rightleftarrows 2\ Fe^{++} + G\text{--}S\text{-}SH + 2H^{+}$$

$$(2)\ Fe^{++} + 2H^{+} + \tfrac{1}{2}\ O_2 \rightleftarrows 2\ Fe^{+++} + H_2O$$

These reactions are intensely interesting, since it shows the possibility of an oxidation-reduction tie-up between iron and glutathione. These findings may have much to do with either chlorophyll formation or its function in plants.

This study has for its purpose the exploration of the possibility of the minor elements, manganese, copper, iron, chromium, zinc influencing the oxidation-reduction equilibriums within the plant. From the greenhouse studies and various field observations, it has been noted that these various elements give marked responses to plant growth, the response depending upon the environmental conditions. These conditions appear to manifest themselves either directly or indirectly as soil temperature. The application of a manganese salt to an alkaline muck in the spring, when the soil temperature is low, will cause chlorotic plants to become normal. If the temperature of the soil were raised to a higher level, these same plants would become chlorotic. At this point, an application of copper salt would cause the plants to become normal again. It follows therefore that two types of chlorosis exist; namely, a minimum temperature chlorosis, characterized by a mottled leaf with prominent dark green veins, and the other, maximum temperature chlorosis, characterized by a yellowish-green leaf with almost transparent veins. In both cases the iron content appears to be affected and the results of staining many leaves indicate that the difference appears to be in the state of oxidation of iron. Since iron is a catalyst to the formation of chlorophyll, it must serve in this function, setting up an unbalanced oxidation-reduction equilibrium which is essential to the living plant. It therefore follows that any factors affecting the oxidation-reduction intensity of the plant will shift the direction of this equilibrium and, if sufficient, might and should cause a physiological disturbance of the living processes of the plant. The extent of this change of direction of the equilibrium is determined by the resistance to such a change. This is analogous to buffering capacity, as related to pH, and may be pictured as follows:



The results of the investigations carried on in greenhouse (oxidation-reduction experiment) and also those made under field conditions support this theory to some degree.

Determination of reduced and oxidized ascorbic acid and reduced and oxidized glutathione should give data which would reflect the effect of minor elements in plant nutrition when it is subjected to conditions which produce a lime-induced chlorosis.

Methods of Analysis

Ascorbic Acid: - The method used was essentially the one used by Bessey and King (9). A two-gram sample of plant material was extracted twice by a solution, which was about eight per cent tri-chloro-acetic acid and two per cent in respect to metaphosphoric acid. The strength of the acid was adjusted, so that the pH of the extract was maintained close to 4.5 and not more than 5.0. The plant material was ground in a mortar under 10 to 12 ml. of the

Table 44, showing yields in order of their percentage of the control. Control is taken as 100 per cent.

6

Date	Febr. 1 - April 7 1939		April 7 - June 5 1939		June 5 - Aug. 19 1939		Sept. 1 - Dec. 1 1939		Jan. 5 - Febr. 26 1940	
Crop	Spinach		Mandarin soybeans		Cayuga soybeans		Spinach		Wolverine oats	
Crop Order	Treatment	%	Treatment	%	Treatment	%	Treatment	%	Treatment	%
1	$MnSO_4$	180	$Mn(C_2H_3O_2)_2$	165	K_2CrO_4	145	$FeSO_4$	125	$MnSO_4$	253
2	$Mn(C_2H_3O_2)_2$	152	$MnSO_4$	147	$CuSO_4$	118	$CuSO_4$	124	$Mn(C_2H_3O_2)_2$	249
3	K_2CrO_4	140	Control	100	$Cr_2(SO_4)_3$	113	Control	100	$Fe_2(SO_4)_3$	125
4	$Cr_2(SO_4)_3$	133	$Zn(C_2H_3O_2)_3$	99	$Fe_2(SO_4)_3$	113	$MnSO_4$	93	$Cr_2(SO_4)_3$	103
5	$FeSO_4$	122	$Cr_2(SO_4)_3$	98	$FeSO_4$	112	$Mn(C_2H_3O_2)_2$	92	K_2CrO_4	103
6	Control	100	$TiCl_3$	98	Control	100	$Cr_2(SO_4)_3$	89	Control	100
7	$CuSO_4$	97	$CuSO_4$	97	$MnSO_4$	93	$Zn(C_2H_3O_2)_2$	89	$Fe SO_4$	97
8	$Fe_2(SO_4)_3$	91	$Fe_2(SO_4)_3$	95	$Mn(C_2H_3O_2)_2$	90	K_2CrO_4	86	$CuSO_4$	93
9	$TiCl_3$	90	$FeSO_4$	90	$Zn(C_2H_3O_2)_2$	85	$TiCl_3$	79	$Zn(C_2H_3O_2)_2$	72
10	$Zn(C_2H_3O_2)_2$	68	K_2CrO_4	87	$TiCl_3$	34	$Fe_2(SO_4)_3$	78	$TiCl_3$	64

acid. A small amount of quartz sand was added to facilitate the grinding of the tissue. After thorough grinding, the mixture was transferred to centrifuge tubes and centrifuged. The clear liquid was poured off and the extraction repeated. The filtrates from both extractions were combined and the ascorbic acid was titrated with a standardized 2, 6, dichlorobenzenoneindophenol, to a pink end point which was permanent. For total ascorbic acid, hydrogen sulfide gas was passed through the combined filtrates until all oxidized ascorbic acid was reduced. The excess hydrogen sulfide was washed from the solution by passing carbon dioxide gas through it. The titration for total ascorbic acid is the same as described for the reduced acid. In colored plant extracts the end point is determined in a chloroform layer described by Lewis (45).

Glutathione: The method used for determining total, reduced and oxidized form of glutathione was a modification of that proposed by Woodward and Fry (82). Two grams of green material were weighed out and to this 16 ml. of distilled water were added in a porcelain mortar. The material was crushed and allowed to stand a few minutes at which time 2 ml. of 22 per cent sulfosalicylic acid was added slowly with grinding. The mixture was transferred to a centrifuge tube and centrifuged. The liquid was decanted and extraction repeated. The combined filtrates contained 2 per cent sulfosalicylic acid and had a pH below 2.0. To the combined filtrates were added 2.5 ml. of 4 per cent sulfosalicylic acid and 2.5 ml. of 5 per cent potassium iodide solution. Two or three drops of a one per cent starch solution were added and the filtrate titrated with a .001 N potassium iodate solution. This gave the reduced form of glutathione.

Total glutathione was determined by taking the combined filtrates of the extraction containing 2 per cent sulfosalicylic acid and adding to it 30-40 mg. of zinc dust. It was then allowed to stand at room temperature for 20 minutes. Excess zinc was removed by centrifuging. Titration was the same as for the reduced form. Total glutathione-reduced glutathione - oxidized glutathione.

$\frac{\text{ml of } KIO_3}{3.26} \times \frac{1}{\text{weight of sample}}$ =mg. of glutathione per gram of plant tissue. If divided by 100 would give per cent of glutathione.

Experimental

Spinach was sown on jars of soils taken from 3 five-inch layers of plat 8 of the Manganese-Sulphur Series of the College plots. These layers corresponded to the horizon layers which were analyzed for various forms of manganese in the soils (Table 4). Three treatments were made; control; 250 pounds per acre of Manganese sulfate and 100 pounds per acre of copper sulfate. This spinach was grown during the fall of the year and showed a response to both copper and manganese salts. On the 10-16 inch layer, however, in the spinach died on the jar receiving manganese sulfate. This layer is acid which may account for such a reaction to the treatment. Ascorbic acid and glutathione were determined on the spinach from these jars and the data are given in Table 45. Copper treatment resulted in an increase in reduced ascorbic acid in every case. There is an increase in the ascorbic acid content of the spinach on the jar of the 0-5 inch layer receiving manganese sulfate but it is not as high as in those receiving copper sulfate. Glutathione was determined in the spinach from the 0-5 inch layer only. The glutathione content of this spinach gives data very similar to that received in the case of ascorbic acid. Plates 3, 4 and 5 show this spinach as it appears at the time of sampling.

Plate 3.

Spinach on the 0-5 inch layer of plot 8 of Mn-S Series. Soil pH = 7.2.

A - 1 Control
A - 2 250 pounds of $MnSO_4$
A - 3 100 pounds of $CuSO_4$

Plate 4.

Spinach on the 5-10 inch layer of plot 8 of the Mn-S Series. Soil pH = 7.3.

B - 1	(center	Control
B - 2	(right	250 pounds of $MnSO_4$
B - 3	(left	100 pounds of $CuSO_4$

Plate 5.

Spinach on the 10-15 inch layer of plot 8 of the Mn-S Series. Soil pH = 6.8.

C - 1 (center) Control
C - 2 (right) 250 pounds of $MnSO_4$
C - 3 (left) 100 pounds of $CuSO_4$

Table 45, showing the content of ascorbic acid (Vit. C) in Long Standing Bloomsdale spinach. Sown Aug. 12, harvested Oct. 16, 1939.

Jar No.	Depth In.	Treatment	Wt./jar gms.	Mg. Vit. C per 100 gms.	Units of Vit. C per gm.	Units of Vit. C per jar.
A - 1	0 - 5	Control	74.3	39.35	7.87	585
A - 2	0 - 5	250# $MnSO_4$	113.8	60.00	12.00	1366
A - 3	0 - 5	100# $CuSO_4$	135.6	73.35	14.67	1989
B - 1	5 - 10	Control	107.3	35.00	7.00	751
B - 2	5 - 10	250# $MnSO_4$	103.6	30.00	6.00	622
B - 3	5 - 10	100# $CuSO_4$	88.0	60.55	12.10	1065
C - 1	10 - 16	Control	19.3	60.10	12.02	232
C - 2	10 - 16	250# $MnSO_4$	died	----	----	
C - 3	10 - 16	100# $CuSO_4$	49.9	72.20	14.44	721

Table 46, showing the content of glutathione (G-SH) in spinach grown in greenhouse. Sown Aug. 12, harvested Oct. 16, 1939.

Jar No.	Depth in.	Treatment	Wt. of spinach / jar gms.	Mg. G-SH/ gm spinach	Mg. G-SH/ jar
A - 1	0 - 5	Control	74.3	2.18	162
A - 2	0 - 5	250# $MnSO_4$	113.8	2.72	310
A - 3	0 - 5	100# $CuSO_4$	135.6	2.48	336

During the fall, samples of fall spinach were collected from various plots of the Manganese-Sulphur Series of the College plots. The plants collected do not represent the treatment, those taken on the plots receiving manganese sulfate, sulphur and the combination of the two were healthy normal plants. Those selected from the control plots were the most chlorotic plants on the plot. This chlorosis would not be considered severe. This analysis should be considered a comparison between normal plants and those exhibiting a mild case of chlorosis. The data show that the chlorotic tissue contains one-half to two-thirds as much ascorbic acid as the normal tissue.

Table 47, showing ascorbic acid (Vit. C) content in units per gram of normal and mildly chlorotic spinach. Samples taken Oct. 22, 1939.

Treatment	Condition	Ascorbic Acid units/gram
Manganese sulfate	Normal green	29.52
" "	" "	30.28
Manganese sulfate & sulphur	" "	30.03
Manganese sulfate & sulphur	" "	31.37
Sulphur	" "	22.72
"	" "	24.08
Control	Mildly chlorotic	14.87
"	" "	16.67

All determinations were made on leaves from the inner part of the rosette.

Cayuga soybean leaves were collected, which showed two degrees of chlorosis, namely, chlorotic leaves which are nearly yellow and leaves which show some mottling of yellow in the interveinal tissue. These were analyzed for reduced glutathione and the data show that glutathione (G-SH) content was reduced with the increased severity of the chlorosis.

Table 48, showing glutathione (G-SH) content in normal and chlorotic soybean leaves.

Condition of leaves	Mgm. G-SH/gm. Sample 1	Mgm. G-SH/gm. Sample 2	Average Mgm. G-SH	Mgm. G-SH per 100 gm.
Normal	4.01	4.10	4.055	406
Chlorotic (mottled)	3.60	3.52	3.560	356
Chlorotic (severe)	3.11	2.88	2.995	300

The content of ascorbic acid in the spinach, reported in Table 41, was determined and the data is given in Table 49. The greatest yield per jar was secured on those jars producing the most spinach, namely, those receiving ferrous and copper sulfates. The highest value per gram was produced by copper sulfate and manganese acetate treatments. Zinc acetate, titanous chloride and ferric sulfate treatment gave lowered yields on both production bases.

Table 49, showing Ascorbic Acid (Vit. C) content in spinach plants grown in greenhouse. Sown Sept. 1, 1939, harvested Dec. 1.

Jar No.	Treatment	Av. of 2 titration units of Vit. C.	Ave.	Mg. Vit. C per 100 gm.	Units of Vit. C per gm.	Units of Vit. C per jar
0-1	Control	7.40				
0-11		11.85	9.62	48.10	9.62	529
0-2	750# $MnSO_4$	10.02				
0-12		10.40	10.21	51.05	10.21	524
0-3	700# $Mn(C_2H_3O_2)_2$	12.20				
0-13		14.45	13.32	66.60	13.32	670
0-4	450# $Zn(C_2H_3O_2)_2$	4.76				
0-14		6.10	5.43	27.15	5.43	266
0-5	2350# $Cr_2(SO_4)_3$	14.39				
0-15		6.67	10.53	52.65	10.53	518
0-6	2250# $FeSO_4$	9.90				
0-16		11.67	10.79	53.95	10.79	741
0-7	1500# $TiCl_3$	7.55				
0-17		8.12	7.83	39.15	7.83	338
0-8	640# $CuSO_4$	9.22				
0-18		14.90	12.06	60.30	12.06	824
0-9	500# $Fe_2(SO_4)_3$	4.90				
0-19		7.17	6.03	30.15	6.03	259

Oxidized and reduced glutathione was determined in the spinach from two of the treatments. The spinach treated with ferrous sulfate was normal in appearance while that receiving zinc acetate was very chlorotic. Very little difference existed in total glutathione, while the reduced form was higher and the oxidized form much lower in those receiving ferrous sulfate.

Table 50, showing content of various forms of glutathione in spinach.

Treatment	Total glutathione in mgm. /gm.	Reduced glutathione mgm. per gm.	Oxidized glutathione mgm. per gm.	Ratio $\frac{G\text{-}SH}{G\text{-}S\text{-}S\text{-}G}$
450 Zn Acetate	1.244	.854	.390	2.19
2250 $FeSO_4$	1.390	1.341	.049	29.48

Reduced and oxidized ascorbic acid and reduced glutathione determined on the Wolverine oats reported in Table 42, is given in Table 51. The manganese treatments which gave the highest yields showed the highest content of reduced ascorbic acid and reduced glutathione and the lowest oxidized ascorbic acid. The zinc acetate and titanous chloride treatments which produced the lowest yields gave the lowest content of reduced ascorbic acid and reduced glutathione and the highest values for oxidized ascorbic acid.

The ratio of reduced to oxidized ascorbic acid indicates some effect of that treatment. Very little difference existed between the other treatments and the controls.

The data appear to support the theory that manganese salts when giving beneficial results also have a favorable effect upon oxidation-reduction systems within the plant. It also appears that any minor element producing a beneficial effect upon the plant growth will affect the oxidation-reduction systems in a favorable manner.

Table 51, showing the reduced and oxidized ascorbic acid content in Wolverine oats on Oxidation-Reduction Experiment. Sown Jan. 5, harvested Febr. 26, 1940. Ascorbic acid reported as units per gram of green oats.

Treatment	Block A-Vit.C (c)		Block B-Vit.C		Block C-Vit.C		Block D-Vit.C		Ave.-Vit.C		Significance	
	Red. (a)	Ox. (b)	Red.	Ox.	Red.	Ox.	Red.	Ox.	Red.	Ox.	Red.	Ox.
Control	12.40	2.60	12.05	2.50	12.20	2.90	10.20	3.80	11.71	2.95		
750#$MnSO_4$	21.40	1.50	20.90	1.30	21.25	1.45	20.05	1.50	20.90	1.44	**	**
700#Mn Acetate	20.50	1.50	24.15	1.40	21.80	1.45	19.50	1.40	21.49	1.44	**	**
450#Zn Acetate	9.70	3.00	8.50	3.45	8.40	3.25	8.05	4.75	8.66	3.61	**	
2380#$Cr_2(SO_4)_3$	14.50	2.00	16.90	1.70	13.80	1.80	12.65	2.35	14.49	1.96	**	*
2250#$FeSO_4$	15.00	3.20	17.20	3.20	13.60	3.90	12.50	3.70	14.58	3.50	**	
1500#$TiCl_3$	8.40	5.50	8.75	3.85	8.00	3.30	7.95	4.30	8.28	4.24	**	**
640# $CuSO_4$	12.10	3.60	11.40	2.90	10.50	1.90	12.20	3.00	11.55	2.85		
500#$Fe_2(SO_4)_3$	14.00	2.70	12.20	4.00	14.30	3.10	10.80	2.70	12.83	3.13		
1380#K_2CrO_4	15.00	2.90	13.10	3.30	14.40	2.20	13.30	2.30	13.95	2.68	**	

(a) Red. = Reduced (b) Ox. = Oxidized (c) Vit. C = Ascorbic Acid.

Analysis of Variance

Reduced Ascorbic Acid

Source	D.F.	S.S.	Var.	S.D.
Total	39	76.40		
Replication	3	19.2		
Treatment	9	711.4	79.04	
Error	27	33.4	1.24	1.11

F - 63.90

Difference for significance between means

1% - 2.19 5% - 1.62

Oxidized Ascorbic Acid

Source	D.F.	S.S.	Var.	S.D.
Total	39	40.68		
Replication	3	1.10		
Treatment	9	31.09	3.454	
Error	27	8.49	.314	.177

F - 11.0

Difference for significance between means

1% - 1.10 5% = 0.82

Table 52, showing average contents of reduced and oxidized forms of ascorbic acid and milligrams per 100 grams of glutathione in Wolverine oats on Oxidation-Reduction Experiment.

Treatment	Red. Ascorbic Acid (a)		Ox. Ascorbic Acid (b)		Ascorbic Acid Ration Red. / Ox.	Glutathione	Glutathione
	Units / gm.	Units / jar	Units / gm.	Units / jar		mg./100 g.	mg./100 g.
Control	11.71	254	2.95	64	3.97	183	40
750#$MnSO_4$	20.90	1150	1.44	79	14.51	321	177
700#Mn Acetate	21.49	1163	1.44	78	14.92	341	184
450#Zn Acetate	8.66	136	3.61	57	2.40	150	23
2380#$Cr_2(SO_4)_3$	14.49	323	1.96	28	7.39	207	46
2250#$FeSO_4$	14.58	306	3.50	74	4.17	230	48
1500#$TiCl_3$	8.28	116	4.24	59	1.95	154	22
640#$CuSO_4$	11.55	234	2.85	58	4.05	215	44
500#$Fe_2(SO_4)_3$	12.83	348	3.13	85	4.10	182	49
1380# K_2CrO_4	13.95	311	2.68	60	5.21	212	47

(a) Red. = Reduced
(b) Ox. = Oxidized

General Discussion

It is very difficult to give a very definite explanation as to the role that manganese plays in soil fertility and plant nutrition. In the face of all the essential nature of manganese in plant nutrition, it would be extremely foolish to say that it is not an essential element. A very comprehensive study of these data and other data strongly suggests the possibility of an accessory function, of an intensity nature. It has been previously pointed out that certain observational facts, yield data, and chemical analysis failed to support the essential element theory. It is therefore necessary to study the data given here and the data of other investigators in order to obtain a comprehensive insight into its possible functions.

In the first place, certain soil conditions have been associated with manganese deficiency, such as alkalinity, high lime content and organic matter supply. It is the contention of this paper that the pH of the soil is not a causative but rather an attendant factor. Such a relationship is hardly tenable, since pH is a resultant of many processes including such reactions as leaching and oxidation - reduction which may alter the availability of, or the essentiality of, the manganese. These may be the resultant of natural processes or subsequent processes initiated by the nature of soil management or by change in environmental conditions.

It generally has been accepted that if the soil is made more acid or if it is water logged, the solubility or availability of the soil manganese is increased. Leeper has pointed out that this is true only if the soils are high in active manganic compounds. His method of determining manganese-deficient soils appears to be very reliable. On the other hand, this work presents a very contradictory phase to Leeper's active manganese theory. In general, the soils made acid by application of sulphur have given either the highest or approximated the highest yields of crops grown on these plots. The plants have been just as completely cured by this treatment as those receiving the manganese salt. The disturbing element is that this treatment does not show an increase in active manganese nor does it show an increase in bivalent manganese over that of the control which has produced chlorotic plants. The analysis of plant material fails to show a consistently higher manganese content in the plants from sulphur tested plots than on those from controls and quite often it appears to be definitely lower. This eliminates the possibility of small increases with an accelerated velocity of the chemical reaction. Such a contradiction does not necessarily refute Leeper's theory. It is highly probable, however, that with a higher hydrogen ion concentration, the need for manganese becomes less. This idea is supported in Harmer's unpublished data (31) which shows strongly acid organic soils which contain but mere traces of manganese upon total analysis and are still capable of producing perfectly normal crops. A survey of the literature shows that there is a great variation in the quantity of manganese sulfate required to be added to the soil for a desired benefit. Further, the amounts considered necessary for toxic effects also vary greatly. It is therefore contended that the soil's need for active MnO_2 is quite variable and it can be concluded that, in the apparent presence of exchangeable hydrogen, the need for manganese may be very small and is not so acute.

The data show that in making these soils more acid, the solubility of the calcium is decreased. The water-soluble and exchangeable calcium are lowered by the application of sulphur.

The analysis of the chlorotic plant tissue shows that the mobility of the iron has become impaired and that the plant suffers from a lack of iron at points where it is vitally necessary. McGeorge (54) has pointed out that, when plants assimilate luxury amounts of calcium iron will be precipitated in the cells. Wilcox and Kelley (76) state that, under such conditions, there is an abundance of calcium oxalate crystals in the plant cells. The data of the seasonal trend tend to support McGeorge's theory in that, during periods of low temperature in soils and with high moisture conditions, the plant takes in excessive amounts of calcium. It is during these periods that severe chlorosis prevails which chlorotic condition is curable by the application of manganese or sulphur. Either of these treatments cause the iron to function properly in the plants. There is no reason why another chemical other than manganese should not be capable of producing the same effect upon iron. The functioning of iron which is affected by the hydrogen ion concentration and the calcium content of the plant tissue is subject to the favorable and unfavorable influence of the ions capable of taking part in oxidation-reduction reactions. It is therefore plausible and very probable that, under different sets of conditions, different members of this group of ions will give favorable responses to plant growth. Excesses of any members of this group will in all probability be expressed in a disturbed state of oxidation of the iron. The results obtained by staining the iron in plant tissue gives very remarkable support of the effect upon the state of its oxidation. The non-responsive crops such as sweet clover, sugar beets and peppermint do not show these effects.

Since the precipitation of iron appears to be the resultant of disturbed oxidation-reduction equilibrium, it would be expected therefore, that the other oxidation-reduction systems within the plant would be affected. In a normal healthy plant, the ascorbic acid and glutathione are predominantly in the reduced form. The fact that they occur in this form substantiates the theory of an effect upon the oxidation-reduction system by the manganese and other minor elements capable of taking part in such reactions.

The part played by climatic factors appears to be indirect in that it probably influences the intake of calcium. It is also plausible that temperature might be capable of playing a part in the direction intensities of oxidation-reduction reactions which is partially supported by the lower ascorbic acid content under higher prevailing temperature ranges. The elements of climatic environment need further investigations under more controlled conditions than has been possible in this study. The observations made of these factors in this study suggest that they are very important.

Oxidation-reduction has been stressed considerably in recent work. The data obtained in this study indicate that oxidation-reduction potential of the soil is a minor factor in the plant growth. The prevailing oxidation-reduction systems may influence the balance of nutrients available to plants which in its natural condition may or may not have an injurious influence upon plant growth. This influence may become greatly aggravated when the condition is altered by treatments, such as liming or by other means. Oxidation-reduction systems within the plant are of great importance to its physiological processes. The kind of ions taken in by the plant and the environmental conditions have an influence upon these systems. The state of oxidation of iron can be altered by an excess of the multivalent ions or precipitated by excessive calcium intake.

The oxidation-reduction experiment supports this hypothesis inasmuch as when manganese gave beneficial results, zinc gave very detrimental effects and on the other hand when manganese salts were detrimental to plants, copper, chromium and other ions were beneficial. It is not to be construed that the ions other than those of the multivalent group cannot produce similar disturbance. A lack of potash in the soil will cause iron to deposit in nodes of corn. Recent work of Schmitt (67) would substantiate this as he found that a lack of potash in plant nutrition produced plants which were low in reduced form of ascorbic acid and high in the oxidized form.

The question naturally arises as to the importance of the above relationships to plant and animal nutrition. In case of the former it gives added weight of the necessity of harmony of nutrients. Chlorosis can be considered the resultant of an unbalanced condition in the nutrients available to the plant. This unbalanced condition may be due to the lack of one or a group of other ions or to the excess of one or more ions. It is reasonable to theorize that many of the toxic conditions described are probably in all reality a failure of iron to function or to the breakdown of other oxidation-reduction systems within the plant. These physiological breakdowns result in plants which are extremely susceptible to disease.

The relationship of this problem to animal nutrition is extremely important as many of the mineral malnutritional diseases are associated with areas where minor element deficiencies occur in plants. Investigators in nutrition research are confronted with the question of available iron in foods. Many foods are high in iron and yet fail to produce hemoglobin when fed to anemic animals. Chlorosis in plants produces a high total iron and a low available iron content. Since mild chlorosis cannot be readily ascertained it would be expected that plants having such a condition would reach the channels of animal nutrition. Spinach is a very good example of such a crop and is also one of the most susceptible crops to lack of proper fertilization. Great emphasis should be given to proper fertilization as it reflects directly upon nutritional quality of the crop.

Finally it is reasonable to speculate from results here that the role of minor elements is that of the activation of iron.

SUMMARY

As a result of a chemical study of the crops produced on an alkaline organic soil, and of the soil itself, as affected by the application of manganese sulfate, sulphur and other chemical materials, the following conclusions were reached.

A. The Soil

1. The application of sulphur decreases the exchangeable calcium and magnesium, the water-soluble calcium and to a lesser degree, the water-soluble magnesium. It also markedly increases the exchangeable hydrogen.

2. The application of manganese salts increases the total, exchangeable and easily reducible manganese in the soil but does not appreciably influence the exchangeable and the water-soluble calcium and magnesium or the exchangeable hydrogen.

3. The soil complex of this alkaline muck is highly saturated with calcium and magnesium with the greater portion of the exchangeable cations as the former.

4. Manganese salts applied to the soil are fixed firmly in the surface layers. There is no evidence of enrichment of subsoil layers by an application of a manganese salt to the surface layer. The fixation appears to be due to the oxidation of the manganese to the manganic oxide.

5. The system of $Mn^{++} \rightleftarrows Mn^{++++}$ is very important in soils. There is a correlation between crop growth and the quantity of the right-hand member of this equilibrium that can take part in a reaction which goes from right to left.

6. The hydroquinone-ammonium acetate extraction is a reliable means of measuring the manganic manganese which takes part in the manganous-manganic equilibrium of alkaline and neutral organic soils.

7. The water-soluble manganese is very low in these soils, even in those receiving manganese salts.

8. The application of sulphur tends to increase the exchangeable more than the reducible manganese in the soil. The application of manganese salts tends to increase the reducible more than the exchangeable manganese.

9. The seasonal trend of the exchangeable manganese in this soil shows a decrease as soil temperature increases and at the same time the easily reducible manganese dioxide increases.

10. The seasonal changes in soil temperature and soil moisture affect the state of oxidation of the manganese. Under low soil temperature and high soil moisture content the manganese tends towards the bivalent form. Under conditions of higher soil temperature and generally lower soil moisture content the equilibrium swings towards the manganic form.

B. The Plant.

1. Chlorotic plants show a higher iron content than normal plants. The application of manganese salts or sulphur produces normal plants which have a lower total iron content.

2. There is a difference in the state of oxidation of the iron in the chlorotic and normal plant tissue. In chlorotic plants, ferric iron is abundant in the veins of the leaves and vascular tissue of the stems. Ferric iron could not be found in similar tissue of normal plants.

3. The hydrogen ion concentration is higher in the normal than in the chlorotic tissue.

4. Minor element deficiency of the multivalenced group has to do with the state of oxidation of the iron in the plant. This being the condition, any factor affecting the oxidation-reduction capacity of the plant will affect the oxidation-reduction equilibrium of the ferrous-ferric system. This system can be and is affected by these elements and, if one is in excess then another producing an opposite condition will counteract it. Further, the state of

oxidation existing in the plant is the resultant of the actions of many physical and chemical processes occurring in the plant; or externally, or lastly indirectly through its localized environment; the result of these forces can be expressed in the oxidation level of the ferrous-ferric system in the plant. It follows, while, under one condition, one element may be much more efficient than another, it does not eliminate from causing the same result.

5. The content of magnesium is higher in chlorotic than in normal plant tissue.

6. The application of manganese salts to the soil increases the manganese content of the plant. A still greater increase is obtained when both manganese salts and sulphur are applied to the soil together.

7. In general, the application of sulphur to the soil did not increase the manganese content of the plants over that of the plants from the control.

8. Climatic factors, precipitation, soil temperature and soil moisture affect the plants intake of calcium. A high soil moisture content and a low soil temperature cause certain plants to take in abnormal amounts of calcium.

C. The Oxidation-Reduction Systems.

1. In a healthy plant ascorbic acid and glutathione are predominantly in the reduced form.

2. In chlorotic plants a marked increase occurs in the proportion of ascorbic acid and glutathione in the oxidized form. The reduced form of ascorbic acid is much lower in chlorotic plants.

3. Total ascorbic acid content of the plant is decreased by a chlorotic condition. The limited data on the content of glutathione does not show a decrease due to chlorosis.

4. Minor elements influence the oxidation-reduction equilibrium within the plant. If the minor element is beneficial to plant growth, the oxidation-reduction equilibriums under consideration are strongly towards the reduced forms. If

the minor element causes a chlorotic condition or causes an existing chlorotic condition to become more severe the direction of these equilibriums is towards the oxidized form.

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