THE IRON-MANGANESE RELATIONSHIP IN THE GROWTH OF OATS

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Clarence Cornelius Gray III

1947

THESIS

This is to certify that the

thesis entitled

"The Iron-Manganese Relationship in the Growth of Oats."

presented by

Clarence C. Gray

has been accepted towards fulfillment of the requirements for

M.S. degree in Soil Science

C. E. Millar
Major professor

Date August 19, 1947

M-795

THE IRON - MANGANESE RELATIONSHIP
IN THE GROWTH OF OATS

By

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A THESIS

Submitted to the School of Graduate Studies of Michigan
State College of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Soil Science

1947

THESIS

ADKNOWLEDGEMENT

The writer wishes to express his sincere appreciation to Dr. Ray Cook for his guidance and cooperation and to Dr. Kirk Lawton for his valuable suggestions relative to the chemical analysis.

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INTRODUCTION

In recent years, considerable attention has been given to the role of trace elements in the growth and development of plants. Though it is generally accepted that a definite mineral balance exists for each plant, the proportions of these trace elements to other recognized nutrient elements has been somewhat overlooked.

In plants, certain metabolic disturbances, the result of a disrupted mineral balance, are evidenced by chlorotic tissue on the stems and leaves. Generally, the visual evidences of a disrupted mineral balance are specific for a particular plant and the mineral element or elements concerned. For instance, "grey-speck" or "halo blight" of oats is a characteristic symptom of manganese deficiency. Recently, it has been shown that the intensity of manganese deficiency symptoms, on certain plants, is affected by the concentration of iron present. This suggests strongly that an inter-relationship exists between iron and manganese. Shive (9)[1] concluded that there must be a proper balance not only between the available iron and manganese in the nutrient substrate but also between the active concentrations within the plant tissues. The purpose of this investigation was to observe the effects of varying amounts of added iron and manganese to soil on the growth and appearance of oats.

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1. Figures in parenthesis refer to "LITERATURE CITED" p. 14.

REVIEW OF LITERATURE

Twyman (12), reviewing the literature on the iron-manganese balance in plant metabolism, reports an investigation on the effect of varying proportions of iron and manganese on oats by Salm-Horstmar in 1848. Although Salm-Horstmar's results were significant, they were obscured by time. He found that manganese was necessary for plants in soils where the iron was above 1:100 and where the iron was in contact with humus.

Experimenting with the effect of manganese on the growth of Chlorella, Hopkins (2) found that manganese would not replace iron but that manganese functions physiologically in an indirect manner by its action on the state of oxidation of iron. He concluded that manganese controlled the ratio of ferrous to ferric iron in the culture or cell.

In 1917, Johnson (3) reported manganese as a cause of the depression of the assimilation of iron by pineapple plants.

After èxtensive investigation into the functions of manganese in plants, Kelly (4) concluded that manganese acted catalytically, increasing the oxidations in the soil and accelerating the auto-oxidations in the plants. Upon analyzing the ash of plants on high manganese soils of Hawaii, he found a disturbance of the mineral balance. Increased amounts of manganese in the plant were accompanied by an increase in the amount of lime and a decrease in the amounts of iron and magnesium.

The fact that iron was shown to be equally available in both manganiferous and non-manganiferous soil types of equal Hydrogen-ion concentrations led McGeorge (7) to believe that cholorsis of pineapple leaves on plants grown on manganese soils was due to a greater assimilation of lime indirectly caused by the presence of manganese in excessive amounts. He maintained that the resulting physiological disturbance was due to the greater immobility of iron caused by the excessive concentration of the lime in the leaves and stalk.

Pugliese (8), growing grain in water culture with Knop's solution with added amounts of manganese and iron observed that certain amounts of manganese were stimulatory when accompanied by proportionate amounts of iron but toxic in the absence of iron. He regarded the function of manganese in the plant to be tied up with oxidation phenomena in such a manner that it acted either directly as an oxidizing agent or as an accelerator of oxidizing enzymes. He also pointed out that the best fertilizing results were obtained when iron and manganese were present in the proportion of 1:2.5 by molecular weight.

Sideris and Kraus (10) considered the toxicity of manganese in plants to be due to its chemical reaction with iron during which the iron is rendered inactive.

The most recent contribution on the role of iron and manganese in plant metabolism is that of Somers and Shive (11). These workers grew soybeans in solution cultures at three different iron levels, at each of which, manganese concentrations were varied through a relatively wide range. They found that the pathological symptoms of iron toxicity were identical with those of manganese deficiency. The optimum ratio of iron to manganese in the nutrient substrate for good growth free from pathological symptoms was around 2, regardless of the total amounts of these elements used. A similar ratio between soluble manganese and soluble iron was found in the plant tissues; however, no relationship existed between the total amounts present.

EXPERIMENTAL

Two Michigan Soil types were selected for the experiment, an alkaline Granby sandy loam, normally manganese deficient, and an acid Fox sandy loam, normally not manganese deficient. The Granby is well supplied with organic matter and has a pH of 7.8. The Fox contains only small amounts of organic matter and has a pH of 5.8. Both soils had been previously screened and were air-dry when obtained. Seven and nine kilograms of the Granby and Fox soils respectively were placed in two-gallon glazed jars. Chemically pure nutrients were added in a circular band about 2½ inches deep at the equivalent rate of 400 pounds per acre of 4-16-8 fertilizer. Where iron and manganese were added, they were mixed thoroughly in the soil before the placement of the fertilizer. The soils were kept at the moisture equivalent with distilled water.

There were twenty-five treatments on each of the two soils and each treatment was replicated three times. Equal amounts of fertilizer were applied to all pots. The treatments, in the equivalent of pounds per acre, were

1. Check
2. 200 lbs. $MnSO_4$
3. 600 lbs. $MnSO_4$
4. 1200 lbs. $MnSO_4$
5. 1800 lbs. $MnSO_4$
6. 200 lbs. $FeSO_4$
7. 200 lbs. $MnSO_4$ + 200 lbs. $FeSO_4$

8. 600 lbs. $MnSO_4$ + 200 lbs. $FeSO_4$

9. 1200 lbs. $MnSO_4$ + 200 lbs. $FeSO_4$

10. 1800 lbs. $MnSO_4$ + 200 lbs. $FeSO_4$

11. 600 lbs. $FeSO_4$

12. 200 lbs. $MnSO_4$ + 600 lbs. $FeSO_4$

13. 600 lbs. $MnSO_4$ + 600 lbs. $FeSO_4$

14. 1200 lbs. $MnSO_4$ + 600 lbs. $FeSO_4$

15. 1800 lbs. $MnSO_4$ + 600 lbs. $FeSO_4$

16. 1200 lbs. $FeSO_4$

17. 200 lbs. $MnSO_4$ + 1200 lbs. $FeSO_4$

18. 600 lbs. $MnSO_4$ + 1200 lbs. $FeSO_4$

19. 1200 lbs. $MnSO_4$ + 1200 lbs. $FeSO_4$

20. 1800 lbs. $MnSO_4$ + 1200 lbs. $FeSO_4$

21. 1800 lbs. $FeSO_4$

22. 200 lbs. $MnSO_4$ + 1800 lbs. $FeSO_4$

23. 600 lbs. $MnSO_4$ + 1800 lbs. $FeSO_4$

24. 1200 lbs. $MnSO_4$ + 1800 lbs. $FeSO_4$

25. 1800 lbs. $MnSO_4$ + 1800 lbs. $FeSO_4$

Marion oats were selected because of their sensitivity to various concentrations of manganese. The oats were planted April 26, 1947. After emergence they were thinned to an even stand of thirteen seedlings per jar. On May 15 all pots received $MgSO_4$ at the rate of 50 pounds per acre. On May 25 all pots received $Ca(H_2PO_4)_2$ at the rate of 100 pounds per acre and KCl at the rate of 50 pounds per acre and the pots in the Fox sandy loam received NH_4NO_3 at the rate of 50 pounds per acre. These nutrients were added to offset the inadequate amount of fertilizer added at the beginning of the experiment. The plants were harvested on June 5, approximately one week after heading.

After harvesting, the plants were placed in paper sacks and allowed to air-dry for two weeks before weighing. Because of the large number of treatments, it was decided to run chemical analyses for iron and manganese on the plant tissue from only one pot in each treatment. There was little variation between replications and an effort was made to pick what was thought to be the most representative of the three.

In preparation for chemical analyses, the samples were ground in a Wiley mill, passed through a 1 m.m. screen, and then oven-dried at 80° C. for 24 hours. Four-gram samples were dry ashed in an electric furnace and the ash dissolved in 10 ml. of 1:1 HCl, heated near boiling for 15 minutes, filtered, and made up to volume in a 100 ml. volumetric flask. Iron and manganese were determined colorimetrically on 25 ml. aliquots with the aid of a photo-electric colorimeter. Iron was determined by the O-phenanthroline method as described by Fortune and Mellon (1) and manganese by the periodate method of Willard and Greathouse (13).

In order to get some idea of the availability of the iron and manganese that had been added in the treatments, it was decided to determine iron and manganese in the soils from which the plant samples had been taken.

The selection of an extracting solution for the determinations presented a problem because of the uncertainty regarding what constituted the "available" fractions of the total amounts of iron and manganese present. Since the amounts of iron and manganese added in the treatments were known, a preliminary investigation was carried out to determine the extracting agent that would take out amounts in keeping with the known amounts present. The assumption was made that a direct relationship existed between the amount available and the amount added. The extracting solution finally selected was 0.1 N HCl. The strength of this solution may be questioned, but it is in line with Leeper's (6) thinking that dilute acid extracts may give an estimate of the active manganese and also of the toxic manganese that may come into solution in an acid soil.

Samples of the soil for analysis were obtained by thoroughly mixing the contents of the jars and then screening the soil through a 2 m.m. copper wire screen. The samples were oven-dried at 110° C. Twenty-gram portions were placed in 125 ml. flasks, brought up to the moisture equivalent, and incubated for 10 days. This procedure was carried out since the availability of iron and manganese is affected by the state of the oxidation-reduction system in the soil. After incubation, the flasks were shaken for fifteen minutes with 80 ml. 0.1 N HCl. The extracts were obtained by filtering through Buchner funnels. The extracts were evaporated to dryness and the organic matter destroyed by using Hydrogen peroxide. The residue was taken up in 0.1 N HCl, heated for fifteen minutes, filtered, and made up to volume in a 100 ml. volumetric flask. Iron and manganese were determined on aliquots in the same manner as that in the plant extracts.

RESULTS

Observations during growth

Fox - Germination was good and the early growth was rapid. Growth was normal until approximately twenty days after planting, when there appeared slight traces of browning on the tips of the newer leaves of the plants on all the treatments, including the check which received no iron or manganese. The browning increased in severity as the amount of applied $MnSO_4$ increased. The normal rate of growth continued on all pots except those treated with 1200 or 1800 pounds of $MnSO_4$ per acre, where a definite retardation of growth occurred. After browning, the leaves began to die-back from the tips. At this point, the lower leaves began to turn brown. As the browning progressed, a purpling appeared. By the time the plants were harvested, the stunted plants had overcome their retardation in growth and were the same size as those in the other pots, as shown by the yield data reported in table I.

Granby - Early growth was similar to that on the Fox soil. There were no indications of any physiological disturbance until twenty-six days after planting when there appeared the characteristic manganese deficiency "grey-specks." These symptoms appeared on several of the new leaves on all of the plants that had not received $MnSO_4$. As growth increased, the appearance of the "grey-specks" was more pronounced on these check plants. Surprisingly, the manganese deficiency symptoms and the retarded growth were less severe on the plants that had received iron. On the plants which had received 200 pounds of $MnSO_4$ per acre, there were very slight traces of manganese deficiency. The plants on those pots which had received 600 pounds or more of $MnSO_4$, exhibited the same symptoms that were in evidence on the oats grown on the Fox soil - browning and a dying-back of the tips, and a gradual purpling. Varying the rate of application of $FeSO_4$, with the rate of application of $MnSO_4$ held constant did not affect the growth of the plants. This is shown at the 600 pound rate of $MnSO_4$ in Plate 1. At harvest, the plants on all pots had headed normally, except that the manganese deficient plants showed only a few undersized heads. In contrast to the others, the manganese deficient plants were darker green and much more succulent. As shown by the data in table II, yields were markedly increased by the 200 pound per acre application of $MnSO_4$, as shown in plate 2, but were not further increased by any additional quantity. There was some indication that $FeSO_4$ caused an increase in yield where $MnSO_4$ was not applied.

Iron and Manganese in the Soil

Fox - As shown by the data reported in table I, iron ranged from 37 p.p.m. in the pots which had not received iron, to 82.5 p.p.m. where 1800 pounds per acre of $FeSO_4$ had been applied. There was considerable variation in the amounts of manganese obtained, even in similarly treated soil. This probably can be attributed to errors in sampling and analysis, and possibly to differences in the amounts of soluble manganese due to a shift in the manganous-manganic equilibrium. The acidity of the soils after the treatments were made varied from pH 5.94 on treatment No. 1 to pH 4.89 on treatment No. 25.

Granby - Reported in table II are the results obtained from analyses of the Granby soil. Iron ranged from a low of 10.8 p.p.m. in the untreated soil to a high of 28 p.p.m. where 1800 pounds per acre of $FeSO_4$ had been applied. The results of the manganese determinations were as erratic as those on the Fox soil. The values ranged from 3.2 p.p.m. to 99 p.p.m. The difference in the two soils as to the availability of iron and manganese can be clearly seen. The untreated Fox soil contained as much manganese as did the Granby soil which received 200 pounds per acre of $MnSO_4$. The depression of the alkalinity by the addition of the sulphates was from pH 7.79 to pH7.25. This, in comparison to the corresponding data for the Fox soil, indicated the difference in the buffer capacity of the two soils.

Iron and Manganese in the Plants

Fox - As indicated by the data reported in table I, the total manganese in the plants increased with an increase of the element in the soil, thus indicating that the absorption of this element was determined largely by the concentration in the soil. The iron content of the plants showed a similar relationship; however, it did not hold in all cases. No attempt was made to establish the optimum ratio of iron to manganese for best growth since all the plants seemed to show manganese toxicity symptoms. The data show that at each of the five levels of added $MnSO_4$, except the 1800 pound per acre application, as the addition of $FeSO_4$ was increased the total manganese in the dry tissue increased until the heaviest application of $FeSO_4$ was reached. This very high application of $FeSO_4$ reduced the manganese in the tissue. Apparently, from the data, no relationship exists between the iron in the plant tissue and the amount of manganese in the soil.

Granby - All of the plants grown on the Granby soil that exhibited manganese deficiency symptoms had, as shown by the results in table II, a total manganese content of less than 0.075 milligrams regardless of the amount of manganese added to the soil. It is interesting to compare the levels of manganese in plants grown on the Granby soil with those grown on the Fox soil. Even where the greatest quantity of $MnSO_4$ was applied the plants grown on the Granby contained less manganese than the control plants grown on the Fox soil. This, of course, explains why manganese deficiency did not occur on the Fox soil. Study of the data shows that there is little or no constant relationship between the ratio of iron to manganese in the tissues and the presence or absence of manganese deficiency symptoms. This is in line with the findings of Somers and Shive (11) that the ratios between total iron and total manganese within the tissues show no definite relation to any deficiency - toxicity condition or to normal plant growth.

DISCUSSION

In planning the experiment it was the intention to try to produce manganese deficiency and manganese toxicity symptoms on the oat plants by varying the concentrations and proportions of iron and manganese in the soil. Little success was attained in this connection. No preliminary investigations were carried out; consequently, the amounts of manganese and iron sulphates applied were purely reasonable approximations. Applications of iron as high as that contained in 1800 pounds per acre of $FeSO_4$ did not produce manganese deficiency on the Fox soil. Assuming that an iron-manganese balance exists for oats and discounting the interference of some unknown factor, then the amounts of iron applied were insufficient. The availability of iron and manganese as determined by the 0.1 N HCl extracts, and as shown in table I serves to substantiate this point of view. Of the amounts applied to the soils only a fraction was available for utilization by the plants. A comparison of the amounts obtained shows that "fixation" in the alkaline Granby soil was much greater than that in the acid Fox soil. This agrees with the findings of other investigators that an alkaline soil reaction favors the conversion of manganese from the active manganous form to the relatively inert, and consequently unavailable, manganic oxides. In regard to iron, the peak of its availability is considered to be reached in an acid medium. The applications of $FeSO_4$ did not seem to influence the availability of the manganese, nor did the $MnSO_4$

influence the availability of the iron.

The growth and appearance of the oats grown on the Fox soil was evidently the result of the presence of available manganese in excessive amounts. The chlorotic symptoms present were similar to those described by Kelly (4) on oats and barley plants grown on manganiferous soils of Hawaii.

The appearance of manganese deficiency symptoms on oats grown on the untreated Granby soil showed that the soil was lacking in adequate amounts of manganese. According to the theory advanced by Somers and Shive (11) these symptoms could be intensified by increasing the amount of available iron in the substrate. In this experiment, however, just the reverse happened; as the amount of added $FeSO_4$ increased the incidence of "grey-speck" decreased. The difference in size of the growth can be seen in the picture of cultures shown in plate 3.

The results obtained are somewhat confusing in the light of the postulated theory of the iron-manganese relationship. The explanation may lie in the fact that soil is a complex system and experimental results obtained from natural soils may be surprisingly different from those obtained with culture solutions.

SUMMARY

Marion oats were grown on two different soils, one an acid Fox sandy loam and the other an alkaline Granby sandy loam. Twenty-five treatments were made on each of the two soils by increasing the amount of applied $MnSO_4$ from zero to 1800 pounds per acre at five corresponding levels of $FeSO_4$. The oats grown on the Fox soil exhibited only manganese toxicity symptoms, apparently the amounts of $FeSO_4$ added were insufficient to produce manganese deficiency. Manganese deficiency

symptoms produced on the plants grown on the Granby sandy loam were corrected by additions of sufficient manganese sulfate but were not intensified by the application of $FeSO_4$. Tests on the soils, made by extracting with 0.1 N HCl showed that greater proportions of the manganese and iron applied were available in the Fox soil than in the Granby soil. The tests showed further that the untreated Fox soil contained as much manganese as did the Granby soil which had received a 200 pound per acre application of $MnSO_4$. Chemical analyses of the plants failed to show a constant relationship between the ratio of iron to manganese in the tissue and the presence of deficiency or toxicity symptoms.

CONCLUSIONS

From the observations and data of this experiment it is apparent that:

1. Response to manganese by Marion oats is dependent largely on the amount of available manganese in the soil.
2. Iron in the form of $FeSO_4$ did not intensify manganese deficiency symptoms on a manganese deficient Granby soil.
3. Applications of $FeSO_4$ up to 1800 pounds per acre did not lessen the effect of manganese toxicity on a Fox sandy loam.
4. Within the limits of this experiment, Marion oats are not responsive to an iron-manganese balance in a Fox sandy loam or a Granby sandy loam.

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TABLE I. The Effect of Manganese and Iron Sulfates applied to Fox sandy loam soil on the yield of oats and the Manganese and Iron contents of the soil and the ash from the tops of the oat plants grown on the soil.

No.	TREATMENT		pH of CULTURE	AIR DRY TISSUE PER POT IN GRAMS	SOIL ANALYSIS		PLANT ASH ANALYSIS	
	$MnSO_4$ lbs/A	$FeSO_4$ lbs/A			MANGANESE ppm	IRON ppm	MANGANESE* Mgms/Gram	IRON* Mgms/Gram
1.	---	---	5.94	25.5	33.5	38.0	0.9	2.8
2.	200	---	5.90	24.5	40.0	37.0	1.4	6.8
3.	600	---	5.55	23.0	49.0	37.5	2.6	8.5
4.	1200	---	5.45	26.1	102.0	37.0	11.5	8.0
5.	1800	---	5.25	23.9	114.0	40.0	11.8	7.3
6.	---	200	5.80	23.9	22.0	39.0	1.0	6.5
7.	200	200	5.65	24.0	28.0	37.0	2.5	5.0
8.	600	200	5.45	22.0	80.0	36.0	3.1	6.8
9.	1200	200	5.38	24.0	126.0	40.0	11.9	7.4
10.	1800	200	5.25	22.0	143.0	45.0	15.0	9.3
11.	---	600	5.55	20.0	29.0	51.0	4.0	7.0
12.	200	600	5.55	23.0	37.0	51.0	6.7	6.6
13.	600	600	5.48	20.0	100.0	50.5	12.2	7.8
14.	1200	600	5.25	23.8	140.0	60.0	12.2	7.0
15.	1800	600	5.25	26.0	162.0	52.5	15.5	8.9
16.	---	1200	5.50	20.9	26.0	70.0	5.8	8.0
17.	200	1200	5.45	24.0	39.0	68.0	9.4	9.0
18.	600	1200	5.25	23.0	48.0	65.0	14.0	9.0
19.	1200	1200	5.15	23.9	92.0	65.0	18.5	8.6
20.	1800	1200	5.05	22.4	148.0	67.0	14.5	8.6
21.	---	1800	5.25	25.0	28.0	80.0	2.5	3.8
22.	200	1800	5.35	25.0	38.0	81.5	2.2	3.6
23.	600	1800	5.19	21.0	47.0	82.5	2.8	6.0
24.	1200	1800	4.99	21.4	138.0	77.0	8.0	2.9
25.	1800	1800	4.49	20.7	152.0	75.5	7.8	6.5

* Based on dry tissue.

TABLE II. The Effect of Manganese and Iron Sulfates applied to Granby sandy loam soil on the yield of oats and the Manganese and Iron contents of the soil and the ash from the tops of the oat plants grown on the soil.

No.	TREATMENT		pH of CULTURE	AIR DRY TISSUE PER POT IN GRAMS	SOIL ANALYSIS		PLANT ASH ANALYSIS	
	$MnSO_4$ lbs/A	$FeSO_4$ lbs/A			MANGANESE ppm	IRON ppm	MANGANESE* Mgms/Gram	IRON* Mgms/Gram
1.	---	---	7.79	6.9	5.8	10.8	0.031	8.60
2.	200	---	7.75	21.0	15.0	11.0	0.051	9.80
3.	600	---	7.60	23.0	63.0	11.0	0.156	6.00
4.	1200	---	7.48	20.0	81.0	11.0	0.156	6.00
5.	1800	---	7.43	21.2	88.0	11.0	0.313	11.40
6.	---	200	7.78	9.5	6.1	13.5	0.060	7.20
7.	200	200	7.59	22.9	3.2	12.0	0.070	6.80
8.	600	200	7.51	23.0	70.8	12.0	0.093	5.80
9.	1200	200	7.38	22.1	74.3	14.3	0.193	6.20
10.	1800	200	7.35	21.0	93.4	13.5	0.563	5.70
11.	---	600	7.61	9.5	5.7	17.0	0.063	4.00
12.	200	600	7.55	22.5	21.6	18.0	0.070	3.90
13.	600	600	7.40	26.0	66.6	17.5	0.084	3.60
14.	1200	600	7.45	23.5	70.8	18.0	0.094	3.60
15.	1800	600	7.32	23.0	90.2	16.0	0.500	3.80
16.	---	1200	7.60	9.0	5.8	21.0	0.063	6.40
17.	200	1200	7.45	24.8	23.0	21.5	0.068	8.70
18.	600	1200	7.40	23.1	68.0	22.0	0.156	4.50
19.	1200	1200	7.32	22.8	71.0	21.5	0.113	4.90
20.	1800	1200	7.30	24.2	99.0	21.0	0.500	3.50
21.	---	1800	7.48	13.0	5.1	28.0	0.050	5.20
22.	200	1800	7.40	21.9	20.1	25.0	0.044	4.00
23.	600	1800	7.35	23.0	66.0	19.5	0.050	4.10
24.	1200	1800	7.30	22.0	72.0	25.5	0.200	3.30
25.	1800	1800	7.25	21.0	86.4	24.0	0.500	3.30

* Based on dry tissue.

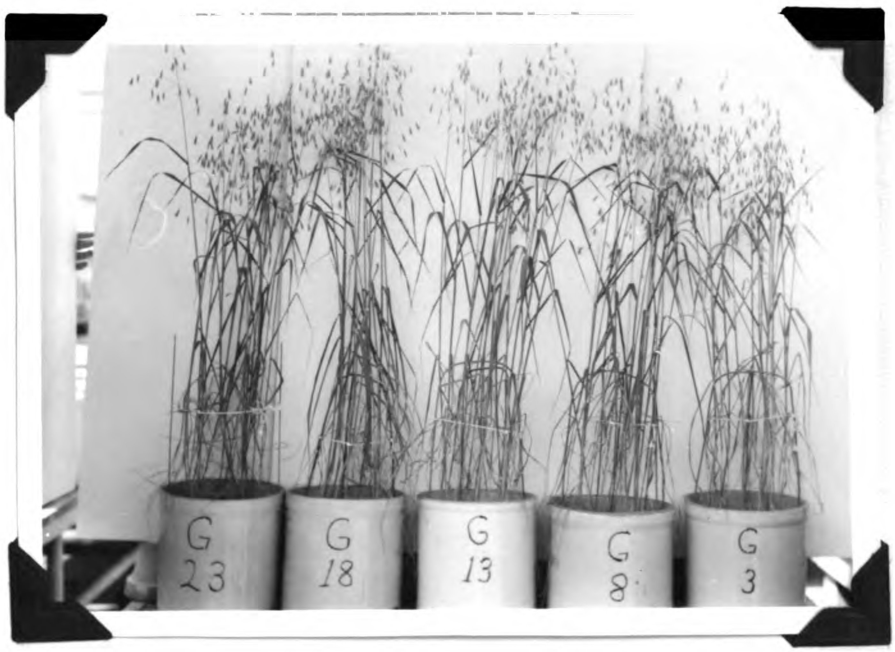

Plate 1. The Response of Oats to 600 pounds per acre of $MnSO_4$ added to Granby sand at Five levels of $FeSO_4$.

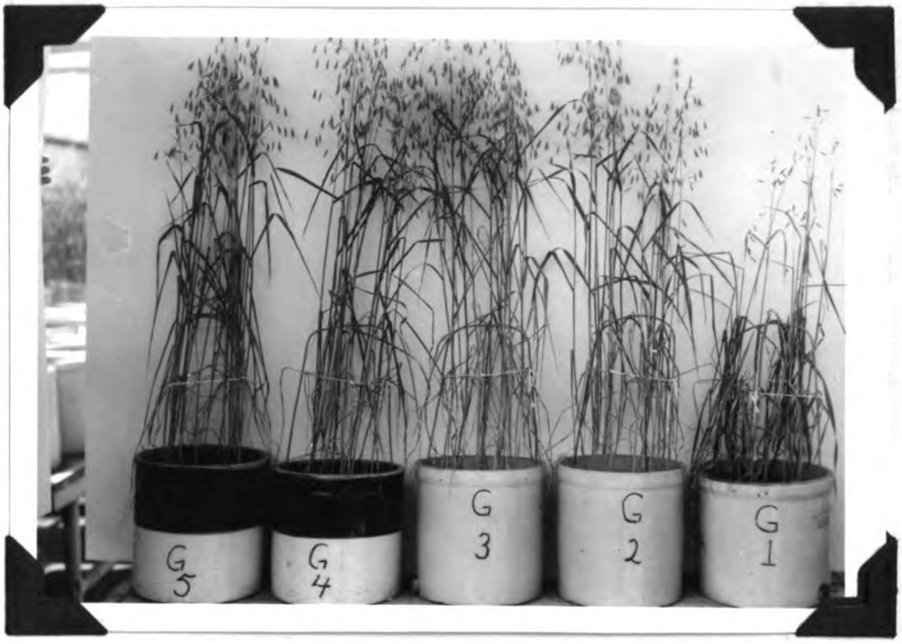

Plate 2. The Response of Oats to increasing amounts of $MnSO_4$ added to Granby sandy loam. No $FeSO_4$ added.

Plate 3. The Response of Oats to increasing amounts of $FeSO_4$ added to Granby sandy loam. No $MnSO_4$ added.