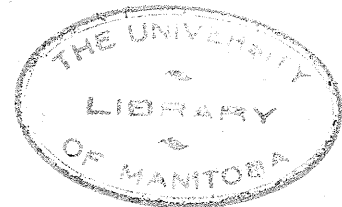


THE EFFECT OF CALCIUM AND POTASSIUM
ON THE ABSORPTION OF
IRON AND MANGANESE BY PLANTS

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INTRODUCTION

Active plant growth is a result of the interaction of the hereditary constitution of the plant with its environment. In addition to light, moisture, heat, oxygen, carbon dioxide and water, plants require from their environment a number of metallic and non-metallic elements to facilitate the proper functioning of their metabolic processes. The elements used by plants have been classified as major and minor elements according to the quantities in which they are used by plants. Among the major elements are found potassium and calcium, both of which are absorbed in fairly large quantities. On the other hand, iron and manganese which are of the minor element group are absorbed to a far lesser extent, but nevertheless they have been shown to be indispensable for normal plant development.

The object of the present investigation was to determine the action of the two major elements (Ca and K) on the absorption of the two minor elements (Fe and Mn). Lime induced chlorosis due to insufficient absorption or improper iron metabolism, as well as manganese deficiency in highly limed soils (5,28,36,15) are practical demonstrations of the action of one element on the absorption and accumulation of an other.

The absorption of iron and manganese in connection with calcium and potassium supply have been studied by a

number of investigators. Swanback, (43) in his studies on the antagonistic phenomena and cation absorption in tobacco in the presence and absence of manganese and boron, has made some observations related to the subject.

In this investigation an attempt has been made to study the absorption by tomato and oat plants of iron and manganese in culture solutions containing different levels of calcium and potassium and to compare the results thus obtained with the conclusions drawn by Swanback ~~in~~ (43) from his studies on Nicotiana. To facilitate adequate comparison the compositions of the solutions used were essentially similar to those of the solutions used by Swanback (43). Some alterations, however, were made, primarily with regard to the microelements used to enable simultaneous investigation on manganese deficiency (grey speck disease). This was done with a view to continuing the work previously carried out on this subject in the Department of Botany, The University of Manitoba, by Miss Ruth Bulman and which has already been described in her thesis(3).

REVIEW OF LITERATURE

Absorption of Manganese by Plants in connection
with Ca and K Supply.

Manganese occurs in the soil in several forms, i.e. as exchangeable manganese, as a cation attached to clay particles, as a part of organic matter and as a constituent of certain minerals. Manganese deficiency symptoms in plants may be due to a scarcity of this metal in the soil in the vicinity of the plant root or the manganese may be present in the soil in a non-available form. To be available to plants manganese must exist as an exchangeable manganese, as part of organic matter or in an inorganic easily reducible form. In nature manganese is very readily oxidizable thus made not available for the vegetation. Therefore factors affecting the manganic-manganous status in the soil are of great importance. Sherman and Harmer (37) stated that the manganic-manganous equilibrium in the soil depends on its acidity, lime and phosphate content, clay content, aeration temperature and the presence of reducing or oxidizing agents. They showed that neutral or alkaline conditions favor the formation of manganous manganese and acid conditions of manganic manganese. Sulfur retarded the oxidation of manganese.

When strongly acid soils which were rich in soluble manganese were limed the oxidation reduction equilibrium was shifted toward the manganous side and the soils became manganese-deficient with respect to available manganese for plant growth. Generally winter conditions favor the formation of the manganous ion and summer conditions the manganic ion. Manganese is leached easily from acid soils but not so from alkaline soils because of its greater solubility in the former.

Gilbert (10) found that manganese deficiency is linked with soil alkalinity, but the pH range in which chlorosis may occur varies with crop and climate and not with the soil type. Generally, however, manganese deficiency symptoms have been found in organic highly limed soils, or sandy soils, as pointed out by Sherman and Harner (37). Gisiger (12) believed on bases of analyses of straw that the availability of manganese is greater in acid and very alkaline soils than in neutral or slightly alkaline soils.

Olsen (31) held that different plant species absorb different amounts of manganese from the same soil and that different species can tolerate different amounts of manganese with varying optima.

Microbiological activity may affect the state of soluble manganese to some extent. Gerretsen (8)

thought that precipitation of manganic oxide in the soil was caused by specific microorganisms when the pH was between 6.5 and 7.8. MacLachland (26) was able to isolate manganese oxidizing bacteria and fungi and attributed the deficiency of available manganese in the soil to microbial activity.

Morris and Pierre (30) carried out experiments with Lespedeza which is very sensitive to manganese and shows manganese toxicity symptoms at a concentration of 5 p.p.m. They found that by increasing the calcium concentration in the culture solutions from 12 p.p.m. to 60 p.p.m. or 300 p.p.m. there was no effect in reducing manganese toxicity. There was even some indication that manganese toxicity was increased in the higher calcium levels. No evidence was found to show that calcium reduced the toxicity of manganese as reported by Hewlitt (15). However, liming has been found to be beneficial to the growth of Lespedeza in acid soils and this probably might be due, at least in part, to the fact that liming decreases the amount of soluble manganese present in the soil. It was found that ~~all~~ water-soluble manganese was diminished on liming soils and bringing the pH up to values of 5.5 to 6.0.

Swanback, (43) in a study of cation absorption by tobacco plants, observed that there was an antagonism

between calcium and manganese. Symptoms of manganese deficiency were very pronounced with high calcium in the culture solutions, less with medium, and not noticeable with low calcium. Manganese deficiency symptoms were found to appear where calcium hindered the absorption and utilization of manganese at low manganese levels. Hewitt (15) thought that calcium as an element clearly antagonized the intake of manganese. His experiments have been done in sand cultures. Sugar cane and cauliflower were affected with manganese toxicity in acid soils; however, the calcium status modified the toxicity effects considerably. With increasing calcium the toxicity effects decreased.

Burström (4) in his work upon the role of manganese in the nitrate assimilation, showed definitely an antagonism between potassium and manganese. In lower potassium concentrations the intake of manganese by wheat increased by increasing potassium concentrations from $K = 1/2000$ mol. to $K = 1/1000$ mol., but fell rapidly when potassium concentrations were increased further to $K = 1/500$ or $K = 1/125$ mol. He called it a typical cation antagonism.

McCool (27) showed that $N/500$ $MnCl_2$ in distilled water prevented the growth of wheat seedlings and $N/200$

$MnCl_2$ was extremely toxic. This deleterious action was overcome by the presence of N/50 KCl in the nutrient solution. A weaker solution of KCl (N/500) was antagonistic to manganese absorption but to a lesser extent than more concentrated solutions. A N/50 KCl solution was found to be very injurious to roots of wheat seedlings. The presence of either N/500, N/2000 or N/500 $MnCl_2$ counteracted this effect, showing that there existed a mutual antagonism between potassium and manganese. In the same paper McCool stated that he found a definite antagonism between calcium and manganese. By means of several experiments he showed that manganese was rendered innocuous by the addition of calcium, e.g. a pure solution of N/4000 $MnCl_2$ was toxic to Canada pea seedlings, but when a stronger solution of N/1000 $MnCl_2$ was mixed with N/2000 $CaCl_2$, injury due to manganese was greatly reduced. Protective action existed as well with various different combinations of calcium and manganese. He further found from repeated experiments that large applications of lime to pots of soil overdosed with manganese resulted in a more vigorous development of the plants in the pots. Swanback's (43) determinations of the manganese content of shoots and roots of tobacco plants, grown in different levels of potassium and calcium and 0.0054 millimol. of manganese per litre, showed that calcium has a reducing effect on both the

intake and translocation of manganese to the leaves, while no such effect was suggested regarding potassium.

Absorption of Iron by Plants in Connection
with Ca and K supply.

Iron is more abundant in the soil than manganese and is at the same time required in greater amounts by plants. It is available to plants in the ferrous condition as soluble iron or in the colloidal state. It may be precipitated in the soil by iron fixing bacteria in the form of ferric-hydrate, or it may be transformed into the ferric state by any oxidation processes possible (38). Arnon in 1943(1) in disagreement with Somers and Shieve's (43) view, expressed the opinion that both the ferrous and the ferric form of iron, and not the ferrous iron alone, should be regarded as metabolically active.

Chapman (5) showed that colloidal iron can be absorbed by plants, but that it can be utilized only under certain conditions. He concluded from this that the "water soluble" iron content of a soil is not directly related to chlorosis in plants, as part of the available iron is probably associated with the soil colloids or is itself colloidal. Colloidal iron will remain available unless it is absorbed by the calcium carbonate in calcareous soils. In vitro, colloidal iron is most easily brought into solution by weak acids in the presence of a

reducing agent, and Chapman (5) believed that by these means it is brought into solution after absorption. The colloidal iron cannot be reduced in chlorotic plants, and this Chapman ascribed to be the result of a breakdown of the oxygen exchange mechanism.

Iron compounds have long been regarded as oxygen carriers in plants and animals. Disturbances in iron nutrition are probably the most commonly recognized nutritional disorders causing chlorosis.

In connection with this Lindner and Harley (23) stated that there are various ways in which the iron nutrition of plants may be affected so as to bring about chlorotic conditions. Four of the most commonly recognised types are: (1) true iron deficiency, (2) an upset in the phosphate-iron balance, (3) an upset in the iron-manganese balance, (4) a lime induced chlorosis. The latter is probably the widest spread disorder of this type and it has assumed serious proportions in the more arid regions where high concentrations of calcium-carbonate and magnesium-carbonate are present in the soil.

True iron deficiency occurs experimentally in water culture solutions or sand cultures, where the iron level of the nutrient solutions is kept low, or the absorption and utilisation of iron is retarded by the pH or the presence of other metals. Since iron is so widely

spread in most soils, and since the amount of iron necessary for normal plant growth is relatively small, this simple type of chlorosis probably occurs frequently under field conditions.

Sideris and Young (40) found that chlorosis, due to iron deficiency, occurred in pineapple plants generally on manganiferous soils and that it could be prevented by application of iron-sulfate. It may, however, develop in non-manganiferous soils with pH values higher than 5.5 especially when treated with fertilizers which have a residual alkaline reaction. Consequently high pH causes oxidation of iron with following precipitation of the latter.

Johnson (21) and Kelley (22) believed that manganese induces iron chlorosis by oxidizing ferrous iron to ferric iron in the soil, a reaction comparable to that occurring at high pH.

It has been found quite often that iron as well as manganese is more soluble in acid than in alkaline soils. The danger of diminishing the iron content in the soil by leaching is therefore greater in acid soils, especially when poor in lime.

McGeorge (28) concluded from Johnson's (20) and Kelley's (22) data that pineapple chlorosis on manganiferous soils was due to a greater assimilation of calcium

and that the principal disturbance was the greater immobility of iron in the plants caused by excessive lime content in the stalk and leaves; since iron was present in equally available form in both, manganiferous and non-manganiferous soils of equal pH.

Gile (11) distinguished between manganese induced chlorosis and lime induced chlorosis, and stated that manganese induced chlorosis in pineapples in Hawaii was in part due to iron deficiency in the plants induced by manganese, while the lime induced chlorosis in Puerto Rico was due to a lack of iron in the plants induced by calcium carbonate diminishing the availability of iron in the soil.

Tunmann (44) found that "high lime" plants displayed an iron immobility characterized by copious precipitation of iron in the roots with a tendency to diminish in aerial parts to such an extent as to introduce iron chlorosis symptoms. Loehwing (24) and Shiøve (38) expressed similar opinions.

Hopkins and Wann (17 , 46) held that iron is removed by adsorption on calcium phosphate, which gradually precipitates when solutions become alkaline. This is a physico-chemical effect which may influence iron availability within the plant as well as in the culture media.

The oxidation of iron in the plants, as suggested by Shiøve (38) was ascribed to the fact that the triva-

lent manganese has a higher oxidation reduction potential than the divalent iron. The same ability to induce chlorosis was stressed for cobalt where valency change was associated with a still higher potential.

Chlorosis due to an upset in the iron-manganese balance has been discussed more often and the general opinion seems to be that it leads to a disturbance in the oxidation-reduction potential whereby the functioning of iron is disturbed. However, in some cases a mutual antagonism between iron and manganese has been suggested; where chlorosis was due to a retarded absorption of one of these metals(3,45).

Lime induced chlorosis was studied more recently by Lindner and Harley (23). They reported that there was no consistent correlation between the total iron content in leaves and lime induced chlorosis. Furthermore there seemed to be no correlation between lime induced chlorosis and the phosphate-iron balance. It would thus appear that lime induced chlorosis is not associated with the other types of iron chlorosis. In some cases a lower iron content may be a contributing factor in lime induced chlorosis but other factors must also be significant. Lime induced chlorosis is probably brought about by a complex of causes whose interrelations are not yet fully established. There was always found a relatively high

potassium-calcium ratio by Lindner and Harley (24) in chlorotic leaves which lead to the suggestion that a relatively high potassium level induces chlorosis by replacing the iron in the enzyme responsible for chlorophyll formation, thereby inactivating the enzyme.

Parsche (32) showed that calcium-chloride, as well as calcium-carbonate, may induce iron chlorosis in lupine even though the calcium chloride did not affect the pH of the soil appreciably.

Bennett (2) stated that potassium deficiency caused a chlorosis of plum trees which appeared to be a disturbance of the functioning of iron. The chlorotic leaves did not respond to iron applications which usually cure lime induced chlorosis, but they became green when large applications were made. In this form of chlorosis potassium-calcium ratios were lower in chlorotic than in green leaves. A disturbed iron functioning here thus seems to be due to a ratio of potassium to calcium opposite to that existing in lime induced chlorosis.

Hartt (14) found a nodular accumulation of iron in sugar cane where plants were starved for potassium. The addition of potassium to those plants caused some of the iron to pass up into the leaf blades. McGeorge (29) stated that both potassium and phosphorus aid in the distribution of iron and aluminum in the nodes of cane.

Iron toxicity symptoms occurred in plants starved of potash although they contained less iron than plants with good growth, thus supporting the view that the toxicity of iron in sugar cane is rather due to its unequal distribution than to its total concentration. Moreover, it must be taken into account that potash starvation may lead to iron toxicity.

Sideris and Young (40), working with different amounts of potassium on growth and ash constituents of pineapple, found that the plants growing in "high potassium" cultures (205 mg K/L) contained more iron than those in "low potassium" cultures (4 mg K/L). The greater accumulation of iron in the "high potassium" cultures had possibly resulted from the higher rate of metabolic activity which in turn increased the absorption of iron. The greater amount of iron which was found in the roots in "high potassium" cultures than in "low potassium" cultures was probably due to the fact that iron was attracted to the root surface by some physico-chemical force generated in the cells almost proportional to the metabolic activity of the cells, as indicated by the better growth of "high potassium" plants. In the same set of experiments the plants given ammonia as a nitrogen source contained more iron than those given nitrates, indicating that the higher hydrogen ion concentration in the ammonium series had

avored the absorption of iron by increasing its solubility.

Rhode (35) stated that potassium-deficient plants contained low amounts of iron but high amounts of manganese. Moreover he asserted that catalytic enzymes which contain iron and influence assimilation either decrease or completely cease their activity in potassium-deficient plants, whereas enzymes containing manganese are able to continue their function in assimilation and in consequence promote certain metabolic changes which cease on account of iron deficiency.

From the statements cited above it seems that potassium does not have a direct effect upon the absorption of iron. In low concentrations of potassium the absorption of iron is inhibited by lower metabolic activities of the plant; a too high concentration of potassium disturbs the enzyme system by replacing iron in these compounds.

As regards calcium, Swanback (43) suggested that absorption of iron is retarded by calcium. The approximate translocation quotient for iron was 1.0 at low calcium concentrations (0.042 g Ca/L) while in the cultures with high amounts of calcium (0.143 g Ca/L and 0.403 g Ca/L) the quotient was lowered to 0.20 or less. He thought it was possible that insoluble iron compounds were formed in the roots and therefore not translocated.

Chapman (5) working with Pinus maritima,

found that excessive calcium combined with manganese was rapidly fatal by producing iron chlorosis, while high calcium without manganese was tolerated.

Haas (13) stated that with calcareous soils excessive soil moisture resulted in high pH values, thus making iron unavailable both inside and outside the plant, and so causing chlorosis.

MATERIALS AND METHODS

1. Methods of Cultivation

The water culture method was used throughout. Although exact duplication of soil conditions was not possible, it was felt that for experimental purposes, particularly in nutritional studies, soil culture methods would entail extensive and elaborate analyses. Ordinary soil is difficult to use because of its complicated physico-chemical structure. In addition, a series of complicating factors would be involved. Therefore the water culture method seemed best adapted to studies on the absorption of salts and elements, and their effects on each other and on the plants. Furthermore the composition of the aqueous nutrient medium is known and can be easily analysed and altered, if necessary, at any time.

Water culture of plants creates certain problems, when normal growth is desired. A proper balance of salts, aeration of the root system, and a satisfactory pH must be provided.

Under greenhouse conditions the required light, humidity and temperature were present. However, only a few of the experiments could be carried out in a greenhouse. Frequently plants were grown in a small laboratory where the culture vessels were placed on tables facing

north-east windows. Additional light was supplied by three 40 watt fluorescent lamps suspended above the plants. On cold days infra red lamps provided heat. Although the temperature in the room was very dry, satisfactory humidity was obtained by keeping the cement floor moist.

The conditions in the small laboratory were far from optimal, which caused many difficulties throughout the course of the studies.

During the summer months the plants were grown in a larger laboratory in front of east and south facing windows where the light conditions were somewhat better. The temperature also was more favourable during this time.

In the spring and summer months fungi and bacteria attacked the roots of the plants so heavily in some cases that the material had to be discarded. Microscopic examination revealed the presence of bacteria, Olpidium brassicae, Fusarium and species of the Mucorales.

Tomato seedlings suffered frequently from the damping off disease which is known to be caused by Pythium. In order to reduce fungus infection, seed-borne organisms on the tomatoes were controlled by a heat treatment. The seeds were kept for 25 minutes in a water bath at 50°C, thereafter they were allowed to germinate under aseptic conditions. Seedling diseases were controlled with mercuric chloride (2 p.p.m. of mercuric chloride added to the

nutrient solution). The plants were kept in such solutions for one week. The presence of mercury even in this concentration markedly depressed the growth of the tomato plants. The fungi appeared, however, to be unable to survive the mercurial treatment as transplanted plants showed normal growth without fungal attack. In some cases it was found that frequent dipping of the tomato roots in an aqueous solution of 10 p.p.m. of mercuric chloride for one or two minutes helped to suppress the growth of fungi.

Oats in general were more difficult to grow in culture solutions than tomatoes. They suffered more severely from fungal and bacterial attacks, and difficulties were encountered in controlling these organisms.

Despite the culture difficulties it was possible to obtain a considerable number of normal, healthy plants with which to carry out the desired experiments, thus making it possible to obtain certain definite results.

2. Nutrient Solutions

The nutrient solutions were prepared from Fisher, T.P. or B.D.H. AnalaR salts and distilled water, prepared in a Barnstead still. The pH of the distilled water was 6.5. A 2000 ml. sample of it was evaporated to a volume of 25 ml. and tested by spectrophotometric analyses for iron and Manganese. The analyses did not indicate the

the presence of either of these elements, and the distilled water was therefore considered to be free from them.

A test of stock solutions did not show the presence of iron or manganese in detectable amounts, and as a result they were not purified. The stock solutions were 10 times the concentration of the working nutrient solutions. These were prepared separately for each of the low calcium, high calcium and potassium series. The low calcium series contained 0.42 g. of calcium per litre; the high calcium series contained 1.43 g. of calcium per litre; while potassium was not included in the potassium series stock but was added as required to each of the nutrient solutions. The compositions of the solutions are given below.

Composition of the salts in the nutrient solutions
of the "low calcium" series.

| Salt | g. per L. of solution |
|--|-----------------------|
| $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ | 0.25 |
| $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ | 0.156 |
| KNO_3 | 0.214 |
| NaNO_3 | 0.037 |
| $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ | 0.32 |
| $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ | 0.32 |

Composition of the salts in the nutrient solutions
of the "high calcium" series.

| Salt | g. per L of solution |
|--|----------------------|
| $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ | 0.847 |
| $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ | 0.156 |
| $\text{Mg SO}_4 \cdot 7\text{H}_2\text{O}$ | 0.308 |
| K_2SO_4 | 0.185 |

Composition of the salts in the nutrient solutions
of the potassium series.

| Salt | g. per L of solution |
|--|------------------------------------|
| $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ | 0.847 |
| $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ | 0.156 |
| K_2SO_4 | A 0.06 B 0.82 C 0.6 D 1.0 |
| $\text{Mg SO}_4 \cdot 7\text{H}_2\text{O}$ | 0.308 |

3. Plants.

The experiment was designed to include studies on mono- and dicotyledonous plants. The monocotyledon used was the strain Tama of Avena sativa L. while the dicotyledon was the garden strain of Marglobe of Lycopersicon esculentum. Seed samples of the oats were provided by Dr. W.H.Hagborg of the Laboratory of Plant Pathology from experimental plots at Oak Bank, Manitoba. The tomato seeds were obtained commercially.

4. Method of Germination:

The seeds were germinated on either moist quartz sand or ashless filter paper. Impurities were removed from the quartz sand before use. This was done by treating it with 10 per cent nitric acid for one day, followed by several rinses with tap water and then distilled water until the pH of the sand remained at 6.5.

Germination of oat seeds on sand or filter paper moistened with distilled water was satisfactory. Seedlings developed within one week to a height of 5-6 inches. The tomato seeds required a longer period of time for germination, and in addition to water a nutrient solution without iron and manganese had to be given. At the end of 3 weeks the tomato seedlings were usually large enough for transplantation into the cultural vessels containing the various nutrient solutions. Both types of plants germinated and grew equally well on

filter paper as on sand. Because of the greater convenience, the seeds were eventually germinated on filter paper. The sheets of paper were easily changed after use, while cleaning the sand was a more complicated procedure.

Before the oat plants were transferred to nutrient solutions the endosperms were removed in order to prevent contamination of the nutrient media with material stored in the seeds. No such precautions were needed with tomatoes because the storage organs of their seeds are the cotyledons.

Two separate micro element solutions were prepared for boron, copper, zinc and molybdenum. Their compositions were as follows:

Micro nutrient solution I (g. per litre)

2.860 g. H_2BO_3

0.220 g. $ZnSO_4 \cdot 7H_2O$

0.080 g. $CuSO_4 \cdot 5H_2O$

Micro nutrient solution II (g. per litre)

0.075 g. MoO_3

One millilitre from each stock solutions was added to one liter of nutrient media thus providing the plants with 0.05 p.p.m. of boron, 0.05 p.p.m. of zinc, 0.02 p.p.m of copper and 0.05 of molybdenum.

Iron and manganese were added in varying concentrations depending upon the experiment.

The manganese was supplied as manganous sulphate. Iron was employed in several ways until a satisfactory method was evolved. At first ferric ammonium citrate was used. However, with this the iron precipitated rapidly, making it necessary to change the solutions very often. In order to confirm the fact that the precipitate contained iron, the solution was filtered, and both the precipitate and the filtrate were tested. The precipitate contained a far higher concentration of iron than the filtrate. Varying the pH within the range of 4 to 7 did not reduce or prevent precipitation.

Iron humate was next tried, although it is known to be a stimulator of bacterial growth. Horner, Burke and Hooven (18) found that humate was more convenient to employ than other forms of iron, such as sulfate, citrate or tartrate.

The following method was used for the preparation of iron humate.

To one litre of 30 volumes per cent sulphuric acid which was brought to boiling, were added 125 g. of sugar. The mixture was allowed to simmer for three hours. After cooling the liquid phase was discarded by filtering. Concentrated potassium hydroxide or sodium hydroxide was added to the solid phase until it partially dissolved and gave an alkaline reaction when tested with phenolphthalein.

The material was re-filtered and the solid phase discarded after one or more washings with dilute alkali. The alkali extract containing relatively pure soluble potassium humate was purified by precipitation with sulfuric acid at a pH of 3-4. The liquid phase was discarded while the solid hydrogen humate was dissolved and neutralized with 1 to 5 per cent potassium hydroxide until the solution had a pH of 7. One litre stock solution of potassium humate was prepared by adding distilled water.

The concentration of the humate in the final solution was determined by precipitating it in a 5 ml. aliquot portion with KOH. The precipitate was dried overnight in a weighed fritted glass filter at 100°C, and reweighed. The total amount of potassium humate contained in 1 litre of solution was 20 grams.

Iron humate was prepared by the addition of ferrous sulfate to portions of potassium humate. KOH was used to raise the pH to 8, thus preventing the solution to flocculate on standing. The K-humate reacted with approximately 2 mg. of iron per milliliter. It was found that plant growth was severely retarded in humate solutions because of the profuse bacterial and fungal growth.

Jacobsen (19) found that iron supply in nutrient solutions could be maintained by the addition of ferric potassium ethylene-diamine-tetra-acetic acid. This

compound forms chelated complexes particularly with di and tri-valent ions. For the preparation of chelated iron 2.6 gms of ethylene-diamine tetra-acetic acid were dissolved in 26.8 ml. of 1 N. potassium hydroxide. To this 2.49 gms of ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) were added directly and a brown precipitate formed. The mixture was diluted to about 400 ml. with distilled water. The remaining precipitate was dissolved by adding drops of concentrated sulfuric acid until the pH 5.5 was reached. A clear yellow solution resulted at this acidity level. The solution was diluted with distilled water to a volume of 500 ml. One milliliter of the solution in one liter of liquid provided 1 p.p.m. of iron. Chelated iron was used in the majority of experiments and was found to be a satisfactory source of iron.

Thoroughly cleaned pyrex glassware was used throughout the experiments. The beakers, etc. were washed first with aerosol and water, then placed in a cleaning solution (made of $\text{K}_2\text{Cr}_2\text{O}_7$ and conc. sulfuric acid) for at least one half hour and then rinsed in five changes of distilled water.

5. Experimental procedure:

In the preliminary experiments the plants were grown in 150 ml. culture vessels. When four days old and about 5 inches high, the seedlings were placed on wet filter paper sheets containing holes for the roots, and supported by plastic discs. The filter paper sheets were kept moist by

suspending strips of filter paper through holes in the discs and paper sheets into the culture solution. The shoot system was kept in $1\frac{1}{2}$ inch glass cylinders closed at the top to maintain the humidity. The cylinders also served as supports for the aerial portions of the plants. No aeration of the nutrient media was provided. According to Eastwood (6) enough oxygen should be supplied to small roots by a $\frac{1}{2}$ to 1 inch moisture saturated air space between the solution level and the base of the shoots. The space gradually had to be increased in depth as the plants grew.

During the preliminary experiment in the calcium series it was found that growing the shoot systems enclosed in cylinders was unsatisfactory unless the conditions were sterile, as moist stagnant air promotes fungal growth. As a result, the internodes and leaves of the plants were attacked by these organisms. In all further experiments the closed cylinders were eliminated. Furthermore, aeration of the nutrient solutions aids in the absorption of salts and thus results in better growth of the experimental plants (16). Consequently in all later experiments vessels of 300 ml. capacity were used and filled with measured quantities of 250 ml. of nutrient solution and aeration was provided for each vessel. The vessels were covered with 5 inch square paraffin coated cardboard plates . $\frac{1}{2}$ inch wide and 1 to $1\frac{1}{2}$ inch long glass tubes were fitted into holes punched in the

the squares. The purpose of the tubes was to give a firm support to the plants. These were held in place by cotton packing around the bases of the shoots.

The arrangement of the aeration system was very convenient. $\frac{1}{3}$ h.p. electric pressure pump forced the air into a series of glass T-pieces which were joined together with rubber connectors and supported by glass rods, stands and clamps. Bent tubes were attached by rubber connectors to each T-piece and these carried the air to the bottoms of the culture vessels. The air pressures were regulated by screw clamps on each of the tubes leading to the culture vessels. Before the air entered the T-tubes from the pump, it was led through a cotton filter and moistened in a bubbler.



PLATE 1

The general arrangement of the water culture experiments.

Oat plants frequently required additional support for their shoot system. Enameled wire loops which enclosed the shoot systems were provided for this purpose. The wire was attached to rods fastened to the stands above the plants. Black paper folded around the vessels excluded light from the roots thus providing more natural conditions and preventing growth of algae. The general arrangement of these experiments is shown in Plate 1.

6. Analytical Methods:

A modification of the wet digestion method for plant analyses recommended by Sandell¹ was employed in the first experiment. The roots and shoots were analysed together in this single case. After the roots were thoroughly rinsed with distilled water, the plants were dried overnight in an oven at 100°C. and were then cooled in a desiccator, weighed, placed in beakers moistened with 1 ml. of distilled water, and digested in 2 ml. of concentrated sulfuric acid and 5 ml. of concentrated nitric acid on a hot plate at boiling temperature. A few drops of nitric acid were added from time to time until the plant material was completely dissolved and a clear solution was obtained. On cooling 5 ml. of distilled water were added and the solution evaporated again until white fumes of sulfuric acid appeared and approximately 1 ml. of the solution was left in the evaporating dish. The solution was then filtered and the

filter paper washed with $6N HNO_3$ into a 50 ml. volumetric flask, then made up to volume with distilled water,

The same wet digestion method as described for plant material was used for analysing solutions containing iron humate as otherwise the dark brown color of these solutes would interfere with the colorimetric determination of iron and manganese. Solutions containing chelated iron were colorless and could be analysed directly. 50 ml. samples were taken whenever the solutions were changed or the experiment discontinued. The volumes of the nutrient solutions were checked before and after the plants were placed in them.

The iron determinations were carried out by the o-phenanthroline method described by Sandell (36). This method is based on the reaction occurring between ferrous iron and the o-phenanthroline resulting in the formation of an orange-red complex $[(C_{12}H_8N_2)_3Fe]^{++}$. Sandell mentioned that the color intensity is dependent on the amount of iron present irrespective of the pH within the range of 2 to 9. Full color development occurs within one hour (36). In the analytical work herein reported it was found, however, that the most satisfactory results were obtained when the pH level was within the higher acidic range (approximately pH 3-5). The method outlined did not result in full color development. Therefore minor modifications of Sandell's

method were made.

The following procedure was employed for analytical work. A 5 ml. sample was taken from the 50 ml. volumetric flask containing the dissolved material or nutrient solution. This was used for determination of the amount of 1:1 ammonium hydroxide needed to bring the pH to approximately 4. One half millilitre of both 1 M. acetic acid and 1 M. sodium acetate were added as buffers, and a few drops of bromokresol green were used as a color indicator. A further 10 ml. of the solution to be analysed were transferred into a 25 ml. volumetric flask. The required amount of buffer substance and 1:1 ammonium hydroxide were added and the solution was made up to volume with distilled water. A 10 ml. portion of the working sample was placed in a test tube, 1 ml. of hydroquinone and 1 ml. of 0.5% o-phenanthroline solutions were added. The hydroquinone solution (composed of 6.5 ml. of 1 M acetic acid and 3.5 ml. of 1 M sodium acetate in 100 ml. of distilled water with 1 g. of hydroquinone) reduced the ferric iron to ferrous iron while the o-phenanthroline formed the orange-red complex mentioned above. Spectrophotometric analyses were conducted based on the principles discussed on pages 35-36.

For manganese determination 35 ml. of the dissolved material were transferred to a 150 ml. beaker along with 5 ml. of concentrated nitric acid and 1 ml. of concentrated

sulphuric acid plus 0.1 gm. of potassium periodate. The solution was heated to boiling point at which the reaction $2\text{Mn} + 5 \text{Io}_4^- + 3\text{H}_2\text{O}^- \rightarrow 2\text{MnO}_4^- + 5 \text{Io}_3^- + 6\text{H}$ takes place. The purple color of the solution is a result of the presence of the MnO_4^- . The color intensity is dependent upon the amount of manganese in the solution. Some difficulties arose in this connection. With low amounts of manganese the color failed to develop, and sometimes though developing, faded upon cooling or by the addition of water.

Richards (34) reported the necessity of accurate control of acidity in order to obtain satisfactory color development with small quantities of manganese. The addition of sulphuric acid prevented color fading within the acidity range of 5-6%. Excess acidity caused inadequate color development, or caused rapid fading. Skinner and Patterson (33) ashed 10-20 gms. of animal material in a muffle furnace at cherry red and were thus able to oxidize the samples directly, with 0.3 gms. of potassium periodate after they had been extracted with 85% phosphoric acid and 30-50 ml. of water and filtered. 0.01 mgs. of manganese in 50 ml. gave a readable color.

As sulphuric acid is very difficult to evaporate, and as spluttering tends to occur when the amount of solution is reduced, it seemed advisable to reduce the acidity in the working sample and to eliminate the sulphuric acid

if possible. In wet digestion it was not possible to omit the sulphuric and nitric acid. Therefore an ashing method seemed to be more suitable.

The method described by Lindner and Harley (23) was attempted. The plant material was dried overnight at 100°C., cooled in a desiccator and the dry weight determined. It was then ground in a synthetic sapphire mortar to facilitate proper ashing. The size of sample used for ashing was determined by the amount of plant material available. Samples varying from 0.2-1 g. were accurately weighed and ashed in silica crucibles in a muffle furnace at 550°C. for 16 hours. Following cooling in a desiccator the ash was weighed and then moistened with one ml. of distilled water and dissolved in 5 ml. of 6N nitric acid by heating for 1-2 hours.

When a residue was present it was necessary to filter the solution through an ashless filter paper which had previously been washed with 6N nitric acid and distilled water. It was found, however, in some cases that the ash had not been completely dissolved, when it was possible to detect traces of iron and manganese on the filter paper. Therefore, 2 ml. of concentrated nitric acid were used in addition to the 6 N nitric acid to dissolve the ash, and drops of the concentrated acid were frequently added to the mixture alternately with some distilled water until a clear solution

free of any residue resulted. Filtering was then not necessary and the material could be transferred directly to a 50 ml. volumetric flask.

The iron determinations were made by the method described above. For the manganese determinations the solution was first evaporated to approximately 15 ml. as better color development occurred at higher manganese concentrations. The procedure followed was otherwise the same as in the previous experiments. Satisfactory manganese determinations could be made at all manganese levels used.

7. Spectrophotometric Analyses.

The model 14 Coleman universal spectrophotometer was used. It operates on 115 volts A.C. and a frequency of 60-25 cycles.

This instrument is a combination spectrometer and photometer. The spectrometer produces colored light of any selected color and is calibrated in terms of wave-length (λ - μ). The photometer measures the intensity of the monochromatic beam produced by the associated monochromator.

The readings were made by photometric measurements, first with a reference (distilled water) which was assumed to have 100% transmittance, and then with a colored sample interposed in the light beam. The ratio of the two intensity measurements gave percentage transmittance of the sam-

ple at the wave-length ($\lambda - \mu\mu$). The transmittance is positively related to the depth of color in the solution. This is determined by the amount of working substance (i.e. Fe or Mn) present in the sample.

The percent transmittance was read directly on a calibrated scale. The instrument was so delicate as to be influenced by the slight differences found in factory made cuvettes. Thus percentage transmission was influenced by the containers used. Therefore only two cuvettes 19 ml. in diameter with equal transmittance were used throughout. The solution had to be perfectly clear and free from bubbles at the time the readings were taken in order to diminish the error which was + 1% when all precautions were taken and requirements met.

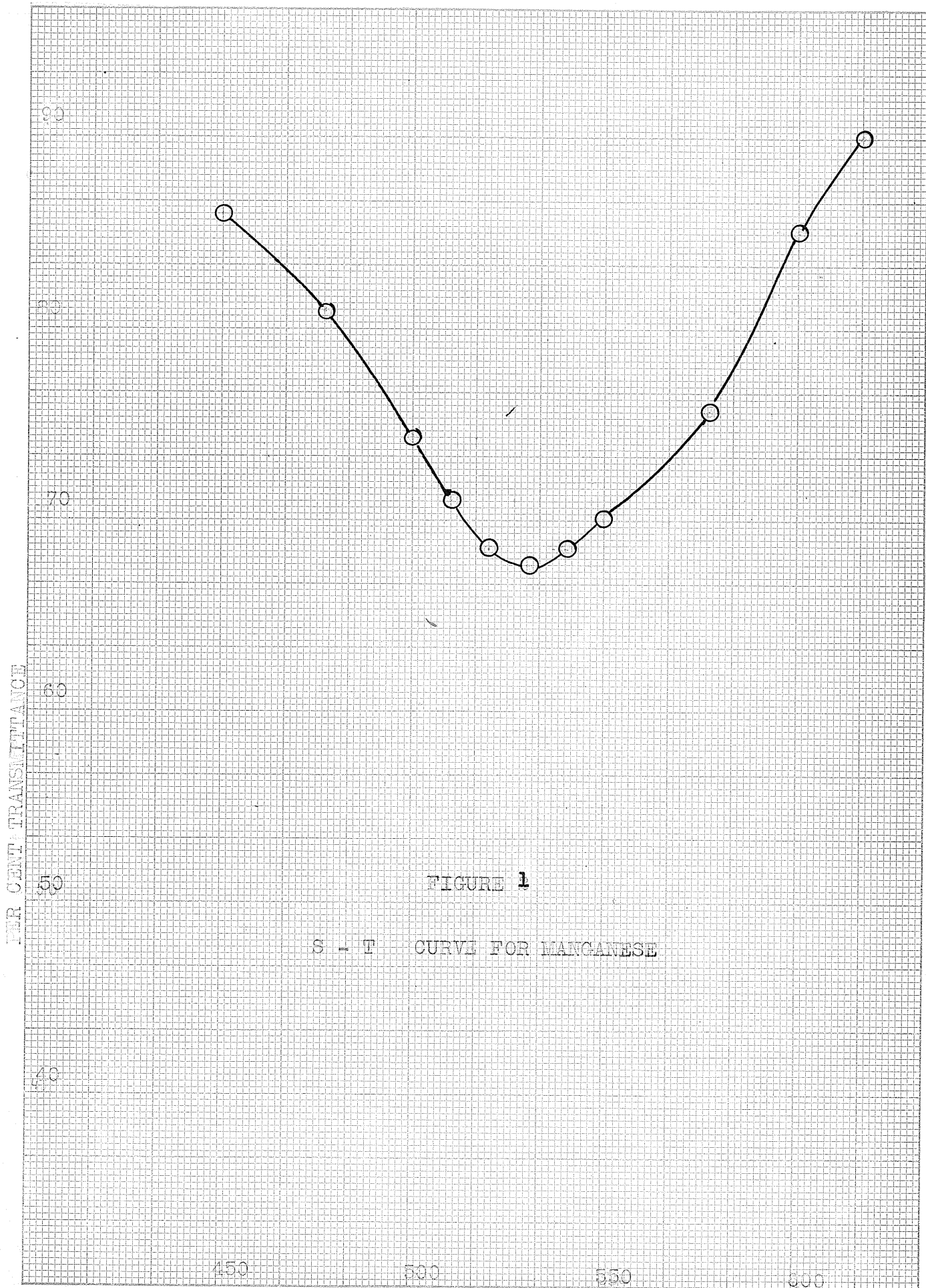


FIGURE 1

S - T CURVE FOR MANGANESE

WAVE LENGTH IN MILLIMICRONS

Per Cent Transmittance

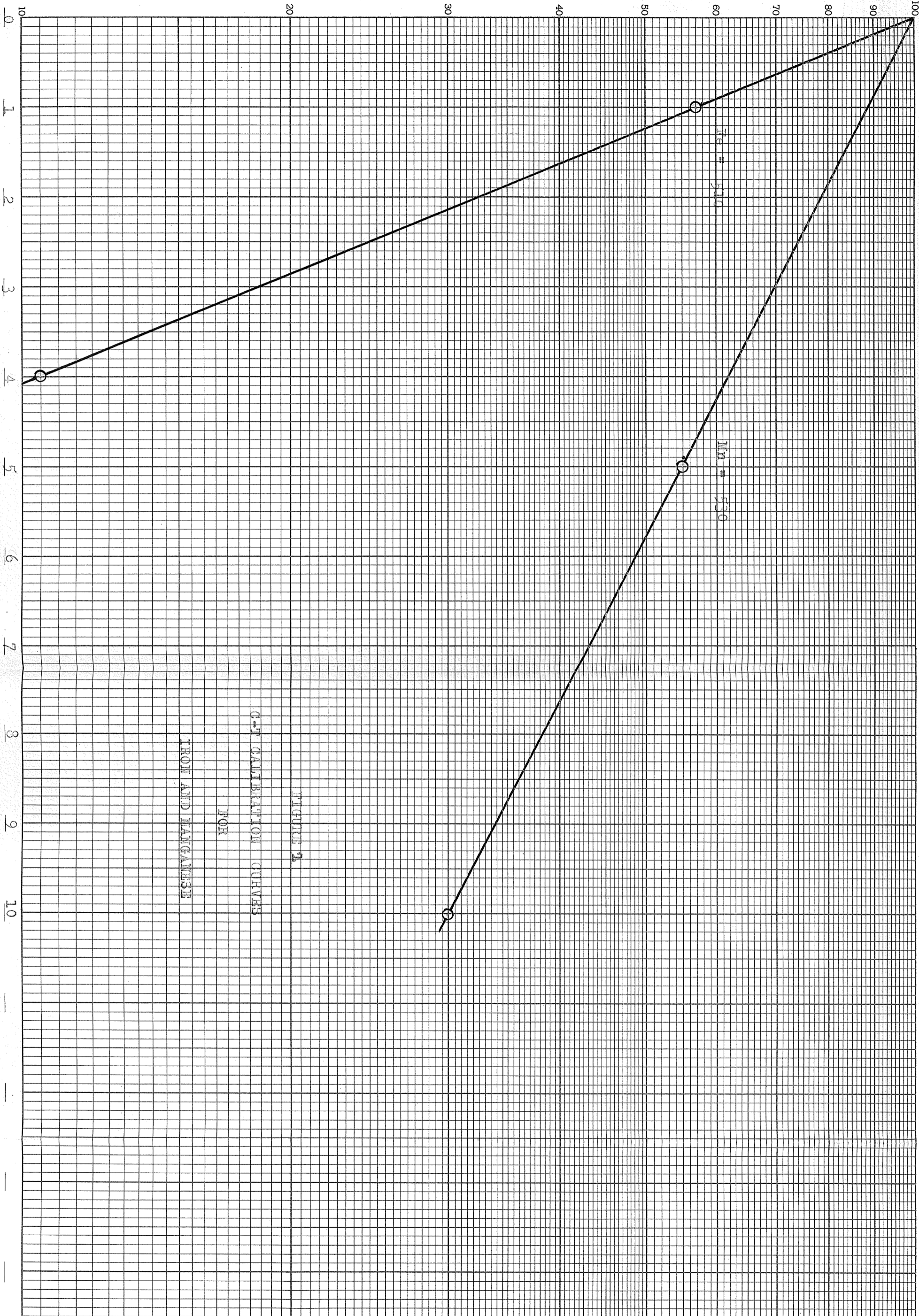


FIGURE 1
C-1 CALIBRATION CURVES
FOR
IRON AND MANGANESE

Concentration in parts per million

MODEL 14 COLEMAN SPECTROPHOTOMETER Serial No. _____

Reference _____
Cell Length mm _____ Remarks _____
Wave Length mμ _____
Slit Width mμ _____

graphs it was possible to determine the concentrations of iron and manganese in p.p.m. when the percent transmittance was determined by the spectrophotometer. The C-T curves have not been changed during the experimental work.

The system of spectrophotometric reading is based on the principles of the Lambert-Beer Law which states that the concentration C is proportional to the logarithms of the reciprocal of the transmittance, T, by a factor K.

$$C = K + \log \frac{1}{T} \text{ or } C = -K + \log T.$$

EXPERIMENTAL RESULTS

Experiment I is preliminary in nature on which some experience in the methods to be used was gained, and it will not be taken into account in the discussion of the experimental results.

The experiments were conducted in two general groups. The series of Experiment II was carried out with different amounts of calcium in the nutrient media, whereas in the series of Experiment III the amounts of potassium were varied. The aim was to determine the effects of calcium and potassium on the absorption of iron and manganese. In both experiments Avena sativa and Lycopersicon esculentum were grown. Each main experiment was subdivided into two series, (A - Avena sativa and B - Lycopersicon esculentum). The experiments for each series were repeated three or four times.

(1) THE EFFECTS OF VARYING QUANTITIES OF
CALCIUM IN THE NUTRIENT SOLUTIONS ON
GROWTH AND ABSORPTION OF IRON AND
MANGANESE.

Calcium was supplied in two different amounts.

In the "low Calcium" level the solutions contained 42 p.p.m. of calcium; in the "high calcium" level 143 p.p.m. of calcium.



Iron and manganese were provided in ratios 5/0; 5/1; and 5/2,5; as indicated individually on the tables.

The experiments were set up in duplicate. The plants and solutions from analogous culture vessels were analysed together.

Preliminary Experiment I - *Avena sativa*
grown in different levels of calcium.

Iron was used in the form of ferric ammonium tartrate.

The experiment was set up with 10 plants for each individual group. It was carried out in a laboratory room.

The nutrient solutions were changed after the first 19 days and analyzed for iron and manganese. The data obtained are given in Table I.

TABLE I. Experiment I

Change in iron and manganese content of nutrient solutions during the first 19 days of experiment.

| Ca level in p.p.m. | Initial amount in nutrient solutions | | Amount lost from solution | |
|-----------------------|---|--------|---------------------------|--------|
| | Fe mg. | Mn mg. | Fe mg. | Mn mg. |
| Low Ca. | 1.00 | 0 | 0.103 | 0 |
| 42 p.p.m. | 1.00 | 0.20 | 0.106 | 0.036 |
| | 1.00 | 0.50 | 0.115 | 0.065 |
| High Ca | 1.00 | 0 | 0.100 | 0.00 |
| | 1.00 | 0.20 | 0.098 | 0.38 |
| | 143 p.p.m. | 1.00 | 0.50 | 0.105 |

The culture solutions in which the plants were grown for the next 10 days were analyzed at the end of the experiment. The results of the analyses are listed in Table II.

TABLE II. Experiment I

Change in iron and manganese content in nutrient solutions during the 10 day period from the 20th to the 30th day.

| Ca level in p.p.m. | Initial amount in nutrient solutions | | Amount lost from solution | |
|-----------------------|---|--------|---------------------------|--------|
| | Fe mg. | Mn mg. | Fe mg. | Mn mg. |
| Low Ca | 1.00 | 0 | 0.042 | 0 |
| 42 p.p.m. | 1.00 | 0.20 | 0.035 | 0.014 |
| | 1.00 | 0.50 | 0.030 | 0.018 |
| High Ca | 1.00 | 0 | 0.056 | 0 |
| | 1.00 | 0.20 | 0.044 | 0.012 |
| | 143 p.p.m. | 1.00 | 0.50 | 0.034 |

From an examination of the data given in Tables I and II it would appear that both elements concerned were absorbed in relatively greater amounts at the younger stages of plant growth. The diminution of the elements in the nutrient solutions, however, probably is not due only to absorption by plants. The filter paper strips

hanging into the solutions (as described on page 27), probably absorbed some of the nutrient salts and especially more at the beginning of the experiment than towards the end.

Table III shows that the plants contained less iron than would be expected from the analyses of the nutrient media; this supports the above mentioned view regarding the filter paper strips. The content of manganese in the plant material could not be estimated due to technical difficulties.

Plants growing without manganese produced less dry weight than those receiving manganese in both treatments. In the "high calcium" level the difference between plants not receiving any manganese and those receiving 2.5 p.p.m. was very small, but it must be taken into consideration that the latter group of plants suffered excessively from fungal attack.

In the "high calcium" the plants showed a somewhat higher average of dry weight than those in the "low calcium" level.

The entire plants (roots and shoot systems) were analyzed together and it was found that plants receiving more calcium contained more iron.

TABLE III. Experiment I.

Dry weight and iron content of *Avena sativa* plants grown in different levels of calcium.

| Ca level | Time and duration of experiment | Content in nutrient solution | Fe p.p.m. | Mn p.p.m. | Dry weight in g. | per plant average | mg Fe/g dry material | dry average |
|------------|---------------------------------|------------------------------|-----------|-----------|------------------|-------------------|----------------------|-------------|
| Low Ca | June to | 5 | 0 | 0 | 0.196 | | 0.098 | |
| 42 p.p.m. | July 1951 | 5 | 1 | 1 | 0.270 | 0.243 | 0.075 | 0.079 |
| | 29 days | 5 | 2.5 | 2.5 | 0.257 | | 0.066 | |
| High Ca | | 5 | 0 | 0 | 0.282 | | 0.100 | |
| 143 p.p.m. | | 5 | 1 | 1 | 0.310 | 0.293 | 0.110 | 0.101 |
| | | 5 | 2.5 | 2.5 | 0.286 | | 0.093 | |

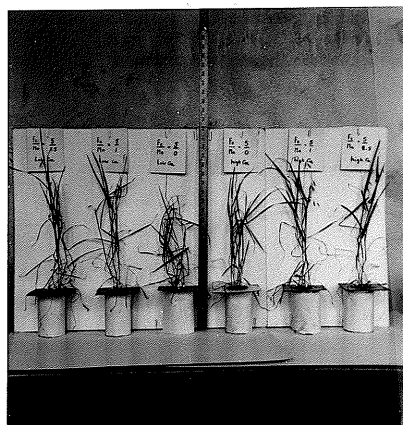


PLATE 2

Avena sativa plants grown in nutrient solutions containing different levels of calcium and different iron/manganese ratios.

Swanback (43) explains it as being due to the fact that calcium causes a shrinkage of pores of the cell walls and thus brings about an accumulation of iron or formation of insoluble iron compounds in the roots.

In the following experiments, therefore, the roots and shoots were analyzed separately.

Experiment II - Series A. *Avena sativa*
grown in different levels of calcium.

The experiments were set up with 10 plants for each individual treatment.

Chelated iron was used in the concentration of 5 p.p.m. throughout.

The pH of the nutrient solutions was 4.5 initially and changed to 5.0 during the first 10 days of use. At this acidity level the solutions remained stable throughout the experimental period.

Group 1. - This experiment was carried out in a laboratory room. The duplicate vessels were inoculated with root tips from grey speck diseased oat plants. All plants were analyzed but the data are given here only for the non-inoculated control vessels, because the inoculum was found to contain iron and manganese and it was not possible to estimate the exact amounts of these elements present in the inoculated nutrient solutions. The nutrient solutions were not changed during the course of the experiment

because an attempt was made to observe the development of grey speck disease in an undisturbed process.

The observations made with regard to grey speck disease, will be discussed separately. At present, attention will be paid only to the absorption of iron and manganese.

The plants grew satisfactorily. Plate 2 shows the plants at the time of discontinuation of the experiment.

Group 2. - This experiment was carried out in a greenhouse.

The culture solutions were changed after the first three weeks. Analyses showed that most of the iron and manganese present in the solutions was absorbed. This was not surprising as vigorous growth was evident. Healthy roots and strong shoots were produced. Plate 3 shows the plants at 40 days of growth in the nutrient media.

Group 3. - This experiment was set up in the laboratory room. Due to the short duration of the experiment a change of the nutrient solutions was not desirable. Many plants suffered from bacterial attack and died. Only normally grown healthy plants with a good root development were selected for analysis.

Analytical data etc. relating to Experiment II are given in Tables IV and V.



Plate 3.

Avena sativa plants grown in nutrient solutions containing different levels of calcium and different iron/manganese ratios.

TABLE IV

Experiment II - Series A

Dry weight and percentage of ash of *Avena sativa* plants grown in solutions containing different levels of calcium.

| Group No. | Ca Level | Time and duration of expt. | Content in nutrient solutions | | Dry weight per plant average | Percent of ash | | Root system age | Average |
|-----------|-----------|----------------------------|-------------------------------|-----------|------------------------------|------------------|-----------------|-----------------|---------|
| | | | Fe p.p.m. | Mn p.p.m. | | Shoot system age | Root system age | | |
| 1 | Low Ca | August to Sept. 1951 | 5 | 0. | 0.095 | 10.26 | 7.4 | 7.4 | |
| | 42 p.p.m | | 5 | 1.0 | 0.117 | 0.107 | 9.49 | 9.91 | 10.6 |
| | | | 5 | 2.5 | 0.111 | 9.98 | 12.6 | 12.6 | 13.5 |
| | High Ca | 55 days | 5 | 0 | 0.091 | 11.64 | 7.4 | 7.4 | |
| | 143 p.p.m | | 5 | 1.0 | 0.133 | 0.126 | 8.09 | 9.55 | 9.0 |
| | | | 5 | 2.5 | 0.153 | 8.93 | 9.4 | 9.4 | 8.6 |
| 2 | Low Ca | December to Jan. 1952 | 5 | 0 | 0.20 | 12.92 | 15.2 | 15.2 | |
| | 42 p.p.m | | 5 | 1.0 | 0.44 | 0.56 | 6.52 | 8.69 | 11.1 |
| | | | 5 | 2.5 | 0.46 | 6.64 | 10.3 | 10.3 | 12.2 |
| | High Ca | 40 days | 5 | 0 | 0.24 | 11.88 | 18.1 | 18.1 | |
| | 143 p.p.m | | 5 | 1.0 | 0.36 | 0.55 | 7.02 | 8.31 | 13.6 |
| | | | 5 | 2.5 | 0.44 | 6.62 | 12.2 | 12.2 | 14.6 |
| 3 | Low Ca | July 1952 | 5 | 2.5 | 0.026 | 13.0 | 5.0 | 5.0 | |
| | High Ca | 15 days | 5 | 2.5 | 0.050 | 16.0 | 16.0 | 16.0 | |

TABLE V. Experiment II - Series A.

Iron and manganese content of *Avena sativa* plants grown in culture solutions containing different levels of calcium.

| Group No. | Ca Level | Time and duration of expt. | Content in nutrient solutions | | | | Content in mg/g dry material | | | |
|-----------|----------|----------------------------|-------------------------------|-----------|------------------|-----------------|------------------------------|-----------------------|------------------|-----------------|
| | | | Fe p.p.m. | Mn p.p.m. | Shoot system age | Root system age | Aver. shoot system age | Aver. root system age | Shoot system age | Root system age |
| 1 | Low Ca | August to Sept. 1951 | 5 | 0 | 0.128 | 1.75 | 0.396 | 0.516 | 0.568 | 1.534 |
| | 42 p.p.m | | 5 | 1.0 | 0.097 | 0.103 | 1.90 | 1.77 | 0.636 | 2.500 |
| | High Ca | 55 days | 5 | 0 | 0.107 | 0.60 | 1.10 | 0.416 | 0.506 | 0.630 |
| 2 | Low Ca | December to January 1952 | 5 | 0 | 0.020 | 2.00 | 0.696 | 1.178 | 0.845 | |
| | 42 p.p.m | | 5 | 1.0 | 0.028 | 0.025 | 1.41 | 1.59 | 0.095 | 0.135 |
| | High Ca | 40 days | 5 | 2.5 | 0.027 | 1.38 | 0.177 | 1.220 | 0.278 | 0.950 |
| 3 | Low Ca | July 1952 | 5 | 2.5 | 0.028 | 1.18 | 0.278 | 0.950 | 0.25 | |
| | High Ca | 15 days | 5 | 2.5 | 0.14 | 0.14 | 0.28 | 0.28 | | |

From Table V it appears that oat plants which grew in solutions with a higher calcium content absorbed less iron in every case, but no such effect of calcium could be noted with regard to manganese absorption.

In the root systems a decreased content of both elements (iron and manganese) was found with plants growing in nutrient media containing more calcium. These results do not bear out the suggestion of Swanback (43) (cited in connection with Experiment I) who believed that calcium caused a diminution of pores in the cell wall and thus leads to an accumulation of iron in the roots. The root analyses have been presented only in order to determine if Swanback's results would apply also to oats. Since there is a possibility of precipitation of substances present in the nutrient solutions on the outer surfaces of the roots, which could introduce errors with regard to absorption, no further stress has been laid on root analysis. For the same reason the results obtained from culture solution analyses are not listed in this thesis.

Experiment II - Series B - *Lycopersicon*
esculentum grown in different levels of
calcium.

The seeds were sterilized by a heat treatment and germinated under aseptic conditions (see pages 18-19).

In the Series B all three sets of experiments were carried out simultaneously in the laboratory.

Three plants were grown for each individual treatment. The plants were supplied with chelated iron. The iron manganese ratio was 5/2.5 throughout.

Initially the pH of the nutrient solutions was 4.5. No pH readings were taken later.

The solutions were changed after the first three weeks. All plants showed good root and shoot development. At the end of the experimental period the shoots were measured and it was found that the plants receiving less calcium were approximately 8 inches tall, while the plants receiving more calcium showed an average of 7 inches. Correspondingly with growth, the dry weight of the plants was somewhat higher in the low calcium treatment than in the high calcium treatment. Table VI gives the dry weight for the individual groups and indicates that the average percentage ash present in the green portions was higher in the higher calcium treatment. Table VII shows that in all three sets it was found that slightly more iron but far less manganese was absorbed or translocated in "high calcium" than in "low calcium". The results contradict those obtained with oats suggesting that no general rule for the absorption of particular elements by plants can be stated. Apparently different plant species behave differently in this as in other respects.

TABLE VI. Experiment II - Series B.

Dry weight and percentage of ash of *Lycopersicon esculentum* plants grown in solution containing different levels of calcium.

| Group No. | Ca level | Time and duration of expt. | Content in nutrient solution | | Dry weight per plant | Average of ash in shoot system | Average percentage of ash |
|-----------|----------|----------------------------|------------------------------|-----------|----------------------|--------------------------------|---------------------------|
| | | | Fe p.p.m. | Mn p.p.m. | | | |
| 1 | Low Ca | May to June | 5 | 2.5 | 0.110 | 19.65 | |
| 2 | 42 p.p.m | June 1952 | 5 | 2.5 | 0.121 | 18.20 | 18.28 |
| 3 | | 44 days | 5 | 2.5 | 0.151 | 16.90 | |
| 1 | High Ca | May to June | 5 | 2.5 | 0.072 | 20.20 | |
| 2 | | June 1952 | 5 | 2.5 | 0.081 | 23.00 | 19.73 |
| 3 | | 44 days | 5 | 2.5 | 0.135 | 16.00 | |

TABLE VII. Experiment II - Series B.

Iron and manganese content of the shoot systems of Lycopersicon
esculentum plants grown in solutions containing different levels
of calcium

| Group No. | Ca level | time and duration of expt. | Content in nut. solutions | | Content in mg/g dry material | |
|-----------|------------|----------------------------|---------------------------|-----------|------------------------------|------------|
| | | | Fe p.p.m. | Mn p.p.m. | Fe average | Mn average |
| 1 | low Ca | May to June 1952 | 5 | 2.5 | 0.094 | 0.500 |
| 2 | | | 5 | 2.5 | 0.125 | 0.560 |
| 3 | 42 p.p.m. | 44 days | 5 | 2.5 | 0.112 | 0.606 |
| 1 | high Ca | May to June 1952 | 5 | 2.5 | 0.150 | 0.393 |
| 2 | 143 p.p.m. | | 5 | 2.5 | 0.151 | 0.384 |
| 3 | | 44 days | 5 | 2.5 | 0.125 | 0.312 |

(2) THE EFFECTS OF VARYING QUANTITIES OF
POTASSIUM IN THE NUTRIENT SOLUTIONS
ON GROWTH AND ABSORPTION OF IRON AND
MANGANESE.

Potassium was used in four different concentrations which were as follows: K - 26 p.p.m.; K - 82 p.p.m.; K - 260 p.p.m.; and K - 450 p.p.m.

Experiment III - Series A - *Avena sativa*
grown in different levels of potassium.

Duplicate vessels, each holding 6 plants, were set up for the individual treatments.

Iron was supplied in the concentration of 5 p.p.m. in the form of chelated iron. The concentration of manganese was 2.5 p.p.m. throughout Series A.

Group 1. The plants were grown in the greenhouse. During the first ten days nutrient solutions were changed twice. The changes were made desirable by precipitation of salts and by the fact that this experiment was conducted during the spring when frosting on the greenhouse panes melted and thus caused contamination of some solutions.

Many plants were attacked quite heavily by fungi and bacteria, however, only healthy, normally growing specimens were selected for analytical determinations.

Group 2. The experiment was carried out in the botany

laboratory.

Nutrient solutions were changed after 14 days. 2 p.p.m. of mercury in the form of mercuric chloride were added to the fresh solutions in order to suppress fungal and bacterial growth. This concentration of mercury, however, seemed to be toxic to oat plants because wilting appeared after three days and the plants did not recover.

Group 3. The experiment was set up in the botany laboratory. The nutrient solutions were not changed during the course of this experiment because of the relatively short duration of the experiment.

Here again some plants suffered from fungal and bacterial attack, but only healthy plants were selected for analysis.

Analytical data relating to Experiment III Series A are given in Tables VII and IX. From a study of the data it will be noticed that increase in potassium supply did not appear to have any definite effect on the dry weights of the experimental plants. Ash content, however, appeared to increase with increased potassium supply.

According to Table IX potassium had a retarding influence on the absorption of iron and manganese by oat plants. A gradual fall in iron content in shoots was obtained with rising potassium concentration in the nutrient solutions above 82 p.p.m. Group 3, where the intermediate

TABLE VIII

Experiment III - Series B.

Dry weights and percentage of ash in the shoot systems of Avena sativa plants grown in different levels of potassium.

| Group No. | K level | Duration of expt and time | Content in nutrient solutions | Dry weight per plant in g. | Percentage of ash |
|-----------|------------|---------------------------|-------------------------------|----------------------------|-------------------|
| | | Fe p.p.m. to Mn p.p.m. | | | |
| 1 | K=26 p.p.m | February 5 | 2.5 | 0.247 | 4.72 |
| | | to | | | |
| | K=82 p.p.m | March 1952 5 | 2.5 | 0.288 | 6.88 |
| | K=260p.p.m | 35 days 5 | 2.5 | 0.193 | 6.18 |
| | K=450p.p.m | 5 | 2.5 | 0.227 | 6.64 |
| 2 | | May to | | | Shoots |
| | K=26 p.p.m | 5 | 2.5 | 0.0347 | 15.6 |
| | | June 1952 | | | Root |
| | K=82 p.p.m | 5 | 2.5 | 0.0397 | 18.7 |
| | | 21 days | | | 12.2 |
| | K=260p.p.m | 5 | 2.5 | 0.0380 | 20.1 |
| | K=260p.p.m | 5 | 2.5 | 0.0380 | 20.1 |
| | K=450p.p.m | 5 | 2.5 | 0.0421 | 23.1 |
| 3 | K=26 p.p.m | July 1952 5 | 2.5 | 0.041 | 14.5 |
| | K=450p.p.m | 15 days 5 | 2.5 | 0.051 | 16.5 |

TABLE IX. Experiment III - Series A.

Iron and manganese content in the shoot systems of Avena sativa plants
grown in different levels of potassium.

| Group No. | K level | time and duration of expt. | Content in nut. solutions | | Content in mg/g dry material | |
|-----------|--------------|----------------------------|---------------------------|-----------|------------------------------|-------|
| | | | Fe p.p.m. | Mn p.p.m. | Fe | Mn |
| 1 | K 26 p.p.m. | Febr. | 5 | 2.5 | 0.040 | 0.155 |
| | K 82 p.p.m. | to | 5 | 2.5 | 0.050 | 0.168 |
| | K 260 p.p.m. | March 1952 | 5 | 2.5 | 0.037 | 0.096 |
| | K 450 p.p.m. | 35 days | 5 | 2.5 | 0.035 | 0.096 |
| 2 | K 26 p.p.m. | May | 5 | 2.5 | 0.310 | 0.370 |
| | K 82 p.p.m. | to | 5 | 2.5 | 0.391 | 0.417 |
| | K 260 p.p.m. | June 1952 | 5 | 2.5 | 0.283 | 0.291 |
| | K 450 p.p.m. | 21 days | 5 | 2.5 | 0.272 | 0.297 |
| 3 | K 26 p.p.m. | July 1952 | 5 | 2.5 | 0.182 | 0.301 |
| | K 450 p.p.m. | 15 days | 5 | 2.5 | 0.133 | 0.231 |

potassium levels were left out, similarly revealed a lower iron content in plants grown in the higher potassium concentration.

Absorption of manganese definitely was retarded by potassium when the latter was present in nutrient solutions in concentrations higher than 82 p.p.m.

Experiment III. Series B, *Lycopersicon*
esculentum grown in different concentrations
of potassium.

In this experiment each group contained three plants. Chelated iron (5 p.p.m.) or iron humate (10 p.p.m.) were used in this series. 2.5 p.p.m. or 5 p.p.m. of manganese were respectively added in order to keep the Fe/Mn ratio at 2/1.

Group 1. The experiment was carried out in a greenhouse and iron was supplied in the chelated form. The nutrient solutions were changed after the first three weeks. Very healthy plants, which are shown in Plate 4, were obtained. It will be observed that the growth increased with increasing potassium content in the nutrient solutions. Plate 5 shows all the plants from the lowest potassium level, and Plate 6 the plants from the highest potassium level. It will be seen that the smallest plant from the highest potassium culture was one inch shorter than the tallest one from the lowest potassium level.

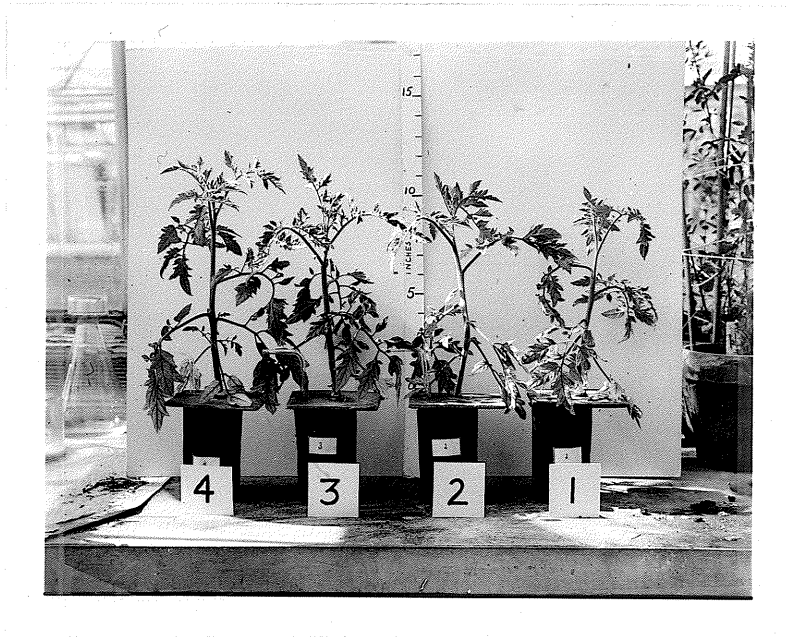


PLATE 4.

Lycopersicon esculentum grown in nutrient solutions containing different levels of potassium.

*Numbers indicate potassium concentrations:

1 = 26 p.p.m.; 2 = 82 p.p.m.; 3 = 260 p.p.m.;

4 = 450 p.p.m..



PLATE 5

Lycopersicon esculentum plants grown in
nutrient solutions containing 26 p.p.m. of potassium



PLATE 6

Lycopersicon esculentum plants grown in
nutrient solutions containing 450 p.p.m. of potassium

Groups 2 and 3.

Both these groups of the experiment were conducted simultaneously in the laboratory and chelated iron was used. In spite of the fact that the plants suffered somewhat from fungal attack in the seedling stage, it was possible to obtain specimens with healthy normal growth. The roots of the diseased seedlings were dipped in a solution containing 10 p.p.m. of mercuric chloride for 1 to 2 minutes in order to suppress fungal growth. However, when the plants were placed in the nutrient media infection reappeared after a short period. Therefore, at the end of the first week, fresh nutrient solutions were prepared which contained 2 p.p.m. of mercury in the form of mercuric chloride. The culture vessels, aerators and cardboard plates holding the plants were sterilised with 10 p.p.m. of mercuric chloride. The heavily diseased roots were removed from the plants and the healthy ones dipped in 10 p.p.m. of mercuric chloride before being transferred to the freshly prepared nutrient solutions. The plants were grown in these solutions for one week, and the only poisoning effect appeared to be a **suppression** of their growth. When transferred to solutions free of mercury the plants started to grow vigorously. At the end of the experimental period the same relationship between plant size and potassium concentration was observed

as appeared in the previous group of the experiment.

For analyses the plants were divided into two groups, according to their location in the room with respect to light conditions during the growth period. Group 2 received more light and the plants in it showed somewhat better growth than those in Group 3. All other factors were equal for both groups.

Group 4. This group of the experiment was carried out in the small laboratory. It differs from the others in so far as the Malgrobe variety of Lycopersicon was used, and the plants were supplied with humate iron in the concentration of 10 p.p.m. Accordingly 5 p.p.m. of manganese were given. The solutions were changed once a month only, but the plants grew very slowly and thus did not absorb much.

Plate 7 shows two plants from each treatment. The photograph was taken at the end of the experimental period. A decrease in the size of the plants in correlation with the decrease of potassium in the nutrient media, could be observed as in the previous groups.



Plate 7.

Lycopersicon esculentum grown in nutrient solutions containing different levels of potassium.

*Numbers indicate potassium concentrations:

1 = 26 p.p.m.; 2 = 82 p.p.m.; 3 = 260 p.p.m.; 4 = 450 p.p.m.

TABLE X. Experiment III - Series B.

Dry weight and percentage of ash of *Lycopersicon esculentum* plants
grown in different levels of potassium.

| Group No. | K level | time and duration of expt. days | Cont. in nut. solut. $\frac{\text{K p.p.m.}}{\text{Mn p.p.m.}}$ | Dry weight per plant in g | Percentage of ash in shoot system | Percentage of ash in root system |
|-----------|---------|---------------------------------|---|---------------------------|-----------------------------------|----------------------------------|
| 1 | K 26 | p.p.m. Febr. to | 5 | 2.83 | 8.51 | 8.80 |
| | K 82 | p.p.m. April | 5 | 3.21 | 8.89 | 7.42 |
| | K 260 | p.p.m. 1952 | 55 | 3.33 | 10.07 | 10.16 |
| | K 450 | p.p.m. 1952 | 5 | 3.57 | 10.09 | 11.20 |
| 2 | K 26 | p.p.m. May to | 5 | 0.289 | 17.05 | |
| | K 82 | p.p.m. June | 5 | 0.543 | 15.00 | |
| | K 260 | p.p.m. 1952 | 45 | 0.383 | 21.10 | |
| | K 450 | p.p.m. 1952 | 5 | 0.571 | 21.60 | |
| 3 | K 26 | p.p.m. May to | 5 | 0.217 | 18.55 | |
| | K 82 | p.p.m. June | 5 | 0.303 | 21.85 | |
| | K 260 | p.p.m. 1952 | 45 | 0.080 x | 23.00 | |
| | K 450 | p.p.m. 1952 | 5 | 0.323 | 23.00 | |
| 4 | K 26 | p.p.m. Aug. to | 10 | 0.134 | 9.04 | 14.60 |
| | K 82 | p.p.m. Okt. 1951 | 10 | 0.103 | 7.04 | 21.20 |
| | K 260 | p.p.m. 1951 | 10 | 0.39 | 11.20 | 16.70 |
| | K 450 | p.p.m. 1951 | 10 | 0.173 | 13.10 | 27.00 |

x one plant especially poorly grown.

TABIE XI. Experiment III - Series B.

Iron and manganese content in the shoot systems of Lycopersicon esculentum
plants grown in different levels of potassium.

| Group No. | K level | time and duration of expt. days | Content in mut. solut. | | Content in mg/g dry material | |
|-----------|---------|---------------------------------|------------------------|-----------|------------------------------|------------|
| | | | Fe p.p.m. | Mn p.p.m. | Fe | Mn |
| 1 | K 26 | Febr. to April 1952 | 5 | 2.5 | 0.0815 | 0.244 |
| | K 82 | | | | 0.1312 | 0.250 |
| | K 260 | | | | 0.1125 | 0.257 |
| | K 450 | | | | 0.1100 | 0.200 |
| 2 | K 26 | May to June 1952 | 5 | 2.5 | 0.156 | 0.580 |
| | K 82 | | | | 0.100 | 0.330 |
| | K 260 | | | | 0.110 | 0.419 |
| | K 450 | | | | 0.093 | 0.313 |
| 3 | K 26 | May to June 1952 | 5 | 2.5 | 0.100 | 0.607 |
| | K 82 | | | | 0.110 | 0.535 |
| | K 260 | | | | 0.175 | 0.200 |
| | K 450 | | | | 0.125 | 0.423 |
| 4 | K 26 | Aug. to Okt. 1951 | 10 | 5 | 0.032 | could not |
| | K 82 | | | | 0.075 | be |
| | K 260 | | | | 0.159 | determined |
| | K 450 | | | | 0.074 | |

Table X shows that dry weight and percentage of ash generally increased in tomato plants when the amount of potassium in the nutrient media was increased. The increase in dry weight and percentage of ash did not strictly follow the increasing concentrations of potassium, but nevertheless a general tendency of potassium, in the levels employed, to aid in growth and ash production could be observed throughout.

Table XI reveals an antagonistic effect of potassium in concentrations higher than 260 p.p.m. upon absorption of iron. Potassium in concentrations from 26 p.p.m. to 260 p.p.m. was not found to have a definite influence on absorption of iron by tomato plants.

The absorption of manganese was in no case as high at the potassium level of 450 p.p.m. as at the levels of 26 p.p.m. or 82 p.p.m. which suggests the possibility of a potassium manganese antagonism in higher potassium concentrations. Experimental data available, however, are insufficient to permit definite conclusions to be drawn regarding this last point.

DISCUSSION

An important fact emerges from the foregoing account of experiments carried out with Avena sativa and Lycopersicon esculentum grown in nutrient media containing different amounts of calcium. This is that species differ markedly from one another in their behaviour when grown under similar experimental conditions.

Oat plants did not show a marked response in growth but did exhibit a slight increase in dry weight generally, when grown in increased concentrations of calcium. The ash content did not show a definite correlation with the calcium content of the nutrient solutions. Iron absorption, however, decreased with increased calcium content; on the other hand no definite effect of calcium in the concentrations employed could be noticed in connection with manganese absorption by oats. It may be that higher concentrations of calcium than those used might have a retarding effect on manganese absorption. This is suggested by the fact that Lundegardh (35) held that there is a definite relation between calcium concentration of the soil and the manganese intake by plants, regardless of the total manganese content in the soil. Gilbert (10) stated that in Rhode Island, where soils were generally poor in manganese, crops have shown manganese deficiency as a result of excessive liming.

Tomato plants revealed an increased iron absorption and ash production when given increased calcium. However, the manganese absorption and dry weight decreased at the same time. The findings obtained with tomato plants agree in part with Swanback's (43) results using tobacco plants. He did not find any retarding influence of calcium in the two concentrations employed here, namely, 42 p.p.m. and 143 p.p.m. on the absorption of iron, but he obtained a considerable decrease in the absorption of manganese .

When we come to consider the effects of various levels of potassium in the culture media, we find that experimental results reveal a more comparable behaviour between oats and tomatoes than has been shown to be the case with varying calcium.

Oats did not show differences in growth and dry weight production in relation to differences in potassium supply. An increase in ash content could, however, be noticed when the plants were given more potassium. Also iron and manganese absorption seemed to be increased when the potassium supply was increased from 26 p.p.m. to 82 p.p.m. On the other hand, the absorption of these two elements was retarded when the potassium concentration in the nutrient media was increased to 260 p.p.m. and 450 p.p.m.

Tomato plants clearly showed a parallel increase in growth (as can be seen on Plates 4 and 7) and a general

increase in dry weight and ash production with rising potassium content in the nutrient solutions. This was especially marked when plants grown in the lowest and in the highest concentrations of potassium were compared.

Potassium was found to retard the absorption of iron when supplied in a concentration of 450 p.p.m.

The absorption of manganese by tomato plants seemed to show some indication of being retarded by increased potassium supply. This could be suggested by the data furnished by Experiment III, Series B (see Table XI). The marked variation in the manganese absorption occurring at the intermediate levels of potassium supply, however, made it impossible to arrive at a definite conclusion with regard to this point.

Swanback (43) observed an increase in manganese content in stems and leaves of tobacco plants grown at potassium levels of 26 p.p.m.; 82 p.p.m. and 260 p.p.m. This agrees with the results obtained by the writer in Group I of Experiment III. He, however, could not find any change in iron content in relation to potassium supply. It will be noted, however, that he did not use potassium concentrations as high as 450 p.p.m.

In conclusion it may be stated that the experimental data here described indicate that Nicotiana (see Swanback 43) and Lycopersicon esculentum, which are both members

of the same family, behave similarly with respect to the absorption of iron and manganese in different levels of calcium and potassium, while Avena sativa, a widely separated species, shows a marked difference of behaviour in this respect.

SUMMARY.

Experiments were carried out to determine the influence of calcium and potassium on the absorption of iron and manganese by tomato and oat plants. Simultaneously some observations on manganese deficiency in oats (grey-speck) disease were made.

Analyses of the shoot systems of the experimental plants were used for the evaluation of the results.

1. The results obtained from the two different plant species are very different with regard to the calcium treatments, but show some similarity with regard to potassium treatments. Thus it appears that results obtained with one species of plant do not necessarily hold for other species.

2. Oats show a slight dry weight increase when grown in nutrient solutions with increased calcium content. The reverse is true for tomato plants.

3. No definite influence on ash content of oat plants is observed in relation to varying calcium supply. Tomato plants, on the other hand, produce more ash at the higher calcium level.

4. Calcium seems to depress absorption of iron in the case of oats, but not in the case of tomatoes.

5. The absorption of manganese by oat plants is not influenced by increase in calcium content of the nutrient solutions, but it is definitely depressed with tomato plants.

6. Dry weight production of oat plants is not influenced by the different concentrations of potassium used but it is increased in the case of tomatoes grown at the higher potassium levels.

7. It is shown that potassium in the concentrations used increases the growth of tomato plants but does not have a similar effect on oats.

8. The percentage of ash increases for both species with increased potassium supply.

9. Iron absorption by oat plants increases with increasing potassium concentrations in the culture solutions up to 82 p.p.m. but falls when the concentrations of potassium are still further increased. With tomatoes a retarding effect on iron absorption is observed with an increase in potassium supply from 260 p.p.m. to 450 p.p.m.

10. Manganese absorption by oat plants at different levels of potassium parallels the absorption of iron under the same conditions. Tomatoes reveal definitely a lower manganese absorption in a potassium concentration of 450 p.p.m. than in a potassium concentration of 260 p.p.m. The results at the intermediate potassium levels fluctuate.

11. Grey-speck does not seem to be a bacterial disease, but rather a physiological manganese deficiency.

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APPENDIX

APPENDIX.

Observations on Manganese Deficiency
(Grey-Speck) Symptoms of Tama Oats.

The first nutritional experiments with Avena sativa in solutions containing different concentrations of calcium were arranged to facilitate simultaneous studies on grey-speck disease.

The disease was already known and described in the beginning of the present century but the name grey-speck only came into use about the year 1920. It probably originated in Sweden, where the term "Gråfläckjucke" was applied.

Generally it is accepted that the first signs of the disease appear in the young oat plants as grey spots on the third or fourth leaves. Very characteristically a line of withering appears transversely across the lower half of the leaf blade, causing the distal portion of the leaf to hang down.

Gerretsen (9) stated that the necrotic spots which appeared on the leaves of manganese deficient plants were of dual origin. Typical brown or yellow spots appeared on the leaf blades, caused by ascending toxic metabolic substances produced by bacteria attacking the root tips of plants which were deficient in manganese. In the advanced stage of the disease necrotic spots of

different origin appeared. A serious necrosis developed then, when the manganese content of the plant was low, and a necrosis of rows of cells situated between the green vascular bundles extended finally toward the top and the base of the leaf blade. This second kind of necrosis Gerretsen claimed to be a direct result of reduced photosynthesis which renders the cells unable to make up for assimilates lost by precipitation and so leads to their death.

In Experiment I, no manganese deficiency symptoms were noticed on any of the plants grown. The reason for this became evident during the course of the ensuing experimental work, where it was observed that very poorly growing plants did not develop manganese deficiency symptoms.

Healthier and stronger plants were obtained in Experiment II, Series A, Group 1, described on page 47 . Here it was observed that the plants, which were standing furthest from the window and which thus received the least amounts of light, developed manganese deficiency symptoms later than those which were in a better illuminated location, near to the window. In this experiment an attempt was made to duplicate Gerretsen's (7) investigation. He inoculated the nutrient media of oat plants with root tips from grey-speck diseased oat plants, and concluded from his findings that typical grey-speck spots were caused by alkaline products

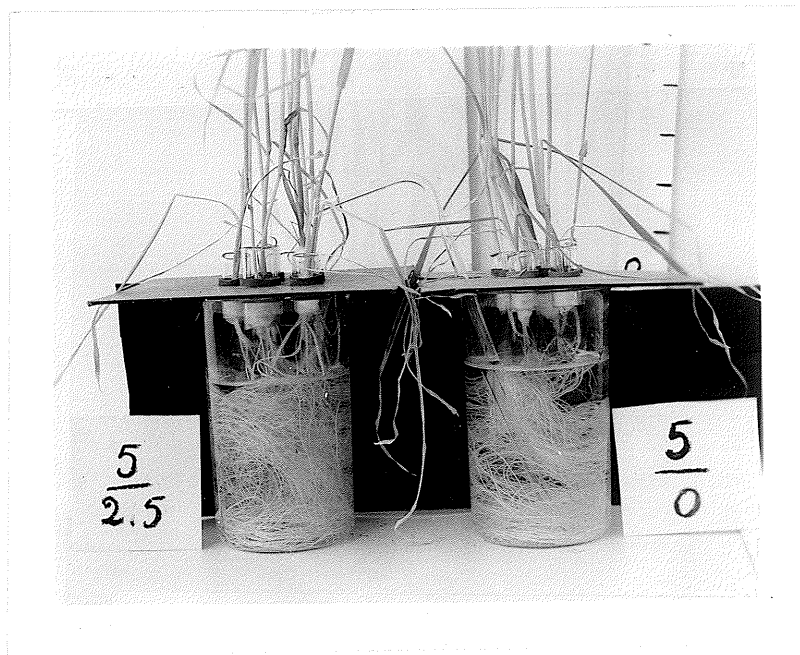


Plate 8.

Roots from manganese deficient plants (Fe/Mn - 5/0)
and healthy plants (Fe/Mn - 5/25)

produced by molds and bacteria in the root tips and transported by the sap stream to the leaves.

All plants, growing in the inoculated culture vessels, showed a slight rotting of the roots in contrast with the noninoculated plants. However, inspite of the presence of bacteria which were introduced into the culture solutions from the grey-speck diseased root tips used for inoculation, the deficiency disease symptoms developed on all plants not receiving manganese including those which were not inoculated.

These findings were contrary to Gerretsen's (75) in which the noninoculated lots failed to develop grey-speck lesions. In addition, in the inoculated plants, not receiving manganese, and showing some root rot, the development of grey-speck was less severe than in the non inoculated plants, having healthier roots. The small amount of manganese introduced in the solutions by the inoculum was probably the cause for the lesser severity of the disease development.

In Experiment II, Series A, Group 2, grey-speck lesions appeared again only on plants not receiving manganese. The roots of plants showing severe signs of grey-speck disease were quite normally developed and healthy. Plate 8 illustrates the comparison of roots from diseased plants (Fe/Mn/-5/0) and healthy plants (Fe/Mn - 5/2.5). The

relative root to shoot development according to dry weight ratios (dry weight of shoot to dry weight of root) was somewhat lower in diseased than in healthy plants, being 39 in "low calcium" - 46 in "high calcium" with diseased plants not receiving manganese; 82 in "low calcium" - 50 in "high calcium" with healthy plants receiving 1 p.p.m. of manganese, and 75 in "low calcium" - 58 in "high calcium" with healthy plants receiving 2.5 p.p.m. of manganese.

It may be noticed that Gerretsen (1935) claimed that roots remain small as a result of manganese shortage, because it causes slower cell division in the root tips with the result that the roots finally succumb to the attack of microorganisms.

He stated further that grey-speck diseased oat plants in the field, in sand, or in water cultures invariably showed signs of microbial disintegration.

In the present experiment the roots and root tips of manganese deficient plants were perfectly healthy in spite of retarded growth. The plants were grown in a greenhouse where the accumulation of parasitic organisms on the plants might be quite high. No sterilisation treatments were given to the seeds or roots at any time of the experiment. Nevertheless there was no evidence of microbial disintegration.

Another experiment was carried out with oat plants

in two different pH ranges, because Gerretsen (8) observed that grey-speck disease in the field was most frequent at the pH levels 6.5 to 7.8. He stated that the precipitation of insoluble manganaous oxide in the soil is caused by specific microorganisms in the same pH limits.

The pH levels chosen for the experiment were 4 and 7.5. Duplicate culture vessels each containing 4 plants were set up adjusted to the above pH levels. 5 p.p.m. of iron humate but no manganese were added to the nutrient solutions (see Table 2). Satisfactory plant growth resulted. The pH of the solutions changed during the first two weeks from 4 to 4.5 and from 7.5 to 6.6 at which point the pH remained stationary until the end of the experiment. The pH was not adjusted after it changed because the new acidity levels still served the purpose. The grey-speck symptoms developed on all the plants in the solutions of pH 6.6 after three weeks, while in the more acid medium the first symptoms appeared after 4 weeks on 4 out of 8 of the specimens.

It may therefore be suggested that the pH range might have an influence on the development of the manganese deficiency symptoms. This seems to be particularly the case in soils, as has been shown by some investigators. (12,37) It is doubtful however that the pH in itself is a determining factor in the production of manganese deficiency symptoms. It seems probable that it is the combined effect

of manganese deficiency or manganese inactivation in some way related to the pH level.

The results of the experiments cited here suggest that it is unlikely that the grey-speck lesions are directly dependent for their development on root infection by bacteria and fungi. It seems rather to be a physiological condition resulting from manganese deficiency and other related factors.

Bulman (3) who studied the grey-speck disease at this university, inoculated Tama oat plants in the same way as has been described here, with grey-speck diseased root tips and determined the disease only in plants starved for manganese regardless of inoculation. She concluded from her data "that grey speck disease of oats is purely a nutritional disease, caused directly by manganese deficiency".