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

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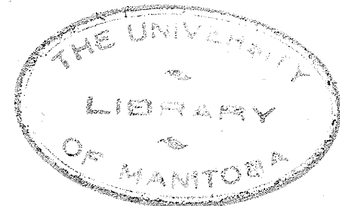

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INTRODUCTION

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 Harmer (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-carbonates and magnesium-carbonates 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 Shive (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 Shive (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

favored 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 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
$Ca(NO_3)_2 \cdot 4H_2O$	0.25
$NaH_2PO_4 \cdot H_2O$	0.156
KNO_3	0.214
$NaNO_3$	0.037
$Mg(NO_3)_2 \cdot 6H_2O$	0.32
$Na_2SO_4 \cdot 10H_2O$	0.32

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

Salt	g. per L of solution
$Ca(NO_3)_2 \cdot 4H_2O$	0.847
$NaH_2PO_4 \cdot H_2O$	0.156
$MgSO_4 \cdot 7H_2O$	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
$Ca(NO_3)_2 \cdot 4H_2O$	0.847
$NaH_2PO_4 \cdot H_2O$	0.156
K_2SO_4	A 0.06
	B 0.82
	C 0.6
	D 1.0
$MgSO_4 \cdot 7H_2O$	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 ($FeSO_4 \cdot 7H_2O$) 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 $K_2Cr_2O_7$ 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 1½ 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 ½ 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 . ½ inch wide and 1 to 1½ 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. A1/3 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. <u>Analytical Methods:</u>

A modification of the wet digestion method for plant analyses recommended by Sandell ([illegible]) 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 $2Mn + 5\ Io_4^- + 3H_2O^- \rightarrow 2MnO_4 + 5\ Io_3^- + 6H$ 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