# STUDIES ON THE MINERAL NUTRITION OF CERTAIN CROP PLANTS WITH SPECIAL REFERENCE TO IRON AND MANGANESE SUPPLY

by

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# ABSTRACT

Studies were made of the effects of growing Phaseolus vulgaris var. humilis, Lycopersicum esculentum, and Allium cepa in culture solutions containing different concentrations of iron, manganese and calcium, and different iron to manganese concentration ratios. A series of spectrophotometric analyses were carried out on the dry plant tissues and culture solutions.

The data suggested that iron and manganese ions of similar valence are competitive or antagonistic in their absorption by these species.

The experimental plants of Phaseolus vulgaris and Lycopersicum esculentum were free from the symptoms of iron and manganese deficiencies when the total content of each of these metals in the leaves of the plants exceeded a certain minimum level. It was found possible to produce the symptoms of both deficiencies simultaneously in individual plants, thus showing that a deficiency of one of these metals does not correspond to a toxicity of the other, as believed by certain investigators.

The data indicated that the absorption of iron and manganese by Phaseolus vulgaris plants was depressed when the calcium content of the culture solution was increased from 42 to 143 p.p.m.

The number of divalent ferrous or manganous

ions absorbed per unit of time by Allium cepa plants from a continuously shaken single salt solution at a constant temperature of 20° C. was constant and independent of the concentration of the cation in the culture solution.

None of the metals titanium, nickel or cobalt could be used as a substitute for iron or manganese in the nutrition of Phaseolus vulgaris var. humilis.

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## I. INTRODUCTION

The complex problems of the relationships between the composition of the nutrient substrate, the physiological functions of nutrient elements, and the characteristics of the plant are of such basic significance that they have, over many years, attracted the attention of investigators. During the past quarter of a century, there has been an increasing awareness of the importance of trace elements in these interrelationships. It is recognized that deficiency or excess in supply of a minor element may lead to an abnormal condition of the plant. More is known of the plant pathological aspect than of the physiological functions of these elements effective in minute quantities in plant growth and metabolism. Moreover, little attention has been given to the effect of their relative proportions in the substrate and within the plant.

The place of manganese among the trace elements is not questioned. It is seldom found in more than minute amounts in field grown plants. In the present work iron also is included among the minor elements, although it is almost always absorbed to a considerably greater extent than other elements of this group.

Manganese and iron have had an important place in qualitative studies because of their association with a group of plant diseases, of agricultural significance, known as chloroses, in which the most pronounced symptom is a chlorotic condition of the leaves. Two of these, lime-induced chlorosis, and the grey speck of oats, occur upon soils adjacent to the city of Winnipeg.

Control of these chloroses may lead to increased crop yields of susceptible species, and to an extension of the number of species which can be grown successfully upon soils where such diseases occur.

There must be an increased understanding of the manner in which chloroses develop before practical procedures for their prevention can be evolved. The necessity for further investigations into the availability of iron and manganese in supply, their absorption, and their physiological functions is apparent.

There is evidence to support the view that a healthy condition in a plant is related to the ratio of iron to manganese in the substrate in which the plant grows. Certain investigators have presented data which suggest that these metals may be functionally interrelated. Others believe that they behave independently. A study of the literature shows that, in general, the results of various workers, concerning the relationships between iron and manganese, in plant nutrition, are apparently conflicting.

The experimental examination of such questions is fraught with certain difficulties. The use of artificial culture methods to achieve an inorganic medium, and to effect comparison with soil cultures, involves a change in the physical environment around the roots, and in the conditions of presentation of inorganic nutrients. However, through the use of colloidal materials, in artificial cultures, one may more truly simulate soil conditions. Despite their weaknesses, artificial culture media, particularly water-culture media, are subject to more exact control than soil cultures. At present, culture on artificial media provides the most important means by which the plant physiologist may extend our knowledge of the fundamental processes related to mineral nutrition.

The investigations here reported were carried out with various plant species in solution culture. Of the experimental materials used the more important were _Phaseolus vulgaris_ var. humilis, _Lycopersicum esculentum_, and _Allium cepa._

The present paper embodies the results of a series of studies into the effects upon these species of varying the concentrations of iron, manganese, and calcium, and the ratio of iron to manganese in the aqueous substrate, which, in certain experiments, was maintained at a constant temperature. Analyses of the plants, and of the nutrient solutions were carried out for the specific purpose of making a systematic study of possible interrelationships between iron and manganese in their effects upon the metabolic processes of plants, the aim being an increased understanding of some of the fundamental processes involved in these interrelationships.

## II. HISTORICAL

The water-culture technique, developed by Sachs in the year 1860, soon became the most important method employed in small-scale, controlled laboratory experiments intended to solve certain fundamental problems of plant nutrition. Refinements in the technique by more recent workers resulted in the discovery that certain elements are required by plants in minute amounts. Manganese is one of the more important of these minor elements. The proportionate amounts of iron found in plant tissues are also low; although it is not generally grouped with the minor elements.

In order to prove the indispensability, for plant growth, of such elements as iron and manganese, it was necessary to demonstrate that a plant could not complete its life cycle unless a certain minimum concentration of the element in question was present in the nutrient substrate. Although the exact manner in which iron functions is still obscure, its indispensability for chlorophyll formation in green plants has long been established. Evidence for the indispensability of manganese is of more recent origin. The work of Bertrand (1897) suggested that manganese increased the oxygen-carrying power of the enzyme laccase. Manganese, however, is not known to be a constituent of this enzyme. Mazé (1914) showed that manganese is necessary to the growth of maize. McHargue (1922) concluded that manganese is an element essential for the normal growth of all plant species.

Analyses of the tissues of normally growing plants have shown that they vary widely with respect to the amounts of iron and manganese which they contain. Bennett (1945) found up to 10,000 parts per million of manganese in the leaves of the tomato, Lycopersicum esculentum, grown in solution cultures.

The present interest in manganese and iron appears to stem mainly from their association with chlorosis symptoms in plants, examples of chlorosis being the already mentioned lime-induced chlorosis, grey speck of oats, and marsh spot of peas.

Toxicities resulting from the occurrence of excessive quantities of iron or manganese within plant tissues have not often been reported. The better known diseases, with which these elements are associated, appear to result from a deficiency of one or the other within the plant. It is becoming increasingly evident that deficiency diseases are seldom the result of a deficiency in the total quantity of the element present in the soil or other nutrient substrate. Frequently, a physiological deficiency results from the insufficient availability of the metal to the plant. This may mean that the element is precipitated or combined in an inactive form within the tissues, or that not enough of it can be absorbed and distributed in the plant for its physiological needs at each phase of growth.

There are many possible reasons for the lack of availability. The chemical and physical problems of availability in soils, and in solid and liquid artificial media, are incompletely understood. Some of the factors which may determine the quantities of an element absorbed by plants from nutrient solutions are the solubility of the compound in which it occurs, a factor which may vary with a change in solution pH; the degree of dissociation, and the valency state of its ion; the antagonistic effect of another ion; selective absorption; chemical interaction, precipitation and fixation; and oxidation to an insoluble form within the solution.

Absorption from the soil involves adsorptive and solid phase phenomena. It is a vastly more complicated process than absorption from solutions.

When iron is insufficiently available to the plant, the terminal leaves become chlorotic. The effect of manganese deficiency is the general development of small chlorotic patches in interveinal areas of leaves. The leaf anatomy of a species determines the pattern. Reduction of growth and the development of necrotic areas follows. Necrosis may appear in the seeds. With some species, there may be a degree of departure from this typical symptom pattern. An example of such a difference is seen in the bush bean, Phaseolus vulgaris var. humilis, in which chlorosis first appears in the petiole and in the basal regions of the lamina.

Linder and Harley (1944) listed four ways in which iron and manganese nutrition may be affected so as to bring about a chlorosis. These are: (1) true deficiencies which occur under experimental conditions, and are not prevalent in the field; (2) an unfavourable ratio of iron to manganese in the substrate; (3) an unfavourable ratio of phosphate to iron in the substrate; and (4) lime-induced chlorosis.

The last type of chlorosis is the most common in the field. It is associated with a high supply of lime in the soil and a high soil pH. It is considered to be an iron deficiency disease. The causes appear to be complex; and to involve the interrelations of several factors and elements.

Olsen (1938) reported that if the supply of phosphate in a nutrient solution were too high in relation to the concentration of iron present, then ferric phosphate was precipitated in the vascular bundles of plants growing in the solution. The result was an iron deficiency chlorosis.

A. P. Withrow and R. B. Withrow (1949) found that the leaves of tomato plants exhibited an interveinal chlorosis when the photoperiod exceeded 18 hours. Data presented by Ingalls and Shive (1931), and by Hopkins, Pagan, and Silva (1944) suggested that a photoperiodic chlorosis may occur if iron, manganese, and light interact to produce a deficiency of iron.

The exact functions of manganese and iron within the plant have not been established. Since deficiencies of these metals induce chlorosis, they have been held to be concerned in chlorophyll formation. However, they are not a part of the chlorophyll molecule. They are regarded, also, as playing a part in oxidations and reductions occurring within the plant. Jones (1920) found iron in the nuclei of plant cells, and in small amounts in areas scattered throughout the cytoplasm. Unlike manganese, iron is comparatively immobile within plants. No appreciable redistribution occurs from one tissue to another.

A posthumous publication by R. W. Thatcher (1934) described an attempt to find a relationship between the biological function of an element and its place in the periodic table. The suggestions presented were an attempt to systematize the subject of mineral nutrition, so that the role of any particular element could be forecast. Thatcher expressed the opinion that manganese and iron were co-ordinating catalysts for oxidation-reduction reactions. The proposed classification had certain weaknesses. Nevertheless, it was supported by Frey-Wyssling (1935) who expressed with greater precision the fact that all the essential elements occur in the first four periods of the periodic table.

So far the functions of iron and manganese in general have been considered. Contributions of considerable interest have been made concerning the specific functions of each, and the deficiency diseases attributable to shortage of one or the other within the plant. A consideration of these follows.

## A. INVESTIGATIONS CONCERNING IRON

One of the earlier experiments concerning the role of iron was conducted by Oddo and Pollacci (1920). They grew plants of Zea mays in nutrient solutions lacking iron, but containing the magnesium salt of pyrrolecarbonic acid. The plants formed chlorophyll. These workers suggested that the formation of chlorophyll in the presence of iron was due to the catalytic action of the element in the formation of the pyrrole nucleus, which is the centre of the chlorophyll complex. They believed that, if this nucleus is already formed, the presence of iron is not necessary for the formation of chlorophyll.

Deuber (1926), and Aronoff and MacKinney (1943) were unable to confirm the work of Oddo and Pollacci.

An investigation similar to that of Oddo and Pollacci was conducted by Sideris (1930). He found that pineapple plants formed chlorophyll when grown in solutions lacking iron, but containing 5 p.p.m. of titanium. Inman, Barclay, and Hubbard (1935), however, found that titanium, supplied to plants as titanium chloride, in amounts of 15 to 20 p.p.m. could not be substituted for iron in the synthesis of chlorophyll.

Investigations of this kind are limited by the difficulties involved in determining the form in which an element exists in the plant.

Oserkowsky (1933) found that there may be less iron present in green pear leaves than in those exhibiting an iron deficiency chlorosis. He suggested that the iron concerned in the synthesis of chlorophyll is in an active form, and is soluble, or otherwise available; while inactive iron is in a precipitated or otherwise unavailable form. McGeorge (1949), also, reported a lack of any apparent relationship between amounts of iron in plant tissues and the appearance of the specific deficiency symptoms for this element in Citrus species and in pear. However, Jacobson (1945) found that surface contamination of leaf samples by dust, clay and other deposits could produce discrepant analytical results. When leaves were washed in dilute hydrochloric acid, a correlation was found between low total iron values and chlorosis. Bennett (1945) confirmed this observation. Smith, Reuther, and Specht (1950) employed several methods of cleansing the leaves of trees of *Citrus* species and found total iron to be consistently lower in chlorotic than in green leaves.

With regard to the functions of iron it is of interest to note the results of an investigation concerning the effects of iron upon certain enzymes. Waring and Werkman (1944) conducted an experiment on the effects of iron deficiency in bacterial metabolism. A study of the enzyme systems of *Aerobacter indologenes* indicated that the catalase, peroxidase, formic dehydrogenase, and hydrogenase activities were suppressed by iron deficiency.

Iron compounds associated with cytoplasmic proteins were studied by Macallum (1895), Sayre (1930), and Noack and Liebich (1941).

Although the physiological functions of iron are incompletely understood, the iron deficiency disease known as lime-induced chlorosis has long been recognized. It was formerly believed that lime-induced chlorosis developed because of iron becoming unavailable as a result of a high soil pH produced by an excessive content of calcium carbonate. It is now recognized that this simple explanation is inadequate to explain completely the causes of the disorder. It does not, for example, explain the observations of Parsche (1940), who showed that calcium chloride, which does not affect soil pH, induces iron deficiency chlorosis in lupine. Unexplained, also, is the frequent observation that some plant leaves may exhibit the symptoms of lime-induced chlorosis while adjacent leaves on the same plant remain green.

Haas (1942) advanced an explanation, for lime-induced chlorosis, which took account of soil moisture levels. From observations of trees of *Citrus* species, and from soil analyses, he concluded that calcareous soils, namely, those containing a high content of calcium carbonate, may or may not produce chlorosis. They are potentially, but not necessarily, alkaline. Calcium carbonate is a soil buffer; it has hydrolytic properties depending upon soil texture, the amount of colloidal matter present, and the amount of moisture present. Moisture permits hydrolysis to proceed, with an attendant rise in pH, and precipitation of such elements as iron, making them unavailable to the plant. If soil moisture is sufficiently reduced, the soil reaction will be acid. It is the continuity of a given moisture percentage, and the time during which roots are subjected to a given pH, which are of importance in iron nutrition. Thus a continuity of high moisture produces chlorosis. Observations similar to those of Haas were reported by Millikan (1943) who found that iron deficiency chlorosis of flax, growing on calcareous soil, was more severe when moisture levels increased.

It is true that ferrous iron oxidizes to the ferric form as the pH of the soil solution rises. Ferric iron is less soluble and, therefore, less available to the plant than ferrous iron. There are, however, many factors which may induce the oxidation of iron. Starkey (1945) suggested that some instances of the precipitation of iron as ferric hydrate, from water solutions, are due to iron bacteria growing by the oxidation of inorganic ferrous compounds.

Linder and Harley (1944) concluded that

lime-induced chlorosis is not related to other iron deficiency diseases. They noted that iron, undoubtedly, forms many different compounds within the tissues of plants. These compounds are associated with nuclei, chloroplasts, and with proteins in cytoplasm. There are, also, the iron porphyrin protein enzymes, which are involved in respiration. They suggested that there may be a dynamic equilibrium in leaf tissue between iron in ionic state, iron salts of organic acids, iron phosphate complexes, iron hydroxide complexes, lipoidal iron, iron-silica complexes, iron nucleoproteins, and a postulated iron-containing chlorophyll enzyme; and a further equilibrium between the two oxidation states of iron. A change, or upset in nutritional factors, shifts, in some way, the equilibria, so that a chlorosis develops as a result of a decrease either of total or of soluble iron in the plant.

It should be noted that the data of Linder and Harley (1944) showed lime-induced chlorosis to be associated with increased quantities of potassium in the leaves of affected plants.

Smith, Reuther, and Specht (1950) advanced the suggestion that, with the plant disorder known as "iron chlorosis", or "lime-induced chlorosis", consideration should be given to manganese as well as to iron nutrition. In support of this recommendation, they provided data showing that leaves exhibiting the symptoms of lime-induced chlorosis are often low in both manganese and iron.

## B. INVESTIGATIONS CONCERNING MANGANESE

Bertrand (1897), as we have seen, first drew attention to the importance of manganese in enzyme systems. Lundegårdh (1939) believed manganese to be of importance in the respiratory system of plants. More recently, with a view to ascertaining the effects of minor elements upon the activities of the enzymes catalase, peroxidase, oxidase, and invertase, Bailey and McHargue (1944) grew tomato and alfalfa plants in nutrient solutions containing varying amounts of manganese, boron, copper, and zinc. In general, enzyme responses were found to exhibit considerable uniformity, indicating that they were not directly influenced by minor elements. There were certain exceptional respones. Oxidase activity, in tomato leaves, increased with increasing additions of copper; and was accounted for by the fact that polyphenol oxidase is a copper-protein enzyme. Peroxidase activity in alfalfa gave a favourable response to 1 p.p.m. of manganese. Peroxidase is an iron-porphyrin-protein enzyme. It was suggested that manganese may have a direct effect on the iron prosthetic group of the peroxidase molecule.

Burström (1939) reported evidence indicating that, in wheat, nitrate is reduced by an enzyme system in which manganese appears to play an essential role. His conclusion was that manganese directly catalyses nitrate assimilation. Leiper (1942), also, investigated the role of manganese in the assimilation of nitrates. Arnon (1937), and Shive (1941) held that, in non-aerated solutions, provided with manganese and nitrate, the plant depends upon nitrates for both oxygen and nitrogen.

The work of Jones, Shephardson, and Peters (1949) with soy bean provided evidence suggesting that manganese is a catalyst without which the reduction of nitrate either fails to occur, or is reduced in speed. In the absence of manganese, but with oxygen of the atmosphere available to plant roots, nitrates accumulated in plants. In the absence of manganese, when an oil layer prevented contact of the nutrient solution with atmospheric oxygen, nitrites accumulated in the plants. Foliage yellowed in both instances. When manganese was added to the solutions the leaves became green.

Working with spinach, oats and Sudan grass, Harmer and Sherman (1943) concluded that manganese plays a role in synthesizing ascorbic acid in chlorophyll-bearing tissue. Plants growing in a soil deficient in available manganese contained significantly less total and reduced ascorbic acid than plants growing in the same soil after an application of manganese, or of sulphur. The latter, presumably, lowered the pH of the soil solution so that the availability of manganese was increased.

Stiles (1946) described certain commonly occurring plant diseases which are thought to result from a deficiency of manganese. He drew attention to the fact that grey speck of oats is most likely to occur on soils of alkaline reaction, especially if they contain large amounts of humus. This disease may affect oats, rye, barley, and wheat. The other diseases described were Pahala blight of sugar cane, speckled yellows of sugar beet, marsh spot of peas, and frenching of tung trees.

Salm-Horstmar (1849) was the first to describe the symptoms of grey speck of oats; the best description of the disease, however, was made by Twyman (1943). Using the water culture technique, Twyman was able to produce the typical symptoms of grey speck disease in oats three weeks after germination. He attributed the results to manganese deficiency. The plants exhibited a slightly yellowish-green appearance. The first signs appeared about half-way along a third leaf. An oval grey-green spot appeared at this point; and the leaf bent over sharply. Within four weeks ten out of fourteen plants, growing in manganese deficient solutions, displayed the symptoms upon the third and, or fourth leaves. Three plants had died. The grey specks soon dried up, and became yellow brown. No typical grey speck symptoms appeared in plants grown in the presence of manganese.

An earlier investigation by Olsen (1934) appeared to show that in basic soils it is the degree of aeration which is decisive for the presence or absence of grey speck disease; for, in neutral or basic soils manganese is quickly oxidized to manganese dioxide when the soil is well aerated.

Gerretsen (1937) reported that oats were stunted, but failed to develop the symptoms of grey speck when grown in sterile culture media of very low manganese content. Upon infecting the sterilized media with bacteria, isolated from infected roots, the symptoms of grey speck developed. He suggested that grey speck is related to the presence of micro-organisms, and not caused by manganese deficiency alone. He found the ammonia content of manganese-deficient plants to be two to three times greater than that of normal plants; therefore he concluded that alkaline products, transported from the roots of the leaves, were responsible for the necrotic leaf spots of grey speck disease; and that the injurious substances were produced as a result of bacterial activity upon the root-tips.

MacLachlan (1941) arrested the development of grey speck in field grown oats by spraying them with a one per cent aqueous solution of manganese sulphate. The soil contained a high content of organic matter. From the soil MacLachlan isolated bacteria capable of manganese oxidation. He concluded, therefore, that the abnormal development of the oats resulted from a decreased availability of manganese caused by its fixation in the soil through bacterial activity. He was able to isolate similar bacteria in fewer numbers from soils on which oats grew normally.

Sherman and Harmer (1941) reported evidence indicating that the development of grey speck of oats on alkaline, organic soils can be prevented by any treatment which increases the exchangeable manganese in the soil to three parts per million. They suggested that neutral and alkaline conditions favour the formation of manganic manganese, and acid conditions that of manganous manganese.

Hewitt (1945) found the symptoms of manganese deficiency in oats to be similar to those of grey speck. Manganese deficiency in the runner bean, <u>Phaseolus coccineus</u>, was characterized by an intervenal mottling of older leaves. He noted that, in acid soils, manganese tended to accumulate to toxicity in these plants. The effects produced by manganese toxicity were different from those produced by manganese deficiency. Manganese toxicity in oats resulted in a slight reduction of growth, an intervenal orange mottling of the leaf tips, and a scorching of the older leaves. Manganese toxicity in the runner bean produced a purple-brown necrosis of the leaves.

An earlier observation with regard to manganese toxicity was made by Olsen (1936). He observed that many species make poor growth in strongly acid soil, and that such plants are often high in manganese content. Olsen ascribed their slow rate of growth to the toxicity of manganese.

## C. INVESTIGATIONS CONCERNING INTERRELATIONSHIPS BETWEEN IRON AND MANGANESE

An analysis of data from experiments on the effects of various calcium and potassium concentrations upon plants in solution culture led Olsen (1942) to infer that these elements are mutually competitive in absorption, and that a high concentration of potassium in leaves suggests a low concentration of available calcium in the soil.

Because the phenomenon is frequently encountered in investigations relating to mineral nutrition, it may be appropriate to discuss further the matter of competition in absorption, or "antagonism", as it was called by Burstrom (1938). The term "antagonism" has been used in a different sense, namely, when it is a matter of a certain ion neutralizing the toxic effect of another ion. Olsen (1942) preferred to restrict the term to its meaning in this sense; and not to use it in reference to the mutual action of ions where one ion apparently retards the rate at which another ion is absorbed by the plant.

Swanback (1939) referred to the competition of two elements in plant absorption as "pseudo-antagonism". He stated that the phenomenon is a result of a difference in ion concentration, and operates through the force of mass action, the element in greatest concentration in the substrate being absorbed to the greatest extent in the plant. If the concentration of this element is increased in the substrate, while that of the other remains constant, proportionately more of the increased ion, and less of the other is absorbed by the plant.

Swanback (1939) found that the absorption of manganese by tobacco plants was depressed when the concentration of calcium was increased in the nutrient substrate. Furthermore, this increase appeared to prevent the translocation of iron from the roots of the plants.

Johnson (1917) reported that manganese, in the dioxide form, in manganiferous soils, of the Island of Oahu, induced a depression in the absorption of iron by pineapples. The resulting chlorosis was removed by applying iron through the leaves. A somewhat similar report was made by Bennett (1945) who investigated the effects upon tomato in water culture when iron and manganese were varied in supply. He found that a large increase in the manganese concentration in the culture solution reduced the absorption of iron by tomato. Morris and Pierre (1947) reported upon the reverse instance of iron depressing the uptake of manganese by *Lespedeza*. They also found that calcium depressed the absorption of manganese by plants.

The importance of the balance between the available iron and the available manganese in the substrate, indicated by the reports above, was reviewed by Twyman (1946).

Hopkins (1930) expressed the opinion that manganese brings about the reoxidation of iron after its reduction to the ferrous state by reducing systems within the plant. Manganese in excess prevents the reduction of ferric iron, or oxidizes iron absorbed in the ferrous state. In either case cell oxidation-reduction processes are disturbed.

If this theory is accepted, it is to be expected that the ratio of iron to manganese in the plant, and in the substrate, is of more importance than their absolute concentrations. The importance of the ratio of iron to manganese in the nutrient substrate has been emphasized by a number of investigators. Pugliese (1913) reported that the optimum for this ratio for wheat was 2.5 : 1.0. Scharrer and Schropp (1934) found that the optimum ratio for maize was 7 : 1.

Shive (1941), Somers, Gilbert, and Shive (1942), and Somers and Shive (1942) expanded the theory of Hopkins. They held that the functional iron, which is the active iron concerned in chlorophyll synthesis and postulated by Oserkowsky (1933), exists in plant tissues in the ferrous state. They pointed out that manganese has a higher oxidizing potential than iron; and supported the oxidation-reduction theory formulated by Hopkins. Oxidized ferric iron they held, is precipitated in an insoluble and inactive form within the tissues of plants. Manganese in excess induces iron deficiency chlorosis by lessening the concentration of active ferrous iron within the plant. The reverse instance produces iron toxicity, which corresponds to manganese deficiency.

Somers and Shive (1942) found that the optimum ratio of the concentration of iron to the concentration of manganese in culture solutions for the growth of soy beans was 2 : 1; and that any appreciable departure from this ratio resulted in the development of pathological symptoms. They claimed that symptoms of iron toxicity correspond to those of manganese deficiency, and vice versa. They stated that ratio values above the effective range resulted in a specific type of chlorosis resulting from excess iron, or deficient manganese, or both; while ratio values below the effective range produced a different chlorosis resulting from excess manganese, or deficient iron, or both. Within the leaf tissues, for normal development, ratios of soluble iron to soluble manganese covered the same range of values as those in the external medium. These soluble, or active fractions were determined from the analyses of expressed cell sap. Insoluble fractions were determined from the residues remaining after extraction. High concentrations of soluble manganese in the tissues were always associated with low concentrations of soluble iron and vice versa. This is an observation with which McHargue (1945) has expressed agreement. Somers and Shive concluded that the optimum ratio of the amount of active iron to the amount of active manganese in the leaf tissues of soy bean must lie between 1.5 : 1 and 2.5 : 1, irrespective of the total amounts of these elements in the tissues, if the plants are to grow normally. Because the oxidation potential of cobalt is higher than that of manganese, it should, on the hypothesis offered, have a greater tendency than manganese to oxidize iron. Somers and Shive apparently were able to substitute cobalt for manganese in the growth of maize. They did not grow the plants through a complete life cycle.

Somers, Gilbert and Shive (1942) working with soy beans, found that a departure from the optimum ratio of iron to manganese in culture solutions resulted in lower respiration rates.

Certain features of the data provided by Somers and Shive (1942) do not parallel their deductions. These discrepancies, however, may be due to biological variation or to other causes, and may not be a sufficient reason for discarding the postulations made. For example, the ratio of iron to manganese in the substrate, for plant health, actually extended, in one experiment, to 12 : 1, which is a wide departure from 2 : 1. The range between between 12 : 1 and 50 : 1 was not tested. At ratio 50 : 1 manganese deficiency symptoms appeared. Therefore, the substrate range of ratios of iron to manganese and the range of ratios of soluble iron to soluble manganese in the leaf tissues do not appear to correspond as stated. The data seem to show that mutual competition in absorption may be displayed by these elements, and that the soy bean may require a minimum total concentration of each of these metals in the tissues before normal development can occur.

In order to study the interaction of iron and manganese on growth, Hopkins, Pagan and Silva (1944) carried out certain water and gravel culture experiments with beans, tomatoes, and pineapples. In general the ratio of iron to manganese in the nutrient substrate was the controlling factor in growth, but the interaction of iron, manganese and light seemed important in regulating the oxidation-reduction potentials of green plants. The experimental results appeared to indicate that the requirements of the plants for iron were augmented as the intensity and duration of illumination was increased. They obtained a normal growth of plants in culture solutions containing iron and manganese in concentrations giving iron to manganese ratios wider than 2 : 1. No evidence was found to support the theory that a rather definite iron to manganese ratio is necessary to normal growth.

Jacobson (1945) published data suggesting that, before chlorophyll can be formed, the total iron content in the leaves of plants must exceed a certain minimum. If these results should prove generally applicable, their importance is evident.

Working with oat plants Twyman (1946) obtained data which substantiated the ratio theory of

Somers and Shive.

Hewitt (1948 and 1948a) working with several species in sand cultures, seriously challenged the oxidation-reduction theory on the grounds that manganese is not unique in its ability to induce iron deficiency symptoms, and that many other trace metals induce an iron deficiency chlorosis regardless of the order of their oxidation-reduction potentials. One of these, moreover, namely zinc, does not undergo the valency change associated with simple oxidation-reduction reactions. Furthermore, in order for the theory to be valid, divalent manganese and cobalt, the forms supplied to the plant by Somers and Shive (1942), would themselves have to be oxidized before being able to oxidize iron in plant tissues. We do not know the form in which these metals exist in the plant. Their ions may be complex, with quite unknown potentials. Again the potential of trivalent cobalt would be so great as to decompose water within plant tissues. Therefore, it is unlikely that it can exist in living tissues.

If Hewitt has successfully refuted the oxidation-reduction theory, it may mean that the data provided by a number of well known experiments will have to be subjected to re-interpretation.

Hewitt found that iron and manganese deficiencies simultaneously appeared upon one leaf of oats. He noted that toxicity symptoms produced by manganese were completely different from manganese deficiency symptoms. This undoubtedly weakens the position of those who propound the theory that iron deficiency corresponds to manganese toxicity, and iron toxicity to manganese deficiency. Furthermore, it suggests independent roles for iron and manganese. However, Hewitt's work does offer evidence to support the belief that a given plant species requires an optimum balance of available iron to available manganese in the substrate.

Hewitt believes that iron has many roles. He explains the appearance of iron deficiency symptoms as due to competition between the chlorophyll producing function and some other role or roles for a limited supply of iron. This competition, he thinks, is accentuated in some manner by the metal which induces the chlorosis.

Morris and Pierre (1949) found that, in Lespedeza, the symptoms of iron deficiency were not identical with the symptoms of manganese toxicity.

Sideris (1950) reported that an increase in manganese in supply in the nutrient substrate depressed the translocation of iron from roots to leaves. This may prove to be an important factor in investigations of the relationships between iron and manganese.

Recently, Ouellette (1951) made a further study of the effects on the growth of soy beans of various iron and manganese concentrations and various ratios between the concentrations of these two metals in nutrient solutions. The solutions were continuously aerated. The results of Ouellette did not entirely parallel those of Somers and Shive. A differential absorption of iron and manganese by soy beans was noted. When the iron concentration was low a concentration of 2.5 p.p.m. of manganese appeared to be toxic, for yields were decreased. No iron toxicity was observed, even with 60 p.p.m. of the element in the substrate. Manganese deficiency was produced by increasing the iron concentration in the nutrient solutions having low manganese concentrations. With respect to the concentrations of iron and manganese in the culture solutions, good plants were produced between substrate ratios of iron to manganese of 5 : 1 and 100 : 1. On the whole, a close interrelationship between the quantitative absorption of iron and manganese was demonstrated.

In view of the many difficulties involved in maintaining the concentrations of soluble iron and manganese constant in culture solutions, it may be suggested here that it is possible for very slight variations in experimental procedure to produce wide variations between the results of similar investigations when the water culture-technique is employed in experiments concerning nutrition. It would seem desirable, for example, that iron and manganese be supplied in similar valency states for such trials. Some of the contradictions and discrepancies shown by the results of the various investigations of the interrelationships between iron and manganese may be attributed to biological variation in the small numbers of plants which must necessarily be used in small-scale laboratory experiments. Therefore, the apparently conflicting results obtained from many of the investigations, concerning the effects of these elements upon plants, should not be permitted to obscure the worth of the work accomplished.

# III. MATERIALS AND METHODS

## A. EXPERIMENTAL PLANTS

The plants used in these experiments were as follows:

Dwarf bean, Phaseolus vulgaris var. humilis (var. Round Pod Kidney Wax);

corn, Zea mays (var. Golden Bantam);

garden pea, Pisum sativum (var. Thomas Laxton);

tomato, Lycopersicum esculentum (var. Vetomold 121);

larkspur, Delphinium ajacis (var. Rocket Larkspur); and

onion, Allium cepa (var. Yellow Globe Danvers.

Plants of a uniform size, for a particular species, were selected for each experiment.

## B. REAGENTS AND STOCK SOLUTIONS

The chemicals and method of preparation of the various solutions and reagents required in the experiments are given below.

### 1. CHEMICALS OF ANALYZED GRADES

The amounts of iron and manganese, or heavy metals and iron or manganese are given when included in lists of maximum impurities on the labels.

a. Baker's Analyzed Chemicals, Chemically Pure (C.P.), Baker Chemical Company, Phillipsburg, N.J.

Ammonium hydroxide, $NH_4OH$, sp.gr.0.9, heavy metals (as Pb) 0.0005 per cent, iron (Fe) 0.0001 per cent.

Calcium carbonate, $CaCO_3$, heavy metals (as Pb) 0.001 per cent, iron (Fe) 0.0003 per cent.

Calcium nitrate, $Ca(NO_3)_2.4H_2O$, heavy metals (as Pb) 0.001 per cent, iron (Fe) 0.0005 per cent.

Magnesium nitrate, $Mg(NO_3)_2.6H_2O$, heavy metals (as Pb) 0.0005 per cent, iron (Fe) 0.0005 per cent.

Magnesium sulphate, $MgSO_4.7H_2O$, manganese (Mn) 0.0005 per cent, iron (Fe) 0.0005 per cent.

Mercuric chloride, $HgCl_2$, i.e., mercury bichloride, iron (Fe) 0.002 per cent.

Paraffin Wax, M.P. 52°C.

Potassium dichromate, $K_2Cr_2O_7$, manganese (Mn) and iron (Fe) 0.0 per cent.

Potassium hydroxide, KOH, heavy metals (as Ag) 0.003 per cent, iron (Fe) 0.0005 per cent.

Potassium nitrate, $KNO_3$, heavy metals (as Pb) 0.0005 per cent, iron (Fe) 0.0003 per cent.

Potassium Phosphate (dibasic), $K_2HPO_4$, iron (Fe) 0.005 per cent.

Sodium acetate, $Na_2C_2H_3O_2.3H_2O$, crystals, heavy metals (as Pb) 0.0005 per cent, iron (Fe) 0.0005 per cent.

Sodium nitrate, $NaNO_3$, heavy metals (as Pb) 0.0005 per cent, iron (Fe) 0.0003 per cent.

Sodium phosphate (monobasic), $NaH_2PO_4.H_2O$, heavy metals (as Pb) 0.001 per cent, iron (Fe) 0.0005 per cent.

Sodium sulphate, $Na_2SO_4.10H_2O$, heavy metals (as Pb) 0.0005 per cent, iron (Fe) 0.0005 per cent.

b. Analyzed Analytical Reagents (AnalaR), British Drug Houses, London

Boric acid, $H_3BO_3$, heavy metals (as Pb) 0.001 per cent, iron (Fe) 0.001 per cent.

Cobalt sulphate, $CoSO_4.7H_2O$, iron (Fe) 0.02 per cent.

Copper sulphate, $CuSO_4.5H_2O$, heavy metals (as Ni) 0.003 per cent.

Ferrous sulphate, $FeSO_4.7H_2O$, anhydrous, ferric iron ($Fe^{+++}$) 0.01 per cent.

Hydrochloric acid (Conc.), HCl, sp.gr. 1.19, iron (Fe) 0.00003 per cent.

Manganese (ous) sulphate, $MnSO_4.4H_2O$, heavy metals (as Pb) and iron (Fe) 0.0 per cent.

Molybdenum trioxide, $MoO_3$, (Molybdic anhydride), heavy metals (as Pb) 0.005 per cent.

Nickel sulphate, $NiSO_4.6H_2O$, iron (Fe) 0.005 per cent.

Nitric acid (Conc.), $HNO_3$, sp.gr.1.42, manganese (Mn) and iron (Fe) 0.0 per cent.

Potassium sulphate, $K_2SO_4$, heavy metals (as Pb) 0.0005 per cent, iron (Fe) 0.0005 per cent.

Sulphuric acid (Conc.), $H_2SO_4$, sp.gr.1.84, manganese (Mn) and iron (Fe) 0.0 per cent.

Zinc sulphate, $ZnSO_4.7H_2O$, manganese (Mn) 0.00003 per cent, iron (Fe) 0.001 per cent.

c. Merck Reagent Grade Laboratory Chemicals, Merck and Co., Ltd., Montreal

Acetone, $CH_3COCH_3$, manganese (Mn) and iron (Fe) 0.0 per cent.

Phenolphthalein, $C_{20}H_{14}O_4$, powder, manganese (Mn) and iron (Fe) 0.0 per cent.

d. Eimer and Amend Laboratory Chemicals, Supplied by Fisher Scientific Co., Ltd., Montreal

(i) Tested purity reagents (T.P.), exact analysis reported.

Acetic acid, $CH_3COOH$, 80 per cent, manganese (Mn) and iron (Fe) 0.0 per cent.

Ethylenediamene tetra-acetic acid, $(HOCOCH_2)_2NCH_2CH_2N(CH_2COOH)_2$, manganese (Mn) and iron (Fe) 0.0 per cent, supplied by Alrose Chemical Company, Providence, Rhode Island.

Manganese, Mn, pure lump metal, carbon free.

o-Phenanthroline, $C_{12}H_8N_2$, manganese (Mn) and iron (Fe) 0.0 per cent.

Sucrose, $C_{12}H_{22}O_{11}$, heavy metals (as Cu) 0.0005 per cent, iron (Fe) 0.0005 per cent.

(ii) Chemically pure (C.P.), high degree of freedom from impurities, suitable for analytical work.

Hydroquinone, $C_6H_4$-1,4-($OH_2$), iron (Fe) 0.0 per cent.

Sodium periodate, $Na_3H_2IO_6$, manganese (Mn) 0.0 per cent.

Titanium(ic) oxide, $TiO_2$, water insoluble.

## 2. PREPARATION OF TITANYL SULPHATE FROM WATER INSOLUBLE TITANIUM(IC) OXIDE

Titanyl sulphate ($TiSO_4.2H_2O$), used in substitution trials, was prepared from water insoluble titanium (ic) oxide ($TiO_2$) by a procedure described by Mellor (1927).

## 3. DISTILLED WATER

Distilled water used throughout the work was prepared in an electrically heated Barnstead water still lined with block tin. Examinations made by means of a Beckman potentiometer showed that the distilled water had a constant pH of 6.5; and spectrophotometric tests showed that it contained no measurable quantity of iron or manganese after evaporation from a volume of 2 liters to 25 ml.

## 4. CLEANING SOLUTION

The glassware used in the experiments was cleaned with a solution of potassium dichromate in sulphuric acid prepared according to the method of Booth and Damerell (1944), and rinsed with distilled water. The cuvettes used in making the analyses were rinsed, in addition, with 6 N hydrochloric acid followed by a rinse of distilled water.

## 5. STOCK SOLUTIONS

The modes of preparation of a number of stock solutions are given below. These contained the various cultural elements, and were added, in different amounts, to distilled water to make the culture solutions in which the experimental plants were grown.

### a. Purified Salts of the Major Nutrient Elements

In order to eliminate the possibility of iron and manganese entering the culture solutions in an uncontrolled manner, 2 liters each of a number of stock solutions of salts of the major nutrient elements, containing 0.5 mole of solution per liter of solution (0.5 M), were purified according to the methods described by Stout and Arnon (1939). The salts were as follows:

Stock solution 1, calcium nitrate ($Ca(NO_3)_2.4H_2O$);

stock solution 2, dibasic potassium phosphate ($K_2HPO_4$);

stock solution 3, magnesium sulphate ($MgSO_4.7H_2O$); and

stock solution 4, potassium sulphate ($K_2SO_4$).

Purification methods. Iron and manganese were adsorbed by calcium carbonate and calcium phosphate particles, and co-precipitated with them after heating the solutions at slightly alkaline reactions. This method of eliminating trace elements from nutrient solutions is less difficult, and more efficient, than conventional methods involving recrystallization.

The solutions were prepared in 4 liter Pyrex boiling flasks. Small amounts of calcium carbonate ($CaCO_3$), calcium nitrate ($Ca(NO_3)_2.4H_2O$), and dibasic potassium phosphate ($K_2HPO_4$) were added to each solution as explained by Stout and Arnon (1939). The solutions, at slightly alkaline reactions, were autoclaved for 90 minutes, at 18 pounds pressure, allowed to settle for 24 hours, filtered, and restored to volume with distilled water. In the case of dibasic potassium phosphate the filtrate was acidified with sulphuric acid to pH 5.5 in order to prevent the formation of a precipitate of calcium phosphate during the mixing of dibasic potassium phosphate and calcium nitrate in the preparation of culture solutions.

Analyses, made with a Hilger spectrograph, showed that all manganese was completely removed, but that some iron remained in the solutions. Analyses made with the Model 14 Coleman Universal spectrophotometer showed that the amount of iron remaining in the 0.5 M stock solutions was not greater than 0.08 p.p.m. This amount may be considered negligible after stock solutions have been diluted in the preparation of culture solutions. Nevertheless, the solutions were further purified by autoclaving with 6.5 gm. of calcium carbonate per liter of solution. The doubly purified stocks were found to be free from iron and, or manganese.

### b. Stock Solutions of Trace Elements

The stock solutions which contained the trace elements were not purified. A salt which contains a trace of another element as an impurity cannot contribute significant amounts of the impurity to a nutrient solution in which the salt itself is present as a trace.

(i) Stock solution 5. This stock solution contained boron, zinc, copper and molybdenum as shown in Table I below. It was added to all of the culture solutions.

TABLE I

Stock Solution 5

| Compound | Gm. in 1 liter of distilled water |
|---|---|
| Boric acid ($H_3BO_3$) | 2.860 |
| Zinc sulphate ($ZnSO_4.7H_2O$) | 0.220 |
| Copper sulphate ($CuSO_4.5H_2O$) | 0.080 |
| Molybdenum oxide ($MoO_3$) | 0.075 |

One ml. of the above micro nutrient stock solution in a liter of culture solution provided the following p.p.m. of the cultural elements:

Boron, 0.5; zinc, 0.05; copper, 0.02; molybdenum, 0.05.

Stock solution 5 was stored in 500 ml. Pyrex volumetric flasks.

(ii) Stock solution 6 (ferrous sulphate, $FeSO_4.7H_2O$). This was prepared by dissolving 2.4890 gm. of the salt in 1 liter of distilled water.

(iii) Stock solution 7 (manganese sulphate, $MnSO_4.4H_2O$). This was prepared by dissolving 2.0303 gm. of the salt in 1 liter of distilled water.

(iv) Dilutions of stock solutions 6 and 7. One ml. of either stock solution 6 or 7 added to 1 liter of a culture solution corresponded to either 0.5 p.p.m. of iron or 0.5 p.p.m. of manganese. Lesser concentrations of iron or manganese were supplied to the culture solutions by first diluting aliquots of the appropriate stock solution. Stock solutions 6 and 7 were stored in 250 ml. Pyrex volumetric flasks.

(v) Stock solution 8 (potassium humate). A synthetic potassium humate was prepared by a method based upon a procedure outlined by Horner, Burk and

Hoover (1934). This material, in alkaline solution, adsorbs iron and manganese. Colloidal humate iron and manganese are thus formed and remain available to plants within the pH range of 3 to 9; unless calcium is present in immoderate amounts, when the colloidal condition of the iron and manganese is destroyed by the replacement of iron and, or manganese by calcium. Phosphates are unable to precipitate humate iron. The first material formed during the preparation of potassium humate is a synthetic humic acid with the empirical formula $(C_6(H_2O)2.1)n$ which is a condensation product of sucrose $(C_{12}H_{22}O_{11})$ with a loss of two-thirds of the molecular water.

## Preparation of Potassium Humate

A stock solution of potassium humate was prepared as follows:

(1) A liter of distilled water and sulphuric acid (30 per cent by volume of concentrated $H_2SO_4$) was put in a 2 liter Pyrex beaker, covered with a watch glass, and brought to the boiling point on an electric hot plate under a fume hood.

(2) One hundred gm. of T.P. sucrose were added to the

solution.

(3) The solution was simmered three hours (at B.P.) with occasional stirring with a glass rod.

(4) Floating material was skimmed off. The preparation was allowed to settle for 24 hours.

(5) The liquid phase was decanted and discarded.

(6) A 40 per cent solution of potassium hydroxide was added to the solid phase until it was partially dissolved and the solution was alkaline to phenolphthalein.

(7) The preparation was filtered, and the solid phase discarded.

(8) Sulphuric acid was added to the solution; and hydrogen humate precipitated at pH 3 to 4.

(9) The liquid phase was discarded after filtration; and the humate re-dissolved in five per cent potassium hydroxide (KOH) to give a neutral solution.

(10) By centrifuging a 5 ml. aliquot at pH 3, discarding the liquid, and weighing the precipitate after drying at 100° C., the total yield of potassium humate was found to be 14.6 gm.

A neutral stock solution was stored in a Pyrex flask and contained 15 mg. of potassium humate per ml. which was capable of adsorbing 1.5 to 2.25 mg. of iron or manganese per ml.

Humate iron and manganese were prepared by adding ferrous sulphate or manganese sulphate directly to the neutral solution of potassium humate diluted five to ten times in distilled water.

(vi) Stock solution 9 (chelated iron). Another form of organic iron was prepared by chelation from 1 N potassium hydroxide, ethylenediamine tetra-acetic acid, and ferrous sulphate, and stored in a 500 ml. Pyrex volumetric flask. In this preparation the ferrous iron becomes part of a complex ion which, for an indefinite period, is extremely stable below pH 6. It is non toxic to plants in concentrations up to 100 p.p.m. The method of preparation was described by Jacobson (1951). One ml. of the stock solution of chelated iron in a liter of culture solution corresponded to one p.p.m. of iron.

## C. CULTURE SOLUTIONS

Three basic culture solutions were used in the experiments.

## 1. CULTURE SOLUTION A

Culture solution A was prepared from solutions of purified salts (see page 42), and was a modification of the solution used by Somers and Shive (1942) for soy bean. Dibasic potassium phosphate here replaced the monobasic salt, which cannot be purified with calcium carbonate. This solution slowly became more alkaline as plants grew in it. The composition of culture solution A is given in Table II.

TABLE II

Culture Solution A

| Compound | Ml. of 0.5 M stock solution per liter |
|---|---|
| Calcium nitrate ($Ca(NO_3)_2.4H_2O$ | 9.00 |
| Dibasic potassium phosphate ($K_2HPO_4$) | 1.16 |
| Magnesium sulphate ($MgSO_4.7H_2O$) | 4.60 |
| Potassium sulphate ($K_2SO_4$) | 1.63 |

## 2. CULTURE SOLUTIONS B AND C

Culture solutions B and C were modifications of solutions designed by Swanback (1939) in an effort to obtain solutions which differed only in their levels of calcium. Culture solutions B and C approached this aim more successfully than Swanback's original solutions, and attained it completely with the exception of the anion $SO_4^{--}$, which remained unbalanced. The salts used in the preparation of these solutions were not purified. The spectrophotometer showed that iron was present in culture solution B in a concentration of 0.09 p.p.m. and in culture solution C in a concentration of 0.01 p.p.m. The addition of treatment iron was adjusted to compensate for this.

Culture solution C was highly buffered. It was found difficult to adjust the pH so that organic forms of iron and manganese would not precipitate in it. Therefore, inorganic forms of iron and manganese were supplied in both culture solution C and culture solution B. These solutions are given in Tables III and IV.

## TABLE III

### Culture Solution B

| Compound | Gm. per liter |
|---|---|
| Calcium nitrate ($Ca(NO_3)_2.4H_2O$) | 0.250 |
| Sodium dihydrogen phosphate ($NaH_2PO_4.H_2O$) | 0.078 |
| Potassium nitrate ($KNO_3$) | 0.214 |
| Sodium nitrate ($NaNO_3$) | 0.037 |
| Magnesium nitrate ($Mg(NO_3)_2.6H_2O$) | 0.320 |
| Magnesium sulphate ($MgSO_4.7H_2O$) | 0.308 |

## TABLE IV

### Culture Solution C

| Compound | Gm. per liter |
|---|---|
| Calcium nitrate ($Ca(NO_3)_2.4H_2O$) | 0.8470 |
| Sodium dihydrogen phosphate ($NaH_2PO_4.H_2O$) | 0.0780 |
| Magnesium sulphate ($MgSO_4.7H_2O$) | 0.6160 |
| Potassium sulphate ($K_2SO_4$) | 0.1850 |
| Sodium sulphate ($Na_2SO_4.10H_2O$) | 0.0701 |

## 3. ADDITION OF TRACE ELEMENTS TO THE CULTURE SOLUTION

### a. Addition of Boron, Zinc, Copper and Molybdenum

One ml. of stock solution 5 (see Table I, page 45) was added to every liter of culture solutions A, B and C.

Concentrations of the elements in the culture solutions. After the addition of stock solution 5 the cultural elements were present in the amounts shown in Table V, page 54.

### b. Addition of Iron and Manganese

Iron and manganese were varied in supply in the culture solutions (see page 44). Manganese was supplied either as the sulphate ($MnSO_4.4H_2O$), or manganese humate. Iron was supplied as the inorganic ferrous sulphate ($FeSO_4.7H_2O$); or in the organic form as iron humate, or chelated iron.

Manganese sulphate is very soluble. Ferrous sulphate, on the other hand, is not stable in

## TABLE V

## Amounts of the Elements in Culture Solutions A, B and C

| Element | P.p.m. in Culture Solution A | P.p.m. in Culture Solution B | P.p.m. in Culture Solution C |
|---|---|---|---|
| Nitrogen | 126.00 | 99.60 | 100.00 |
| Phosphorus | 18.00 | 17.60 | 17.60 |
| Potassium | 109.20 | 82.00 | 82.00 |
| Calcium | 180.00 | 42.00 | 143.00 |
| Magnesium | 55.20 | 60.00 | 60.00 |
| Sulphur | 94.80 | 40.00 | 120.90 |
| Sodium | | 22.90 | 22.90 |
| Boron | 0.50 | 0.50 | 0.50 |
| Zinc | 0.05 | 0.05 | 0.05 |
| Copper | 0.02 | 0.02 | 0.05 |

solution. It is oxidized to the ferric form, and is precipitated from solution as ferric hydroxide, ($Fe(OH)_3$) and ferric phosphate ($FePO_4$), although low concentrations of ferrous sulphate, in solutions of low pH, have been found to remain stable for 48 hours. However, in certain of the present experiments, it was necessary to maintain high concentrations of iron in solution. In order to achieve this, organic forms of iron were prepared; namely, humate iron and chelated iron. Humate manganese was prepared so that iron and manganese could be supplied in similar valency and chemical states.

#### c. Adjustment of pH

So that no new ions would be added, the pH of all solutions was adjusted with sulphuric acid and potassium hydroxide.

## D. CULTURE METHODS

### 1. GENERAL WATER-CULTURE METHODS

#### a. Germination Procedure

The seeds were handled by means of forceps dipped in hot paraffin. They were sterilized by soaking for 2 minutes in a 0.1 per cent aqueous solution of mercuric chloride ($HgCl_2$), placed between No. 40 Whatman filter papers moistened with distilled water, and germinated in petrie dishes at 25°C. in an incubator, where they remained for 48 hours. The radicles from viable seeds were then evident.

### b. Removal of Plant Sources of Manganese

McHargue (1914) found that the seed coats of beans contained an accumulation of manganese. Therefore, these were removed. In order to eliminate other sources of manganese, the endosperm of the corn and garden peas was removed 48 hours after germination. The cotyledons of the beans were removed as soon as the true leaves appeared.

### c. Culture of Seedlings Prior to Experimental Treatments

Until the experimental treatments were begun the seedlings were cultured by one or more of the following methods:

1. The seedlings were put on a paraffined cheese cloth germination net tightly stretched over a circular, glass vessel filled with distilled water.

2. The seedlings were grown with their roots passing through one-fourth inch holes in paraffined, 24 cm. Cenco filter paper on corks floating in distilled water, in a glass vessel.

3. The seedlings were grown in sterile glass quartz sand, of particle diameters 0.2 to 1.0 mm., kept just moist with distilled water.

### d. Purification of Glass Quartz Sand

The latter medium was freed of manganese and iron by soaking in ten per cent cold nitric acid for 14 days, leaching under a hot water tap for 48 hours, soaking in distilled water 48 hours and rinsing in distilled water 6 times. If distilled water, drained 6 times through the sand, exhibited a pH of less than 4, the rinsings were repeated. Aliquots of the purified sand were tested for iron and manganese by extracting in 2 per cent AnalaR hydrochloric acid for one hour. The hydrogen ions were liberated and removed from the sand by leaching with the purified basic culture solution A, which contained no iron or manganese, until the pH of the culture solution remained unchanged on standing over night in contact with the sand.

## 2. METHODS USED AFTER SEEDLINGS OF REQUIRED SIZE WERE OBTAINED

### a. Water Culture Experiments

(i) Containers. The culture vessels were 600 ml. Pyrex, Berzelius beakers, because Pyrex glassware does not supply iron or manganese to culture solutions, although it may yield insignificant traces of arsenic, zinc or lead. In order to exclude contaminating elements the beakers were fitted with square, paraffined, fibre-board covers. The plants were supported by non-absorbent cotton in Pyrex glass tubes of 1 cm. bore and 6 cm. long. These were pushed through holes in the covers, and held in place by rubber washers soaked in paraffin. Additional support was provided, for large plants, by passing loops of paraffined, white, linen thread around glass rods 1 mm. in diameter. To inhibit the growth of algae the Berzelius beakers were covered with black paper, and a layer of white paper on the outside, to exclude light.

(ii) Aeration of the culture solution. Air was delivered by a one-third h.p. electric pressure pump into distilled water in a 250 ml. gas washing bottle through a fritted glass disc, and passed to the bottom of a bubbling vessel, a glass cylinder 45 cm. in height, through a tube formed by the horizontal arms of Pyrex T-tubes linked by rubber tubing and attached by copper wire to a solid glass rod, 6 feet long, supported by clamps. An extension was added to this tube when the experiment included more than 20 culture vessels.

Pyrex aeration tubes of 3 mm. bore delivered air from finely drawn tips into the culture solution in each container at the rate of one bubble of air per second. The rate of delivery was regulated by aluminum tubing clamps on 8 cm. lengths of pressure tubing joining the vertical arms of the T-tubes to the horizontal arms of the aerators.

A constant pressure was maintained in the system which was just sufficient to bubble air through a column of water 30 cm. in height in the bubbling vessel. The pressure was controlled by manipulating glass stopcocks on the entry from the pump, and on the entry to the bubbler. A pressure release was obtained by adjusting a clamp on a short length of pressure tubing on the vertical arm of a T-tube inserted into the tube entering the gas washing bottle.

The aeration system is shown in Appendix Figure 3, page iii of Appendix I.

(iii) Supplementary lighting. In certain experiments the normal sunlight of the laboratory was supplemented for 8 hours daily with three 40 watt fluorescent lamps, one daylight and two white, which were suspended 2 feet above the plants by chains of adjustable length.

### b. Special Absorption Experiments at Constant Temperature

(i) Containers. In certain absorption experiments, ordinary 500 ml. Mason jars were employed as culture vessels. These were fitted with circular, paraffined fibre-board tops in which were bored two circular openings. One in the centre, of 3 cm. diameter, served as a plant holder. A 1 cm. opening, near the edge, was plugged with non-absorbent cotton. This opening permitted the removal, by a pipette, of samples for solution analysis. In other absorption experiments a similar top was fitted to 600 ml. Pyrex Berzelius beakers.

(ii) Shaker and constant temperature bath. A shaker, designed by Dr. W. Leach, was used in certain absorption experiments. This apparatus consisted of a galvanized steel tank, duco enamelled black inside and having a height of 20 inches, a length of 34 inches, and a width of 26 inches. A steel plant tray, with perforated sides, and a carrying capacity of 14 Berzelius beakers, was submerged to a depth of 1 inch in water in the tank. Mounted on wheels on brass tracks, the tray was attached, to a crank disk, near its outer circumference, by a connecting rod. A one-third h.p. electric motor, belted to a wheel on the opposite end of the axis of the crank disk, gave the tray an excursion of 2.5 inches through the water. This apparatus is shown in Plate 1b, page iv of Appendix I.

The culture solutions were maintained at a constant temperature by the water bath, which was heated electrically by two fine-wire uniform-resistance heating coils of nickel-chrome enclosed in a U-shaped 5 mm. Pyrex tubing immersed in the tank water. A mercury thermostat and mercury weighted electromagnetic relay controlled the temperature, which was not observed to vary more than 0.5 degrees from 20° C.

## E. ANALYTICAL METHODS

### 1. MODEL 14 COLEMAN UNIVERSAL SPECTROPHOTOMETER

All estimations of the amounts of iron and manganese in plant tissues and culture solutions were made with a model 14 Coleman Universal spectrophotometer with the galvanometer used as a null device, as described in "Operating Directions for the Model 14 Coleman Universal Spectrophotometer." With this method of operation the error of the instrument was only 0.1 per cent. The overall precision was limited by the personal factor, but was better than 0.5 per cent. This instrument is shown in Plate la, page iv of Appendix I.

## 2. PRELIMINARY STUDIES OF COLORIMETRIC METHODS OF ANALYSIS

### Colorimetric Determination of Small Amounts of Manganese

(i) Benzidine method. Several trials were made in an effort to develop a good technique for the spectrophotometric analysis of manganese. The benzidine procedure, recommended as a quantitative method by Stratton, Ficklen and Hough (1932), and by Wiese (1939), failed at very dilute concentrations, when the blue colour, resulting from the presence of manganese, did not develop full intensity and rapidly faded.

(ii) Tetramethyldiaminodiphenylmethane method. An extremely sensitive method used by Nicholas (1946), which utilized tetramethyldiaminodiphenylmethane, appeared promising; but failed under the same circumstances as the benzidine procedure.

## 3. COLORIMETRIC METHODS OF ANALYSIS USED IN THE PRESENT INVESTIGATIONS

### a. Method for the Determination of Iron

Iron, in terms of mg. per gram of dry weight, or parts per million of solution, was determined by the apectrophotometric method of Sandell (1944), using o-phenanthroline to develop a red-brown colour and hydroquinone to reduce the iron to the ferrous state. The colour intensity was found to be independent of the acidity, in the pH range 2 - 9, and showed no change after many months. Maximum absorption was found to occur at 510 millimicrons, (see pages i and ii of Appendix I for Spectral-Transmittance, and for Concentration-Transmittance graphs). Beer's law was closely followed. The method is very sensitive. It was found possible to estimate, with accuracy, 0.05 p.p.m. of iron in solution. The presence of iron was indicated by colour at lower concentrations, but quantitative estimations were less dependable.

### b. Method for the Determination of Manganese

The permanganate method for the colorimetric estimation of manganese is undoubtedly the most satisfactory yet devised; and yields results of a high degree of accuracy. If used properly, few substances interfere. The pink colour, once produced, is extremely stable. The method was used essentially as described by Sandell (1944a), and Yoe (1928). The method involves the oxidation of manganese to permanganate in acid solution.

As used here, 10 ml. of solution ready for analysis ordinarily contained equal proportions of concentrated sulphuric acid and concentrated nitric acid, equivalent to 5.0 per cent of the volume, and 0.05 - 0.1 gm. of sodium periodate. The solution was heated for 2 hours in a boiling water bath to develop the colour. When the concentration of manganese was lower than 0.4 p.p.m. in the solution, the colour faded, unless the acid content was reduced below 5.0 per cent. When the acid content was sufficient to prevent the precipitation of iodates or periodates of manganese, but did not exceed 2 per cent, it was possible to determine 0.1 p.p.m. of manganese in solution. It was possible to estimate 0.01 mg. of manganese in 1 gm. of dry plant material. The wavelength at which manganese absorbed most strongly was found to be 530 millimicrons, and it was used in making the succeeding analyses.

## 4. PLANT ANLAYSIS METHODS

### a. Preparation of Plant Tissues for Analysis

All plant material selected for analysis was washed at harvest in distilled water. The leaves were collected and preserved in Pyrex test tubes as they abscissed and at the termination of individual experiments. When the roots and stems were analyzed, they were treated as a separate plant fraction. According to Somers and Shive (1942) iron precipitated upon the roots of plants in water cultures, although not always visible, may be a source of error when estimating the iron content of plant tissues. Therefore, in these experiments, in order to remove any precipitation of this nature, the roots were rinsed for one-half minute in 1 per cent hydroquinone solution in sodium acetate-acetic acid buffer of pH 4.5 (65 ml. of 0.1 M acetic acid and 35 ml. of 0.1 M sodium acetate mixed). This was followed by a distilled water rinse. The plant tissues were oven dried at 85°C. to constant weights, macerated for 2 minutes in a Waring Blendor, and the aliquots weighed for analysis. With respect to the preparation of the plant tissue sample for analysis, Schlenker (1943) found grinding adequate when compared with other procedures. During the present investigations, preliminary trials with materials of known iron and manganese content showed that the stainless steel blades did not add these metals to plant tissues.

### b. Leaf Analysis

Leaf iron and manganese were arbitrarily fractioned into sap soluble and insoluble fractions; the former extractable by acetone, but not the latter. Preliminary trials were made with dried and ground lilac leaves. Sideris and Young (1949) believed that the acetone extractable fraction closely approximated a true sap.

(i) Separation of sap soluble and insoluble fractions of leaf iron and manganese. The sap fractions were extracted by pouring 16 ml. of metal free acetone per gm. of dried tissue through leaf samples on No.30 Whatman filter papers washed with hydrochloric acid and folded into small, glass funnels. A gram was the minimum weight of sample necessary for good estimations. The filtrates, collected in 250 ml. Pyrex Erlenmeyer flasks, were assumed to contain the sap soluble fractions of iron and manganese; and the residues the insoluble fractions. The residues were washed, with distilled water, into similar flasks. The volumes of the filtrates were doubled with distilled water. They were placed upon an electric hot plate, under a fume hood, and the acetone removed by evaporation.

(ii) Digestion. Both filtrates and residues were wet-digested with nitric and sulphuric acids, according to the method described by Sandell (1944) for iron determinations. Small glass funnels were placed in the necks of the 250 ml. Erlenmeyer flasks, and the digestions carried out directly in these vessels upon an electric hot plate under a fume hood. When the contents of the flasks had become completely colourless, after boiling off the nitric acid, the sulphuric acid was fumed to one-half its original volume in order to facilitate acid neutralization in the iron determination, and to permit the estimation of minute quantities of manganese. In a few instances precipitates of silica and gypsum had to be removed by filtration through Whatman filters, after the addition of 15 ml. of distilled water.

(iii) Preparation of solutions for analysis. The solutions were finally diluted with boiled, distilled water to 25 ml. in volumetric flasks. Each solution was fractioned into five parts. The volume of ammonia required for neutralization was determined upon a 5 ml. fraction. Iron and manganese were determined, in duplicate, upon the remaining four fractions.

(iv) Estimation of iron. Iron was read from 25 ml. volume, in volumetric flasks, with further dilution for high concentrations.

(v) Estimation of manganese. The clear solutions resulting from the acid digestion of plant tissues were analyzed for manganese without the addition of more acid; the solutions having already been evaporated with nitric acid, and the remaining sulphuric acid supplying the necessary concentration of acid. Colour for manganese was developed in 10 ml. calibrated test tubes. Manganese was estimated directly from 10 ml. volume, if the concentration were low, and from further dilution when the concentration was sufficiently great.

### c. Root and Stem Analysis

The root and stem of each plant were analyzed as a combined plant tissue fraction. Their average total contents of iron and manganese per gram of dry weight were estimated.

## 5. CULTURE SOLUTION ANALYSIS METHODS

The culture solutions were analyzed directly without digestion, otherwise, the procedure was the same as that used with dried plant material.

# IV. EXPERIMENTAL RESULTS

## EXPERIMENT 1

### Preliminary tests to ascertain the nature of deficiency symptoms produced in various plant species when grown in culture solutions containing various concentrations of iron and manganese

Purpose. The purpose of this experiment, begun October, 1949, was twofold in character. In the first place it was considered desirable to produce the deficiency symptoms under experimental conditions so that the symptoms could be studied directly. In this way it was assured that the symptoms in their characteristic form could be readily recognized when they appeared in later experiments. Secondly, this experiment indicated clearly which species showed distinctly defined symptoms of iron and manganese deficiencies respectively, and thus revealed the most suitable species for further and more critical experiments.

Experimental Plants. The species used in these tests were as follows:

Dwarf bean, Phaseolus vulgaris var. humilis;

corn, Zea mays;

garden pea, Pisum sativum;

tomato, Lycopersicum esculentum;

larkspur, Delphinium ajacis.

Procedure. Seven day old seedlings which had been grown on germination nets and supplied only with distilled water were transferred to the water-culture vessels (600 ml. Berzelius beakers), four plants to each vessel. Each culture vessel contained 500 ml. of the nutrient solution. Culture solution A (see page 50) was used and iron and manganese were supplied as ferrous sulphate ($FeSO_4.7H_2O$), and manganese sulphate ($MnSO_4.4H_2O$). In order to maintain iron in solution as long as possible the pH of the culture solutions was adjusted to pH 4.5 at the beginning of each run; the solutions being renewed at intervals of 72 hours. Aeration and supplementary lighting were supplied for 8 hours each day (see pages 59 and 60).

Data. Information relating to experiment 1 is given in Table VI (see page 78).

It will be noted from the data shown in Table VI that the omission of iron or manganese from the culture solution, in all cases, produced deficiency symptoms. These were of a chlorotic nature. No attempt was made to devise a system for the rating of the severity of the symptoms. In general the length of time required for the symptoms to develop would appear to be a satisfactory index of this.

Confirmation of the diagnoses made concerning deficiency symptoms were obtained in the case of each species in the following manner. Several large plants were grown in nutrient solutions deficient in iron or manganese until pathological symptoms appeared. When the metal originally omitted was put in the solution the symptoms ceased to develop in the case of manganese deficiency and were removed in the case of iron deficiency.

Below are described the observed effects of deficiencies in iron and manganese for the different species used in this experiment. These effects are shown in the Plates in Appendix I.

*Iron deficiency in dwarf bean, Phaseolus vulgaris* var. *humilis*. The early symptoms of an iron deficiency chlorosis in this species were exhibited by the terminal leaves, which turned a uniform yellow in colour. The chlorosis progressed slowly downward to the lower leaves; and became more pronounced as the leaves enlarged. Eventually the new growth was white when it opened. The leaves did not absciss; but the affected plants grew very slowly. The roots did not appear to be abnormal.

*Manganese deficiency in dwarf bean, Phaseolus vulgaris* var. *humilis*. Manganese deficiency in this species was also manifest as a chlorosis. The early effects were a mottled, interveinal yellowing of the basal regions of the lower leaves. Widely scattered, small, brown, circular necrotic areas appeared within two or three days. The petioles became yellow. The affected leaves abscissed. The disorder started at the base and extended gradually toward the apical region of the plant. In the most severe cases the stem growing points died. The growth of roots was greatly retarded. Manganese deficient plants of the dwarf bean were taller than iron deficient plants of the same age, but not so tall as healthy plants.

Iron deficiency in garden pea, *Pisum sativum*. The symptoms of iron deficiency in *Pisum sativum* appeared first on the terminal leaves, and were similar to the symptoms of iron deficiency displayed by *Phaseolus vulgaris.*

Manganese deficiency in garden pea, *Pisum sativum*. The terminal leaves were mottled with yellow. Small, brown, necrotic spots later appeared in the chlorotic areas. The tendrils became brown and withered. Subsequently, in instances of severe deficiency, the new leaves failed to expand. The growth of the roots was retarded.

Iron deficiency in corn, *Zea mays*. The new growth was yellow between the vascular bundles. As the severity of the chlorosis increased the new growth was entirely yellow or white; while the leaves immediately below presented a striped appearance, with alternating green and yellow longitudinal bands. The growth of *Zea mays* was retarded by iron deficiency.

Manganese deficiency in corn, *Zea mays*. Manganese deficiency sumptoms differed from iron deficiency symptoms in two respects only; namely, the leaf tips remained green, and bands of necrosis appeared in the yellow areas.

*Iron deficiency in tomato, Lycopersicum esculentum*. The first effect was a yellowing between the veins of terminal leaves. These leaves rapidly became completely yellow; and the chlorosis progressed down towards the lower leaves. There was a limited amount of necrosis in the yellow areas. Growth of the plants was greatly retarded.

*Manganese deficiency in tomato, Lycopersicum esculentum*. The most characteristic symptom was the development of an intervenal chlorosis which appeared first upon the second or third leaves of the plant. As the terminal leaves developed and expanded the variegated, intervenal pattern of numerous, yellow or white dots gradually appeared on them also. There was no leaf abscission.

*Iron and manganese deficiencies in larkspur, Delphinium ajacis*. It was found impossible to distinguish visually between manganese and iron deficiencies in *Delphinium*. In both cases the new growth was a uniform yellow. However, it was found possible to restore the normal green colour by adding the absent metal (iron or manganese) to the culture solution.

## TABLE VI

### Experiment 1

Culture experiments with various concentrations of iron and manganese in the culture solution

| Species used | Number of plants | P.p.m. in solution | | Ratio Fe : Mn in Solution | Symptoms* | Time, days, required for symptoms to appear | Duration of expt. days |
|---|---|---|---|---|---|---|---|
| | | Fe | Mn | | | | |
| Zea | 8 | 0.00 | 2.50 | | -Fe | 6 | 30 |
| | 8 | 0.00 | 0.25 | | -Fe | 9 | 55 |
| | 8 | 5.00 | 2.50 | 2.0 | N | | 30 |
| | 8 | 0.50 | 0.25 | 2.0 | N | | 55 |
| | 8 | 5.00 | 0.00 | | -Mn | 10 | 30 |
| | 8 | 0.50 | 0.00 | | -Mn | 12 | 55 |
| Pisum | 8 | 0.00 | 2.50 | | -Fe | 2 | 30 |
| | 8 | 0.00 | 0.25 | | -Fe | 5 | 55 |
| | 8 | 5.00 | 2.50 | 2.0 | N | | 30 |
| | 8 | 0.50 | 0.25 | 2.0 | N | | 55 |
| | 8 | 5.00 | 0.00 | | -Mn | 11 | 30 |
| | 8 | 0.50 | 0.00 | | -Mn | 14 | 55 |
| Phaseolus | 8 | 0.00 | 2.50 | | -Fe | 3 | 30 |
| | 8 | 0.00 | 0.25 | | -Fe | 7 | 55 |
| | 8 | 5.00 | 2.50 | 2.0 | N | | 30 |
| | 8 | 0.50 | 0.25 | 2.0 | N | | 55 |
| | 8 | 5.00 | 0.00 | | -Mn | 8 | 30 |
| | 8 | 0.50 | 0.00 | | -Mn | 14 | 55 |
| Lycopersicum | 8 | 0.00 | 2.50 | | -Fe | 3 | 30 |
| | 8 | 0.00 | 0.25 | | -Fe | 7 | 30 |
| | 8 | 5.00 | 2.50 | 2.0 | N | | 30 |
| | 8 | 0.50 | 0.25 | 2.0 | N | | 30 |
| | 8 | 5.00 | 0.00 | | -Mn | 14 | 30 |
| | 8 | 0.50 | 0.00 | | -Mn | 18 | 30 |
| Delphinium | 8 | 0.00 | 2.50 | | -Fe | 4 | 30 |
| | 8 | 0.00 | 0.25 | | -Fe | 5 | 30 |
| | 8 | 5.00 | 2.50 | 2.0 | N | | 30 |
| | 8 | 0.50 | 0.25 | 2.0 | N | | 30 |
| | 8 | 5.00 | 0.00 | | -Mn | 9 | 30 |
| | 8 | 0.50 | 0.00 | | -Mn | 12 | 30 |

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* Symptoms indicated as N, normal; -Mn, manganese deficiency; -Fe, iron deficiency.

## EXPERIMENTS 2 AND 3

### The effects of growing Phaseolus vulgaris var. humilis (dwarf bean) in culture solutions containing different concentrations of iron and manganese and different iron to manganese concentration ratios

Purpose. In order to determine how far the findings of Somers and Shive (1942) for soy bean (see page 27) held in the case of *Phaseolus vulgaris* var. humilis, a number of cultures of this species were prepared according to the general procedure described below. These cultures comprised experiments 2 and 3.

General Procedure. Seedlings of *Phaseolus vulgaris* var. humilis were grown for 5 days upon a float of paraffin filter paper (see page 57) in distilled water, and transferred, for a period of 10 days, to purified, white, quartz sand to which was added culture solution A as described on page 57, and kept moist by adding distilled water when necessary. The fifteen day old seedlings were transferred to the water culture vessels; namely, the 600 ml. Berzelius beakers, each of which contained 350 ml. of the nutrient solution, adjusted to pH 5 at the beginning of each run, the solutions being renewed at intervals of 24 hours. Culture solution A (see page 50) was used. Aeration was supplied for 8 hours each day (see page 59).

### Experiment 2

Procedure. The general procedure has been described above. This experiment was conducted, from February 28, 1950 to March 30, 1950, with 18 single plant cultures. Iron and manganese were supplied in the culture solution as ferrous sulphate ($FeSO_4.7H_2O$) and manganese sulphate ($MnSO_4.4H_2O$). The nine different treatments of iron and manganese were duplicated (see Table VII, page 84). Supplementary fluorescent lighting was provided daily for 8 hours (see page 60).

In order to estimate the quantities of sap soluble and insoluble iron and manganese (see page 67), a series of analyses were carried out on the leaves of the plants used in this experiment. The small amounts of dried leaf tissues, provided by the plants individually, made it expedient to combine the material from duplicate cultures in order to have samples sufficiently large to analyze by spectrophotometric methods.

Data. The data obtained in experiment 2 are presented in Tables VII, VIII, and IX (pages 84, 85 and 86). The results may be described as follows:

(1) In all instances the plants used in this experiment were free from iron and manganese deficiency symptoms when the total amounts of iron and manganese in the oven dried leaves were 0.2836 or more and 0.2235 or more mg. per gm. respectively. When these values fell to 0.1 mg. of iron and 0.117 mg. of manganese per gm., or lower, in all instances there occurred symptoms of iron or manganese deficiency. This suggests that Phaseolus has specific requirements with regard to minimum amounts of total iron and manganese in the leaves if healthy plants free from the symptoms of iron and manganese deficiencies are to be produced.

(2) Minimum plant requirements for soluble iron and manganese as determined by extraction from

the leaves with acetone, also, are suggested.

(3) In contrast with the finding of Somers and Shive (1942), Table IX shows that the ratios in the leaves of soluble iron to soluble manganese did not correspond to the iron to manganese concentration ratios in the culture solutions.

(4) Table VII shows that healthy plants occurred only when the iron to manganese concentration ratio in the culture solution lay within the range 1.5 to 2.0 inclusive. Deficiency symptoms of iron or manganese, respectively, occurred when this ratio was 0.5 or lower, or 4.0 or higher.

(5) Within the range of iron to manganese concentration ratios which appeared effective for plant health, there were no marked differences between the average dry weights of the plants. However, these values declined sharply with successive departures from the effective ratio range. It thus seemed probable that a definite relationship existed between the average dry weights of the experimental plants and the iron to manganese concentration ratios in the culture solutions. This relationship is illustrated graphically in Figure 1 page 120.

(6) The analytic data of Table VIII indicate that the total quantity of one of the metals (iron or manganese) in the leaves was depressed when its concentration in the culture solution remained constant while that of the other was increased. This is graphically represented in Figures 3 and 4, pages 122 and 123.

(7) It will be observed that the data relating to cultures 7, 8, 9 and 10 show that when the concentrations of iron and manganese in the culture solution were doubled while the ratio between them was maintained at 2.0, the total quantities of these metals in the leaves of the plants were increased by amounts which were less, proportionately, than their increases in concentration in the culture solutions.

## TABLE VII

### Experiment 2

The effects of growing <u>Phaseolus vulgaris</u> var. humilis in culture solutions containing different concentrations of iron and manganese and different iron to manganese concentration ratios

| Culture No. | P.p.m. in solution Fe | P.p.m. in solution Mn | Ratio Fe : Mn in solution | Symptoms* | Time, days, required for symptoms to appear |
|---|---|---|---|---|---|
| 1 | 0.0 | 5.00 | ——— | -Fe | 4 |
| 2 | 0.0 | 5.00 | ——— | -Fe | 4 |
| 3 | 5.0 | 10.00 | 0.5 | -Fe | 10 |
| 4 | 5.0 | 10.00 | 0.5 | -Fe | 7 |
| 5 | 6.0 | 4.00 | 1.5 | N | |
| 6 | 6.0 | 4.00 | 1.5 | N | |
| 7 | 10.0 | 5.00 | 2.0 | N | |
| 8 | 10.0 | 5.00 | 2.0 | N | |
| 9 | 20.0 | 10.00 | 2.0 | N | |
| 10 | 20.0 | 10.00 | 2.0 | N | |
| 11 | 10.0 | 2.50 | 4.0 | -Mn | 17 |
| 12 | 10.0 | 2.50 | 4.0 | -Mn | 17 |
| 13 | 12.0 | 2.00 | 6.0 | -Mn | 11 |
| 14 | 12.0 | 2.00 | 6.0 | -Mn | 14 |
| 15 | 10.0 | 1.25 | 8.0 | -Mn | 10 |
| 16 | 10.0 | 1.25 | 8.0 | -Mn | 9 |
| 17 | 10.0 | 0.50 | 20.0 | -Mn | 9 |
| 18 | 10.0 | 0.50 | 20.0 | -Mn | 7 |

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*Symptoms indicated as N, normal; -Mn, manganese deficiency; -Fe, iron deficiency.

## TABLE VIII

### Experiment 2

Analyses of Phaseolus vulgaris var. humilis plants grown in the culture experiments described in Table VII

Data are given for the analyses made upon two aliquots of the combined material of duplicate cultures as indicated in column 1. Below each pair of analyses for each sample is given their average*. Accuracy of analytical data: No error greater than ±5 per cent.

| Culture numbers | Average plant dry wt., gm. | Fe in dry leaf tissue, mg. / gm. | | | Mn in dry leaf tissue, mg. / gm. | | |
|---|---|---|---|---|---|---|---|
| | | Soluble | Insol. | Total | Soluble | Insol. | Total |
| 1, 2 | 0.67 | 0.0150 | 0.0000 | | 0.0715 | 0.2610 | |
| | | 0.0150 | 0.0000 | | 0.0710 | 0.2610 | |
| | | 0.0150* | 0.0000* | 0.0150 | 0.0713* | 0.2610* | 0.3323 |
| 3, 4 | 0.78 | 0.0650 | 0.0350 | | 0.1010 | 0.2380 | |
| | | 0.0600 | 0.0400 | | 0.1010 | 0.2390 | |
| | | 0.0625* | 0.0375* | 0.1000 | 0.1010* | 0.2385* | 0.3395 |
| 5, 6 | 2.17 | 0.0650 | 0.2530 | | 0.0355 | 0.2200 | |
| | | 0.0700 | 0.2530 | | 0.0390 | 0.2210 | |
| | | 0.0680* | 0.2530* | 0.3210 | 0.0393* | 0.2205* | 0.2598 |
| 7, 8 | 2.05 | 0.0665 | 0.2155 | | 0.0410 | 0.1830 | |
| | | 0.0700 | 0.2150 | | 0.0420 | 0.1810 | |
| | | 0.0683* | 0.2153* | 0.2836 | 0.0415* | 0.1820* | 0.2235 |
| 9, 10 | 2.61 | 0.1115 | 0.2780 | | 0.1190 | 0.1990 | |
| | | 0.1110 | 0.2785 | | 0.1295 | 0.1985 | |
| | | 0.1113* | 0.2783* | 0.3896 | 0.1198* | 0.1988* | 0.3185 |
| 11, 12 | 1.13 | 0.1130 | 0.1745 | | 0.0290 | 0.0870 | |
| | | 0.1135 | 0.1730 | | 0.0300 | 0.0880 | |
| | | 0.1133* | 0.1738* | 0.2871 | 0.0295* | 0.0875* | 0.1170 |
| 13, 14 | 0.99 | 0.1960 | 0.1965 | | 0.0205 | 0.0930 | |
| | | 0.1960 | 0.1975 | | 0.0195 | 0.0920 | |
| | | 0.1960* | 0.1970* | 0.3931 | 0.0200* | 0.0925* | 0.1125 |
| 15, 16 | 0.86 | 0.1020 | 0.2940 | | 0.0190 | 0.0840 | |
| | | 0.1020 | 0.2945 | | 0.0180 | 0.0835 | |
| | | 0.1020* | 0.2943* | 0.3963 | 0.0185* | 0.0838* | 0.1033 |
| 17, 18 | 0.79 | 0.2095 | 0.4130 | | 0.0180 | 0.0105 | |
| | | 0.2090 | 0.4135 | | 0.0180 | 0.0100 | |
| | | 0.2093* | 0.4133* | 0.6226 | 0.0180* | 0.0103* | 0.0283 |

## TABLE IX

### Experiment 2

### Iron to manganese concentration ratios

| Combined cultures, numbers | Ratio Fe : Mn in culture solution | Ratio soluble Fe : soluble Mn in leaves | Ratio total Fe : total Mn in dry leaf tissue |
|---|---|---|---|
| 1 and 2 | ___ | 0.21 | 0.05 |
| 3 and 4 | 0.5 | 0.61 | 0.29 |
| 5 and 6 | 1.5 | 1.73 | 1.23 |
| 7 and 8 | 2.0 | 1.64 | 1.27 |
| 9 and 10 | 2.0 | 0.93 | 1.22 |
| 11 and 12 | 4.0 | 3.84 | 2.45 |
| 13 and 14 | 6.0 | 9.80 | 3.49 |
| 15 and 16 | 8.0 | 5.51 | 3.87 |
| 17 and 18 | 20.0 | 11.62 | 22.00 |

### Experiment 3

Procedure. The general procedure has already been described (see page 79). This experiment was conducted, from August 15, 1950 to September 15, 1950, with 30 cultures, each of which contained 5 plants of Phaseolus vulgaris var. humilis. The 15 different treatments of iron and manganese were duplicated (see Table X page 91) and were supplied in the culture solution in a wider concentration range than in experiment 2, and in the form of humate iron and humate manganese (see page 47), because ferrous sulphate in a concentration of 100 p.p.m. in the culture solution precipitated within 24 hours in the culture vessels and upon the roots of the plants.

The leaves of the plants in this experiment were analyzed for sap soluble and insoluble iron and manganese (see page 67). The roots and stems from each culture were analyzed as a combined fraction (see page 70) for total iron and manganese.

Data. The data from experiment 3 are shown in Tables X and XI (pages 91 and 92). The following results were obtained.

(1) In all instances the plants used in this experiment were free from iron and manganese deficiency symptoms when the total amounts of iron and manganese in the oven dried leaves were 0.2520 or more and 0.1538 or more mg. per gm. respectively. When these values fell to 0.2310 mg. of iron and 0.1310 mg. of manganese per gm., or lower, in all instances there occurred symptoms of iron or manganese deficiencies. A similar relationship did not hold for the total iron content of the combined tissues of the roots and stems; but the plants, in all instances, exhibited manganese deficiency symptoms when the total manganese content in combined root and stem did not exceed 0.1770 mg. per gm. of dry material.

(2) No particular conclusions appeared as a result of a study of the quantities of soluble iron and manganese, extracted from the leaves by acetone in this experiment.

(3) Healthy plants occurred in cultures with wide variations in both the iron and manganese concentrations of the culture solutions, but none occurred when the ratios of their concentrations in the culture solutions lay outside the range 1.5 to

3.0 inclusive. Ratios above, below, and within this range, in a number of cultures in which the culture solutions did not contain more than 0.05 p.p.m. of iron and 0.10 p.p.m. of manganese, resulted in the simultaneous occurrence of both iron and manganese deficiencies in the same individual plants.

(4) The average dry weights of the plants in the various cultures exhibiting health and normal growth did not differ markedly. In general the dry weights were lowest in those cultures exhibiting the symptoms of iron and manganese deficiencies simultaneously, and in those in which the iron to manganese concentration ratio in the culture solution departed widely from the ratio range within which healthy plants occurred. However, the relation between the average dry weights of the plants and iron to manganese concentration ratios in the culture solutions was not so consistent as in experiment 2, and failed completely at extremely low levels of iron and manganese in the substrate. The data indicate that, in this experiment, the absolute concentrations of these metals in the culture media had greater significance than their concentration ratios.

(5) Certain results arriving out of

experiment 2 are corroborated by a study of the analytic data of Table XI, which clearly indicate that the proportionate relationship between the concentrations of iron and manganese in the culture solution consistently influenced the quantitative accumulation of these metals by Phaseolus. In the leaves and in the roots and stem; i.e. in the plant as a whole, the total quantity of one of the metals (iron or manganese) was depressed when its concentration in the culture solution remained constant while that of the other was increased.

(6) The data also show in harmony with those of Table VIII of experiment 2, that there were increased amounts of iron and manganese in the leaves, and in the plant as a whole, when their concentrations in the culture solution were increased while the ratio between their concentrations remained constant. However, the increased accumulations of iron and manganese in the plants were less, proportionately, than the increases in the quantities of these metals in the substrate.

## TABLE X

### Experiment 3

The effects of growing <u>Phaseolus vulgaris</u> var. humilis in culture solutions containing different concentrations of iron and manganese and different iron to manganese concentration ratios

| Culture No. | P.p.m. in solution | | Ratio Fe : Mn in solution | Symptons* | Time, days, required for symptons to appear |
|---|---|---|---|---|---|
| | Fe | Mn | | | |
| 1 | 0.000 | 0.000 | — | -Fe and -Mn | 5 |
| 2 | 0.000 | 0.000 | — | -Fe and -Mn | Fe5, Mn8 |
| 3 | 1.000 | 10.000 | 0.1 | -Fe | 4 |
| 4 | 1.000 | 10.000 | 0.1 | -Fe | 4 |
| 5 | 10.000 | 100.000 | 0.1 | -Fe | 3 |
| 6 | 10.000 | 100.000 | 0.1 | -Fe | 5 |
| 7 | 0.050 | 0.100 | 0.5 | -Fe and -Mn | 6 |
| 8 | 0.050 | 0.100 | 0.5 | -Fe and -Mn | 7 |
| 9 | 50.000 | 100.000 | 0.5 | -Fe | 6 |
| 10 | 50.000 | 100.000 | 0.5 | -Fe | 8 |
| 11 | 10.000 | 10.000 | 1.0 | -Fe | 11 |
| 12 | 10.000 | 10.000 | 1.0 | -Fe | 10 |
| 13 | 6.000 | 4.000 | 1.5 | N | |
| 14 | 6.000 | 4.000 | 1.5 | N | |
| 15 | 0.002 | 0.001 | 2.0 | -Fe and -Mn | Fe4, Mn11 |
| 16 | 0.002 | 0.001 | 2.0 | -Fe and -Mn | Fe6, Mn12 |
| 17 | 0.050 | 0.025 | 2.0 | -Fe and -Mn | Fe12, Mn10 |
| 18 | 0.050 | 0.025 | 2.0 | -Fe and -Mn | Fe7, Mn14 |
| 19 | 100.000 | 50.000 | 2.0 | N | |
| 20 | 100.000 | 50.000 | 2.0 | N | |
| 21 | 0.500 | 0.200 | 2.5 | N | |
| 22 | 0.500 | 0.200 | 2.5 | N | |
| 23 | 6.000 | 2.000 | 3.0 | N | |
| 24 | 6.000 | 2.000 | 3.0 | N | |
| 25 | 20.000 | 5.000 | 4.0 | -Mn | 17 |
| 26 | 20.000 | 5.000 | 4.0 | -Mn | 9 |
| 27 | 0.050 | 0.005 | 10.0 | -Fe and -Mn | 12 |
| 28 | 0.050 | 0.005 | 10.0 | -Mn | 10 |
| 29 | 100.000 | 1.000 | 100.0 | -Mn | 6 |
| 30 | 100.000 | 1.000 | 100.0 | -Mn | 5 |

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*Symptons indicated as N, normal; -Mn, manganese deficiency; -Fe, iron deficiency.

## TABLE XI

Experiment 3

Analyses of <u>Phaseolus vulgaris</u> var. humilis plants grown in the culture experiments described in Table X

| Culture No. | Average plant dry wt., gm. | Fe in dry leaf tissue, mg. / gm. | | | Mn in dry leaf tissue, mg. / gm. | | | Fe and Mn in dry roots and stems mg. / gm. | |
|---|---|---|---|---|---|---|---|---|---|
| | | Soluble | Insol. | Total | Soluble | Insol. | Total | Fe | Mn |
| 1 | 0.59 | 0.0270 | 0.0000 | 0.0270 | 0.0180 | 0.0000 | 0.0180 | 0.0000 | 0.0000 |
| 2 | 0.72 | 0.0190 | 0.0000 | 0.0190 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 3 | 0.76 | 0.0400 | 0.0875 | 0.1275 | 0.1830 | 0.2100 | 0.3930 | 0.0923 | 0.3948 |
| 4 | 0.84 | 0.0120 | 0.0790 | 0.0910 | 0.0433 | 0.3120 | 0.3553 | 0.0848 | 0.3600 |
| 5 | 0.92 | 0.0235 | 0.1210 | 0.1445 | 0.1348 | 0.4420 | 0.5768 | 0.1560 | 0.7900 |
| 6 | 0.85 | 0.0460 | 0.1110 | 0.1570 | 0.1200 | 0.5260 | 0.6460 | 0.1438 | 0.8850 |
| 7 | 0.78 | 0.0170 | 0.0000 | 0.0171 | 0.0200 | 0.0000 | 0.0200 | 0.0000 | 0.0000 |
| 8 | 0.94 | 0.0100 | 0.0000 | 0.0100 | 0.0120 | 0.0000 | 0.0120 | 0.0000 | 0.0140 |
| 9 | 1.45 | 0.0830 | 0.1020 | 0.1850 | 0.1910 | 0.2918 | 0.4828 | 0.2913 | 0.5228 |
| 10 | 1.64 | 0.1310 | 0.1000 | 0.2310 | 0.1080 | 0.3208 | 0.4288 | 0.2600 | 0.5980 |
| 11 | 1.49 | 0.0710 | 0.1300 | 0.2010 | 0.0280 | 0.2103 | 0.2383 | 0.3108 | 0.3110 |
| 12 | 1.75 | 0.0520 | 0.1510 | 0.2030 | 0.0430 | 0.2020 | 0.2450 | 0.2660 | 0.2970 |
| 13 | 2.20 | 0.0260 | 0.2670 | 0.2930 | 0.0410 | 0.2220 | 0.2630 | 0.3348 | 0.2910 |
| 14 | 2.85 | 0.0243 | 0.3003 | 0.3246 | 0.0360 | 0.2013 | 0.2373 | 0.3350 | 0.3018 |
| 15 | 0.48 | 0.0395 | 0.0000 | 0.0395 | 0.0000 | 0.0000 | 0.0000 | 0.0168 | 0.0000 |
| 16 | 0.65 | 0.0488 | 0.0000 | 0.0488 | 0.0000 | 0.0000 | 0.0000 | 0.0198 | 0.0000 |
| 17 | 0.61 | 0.0220 | 0.1030 | 0.1250 | 0.0000 | 0.0000 | 0.0000 | 0.0360 | 0.0000 |
| 18 | 0.67 | 0.0310 | 0.1210 | 0.1520 | 0.0000 | 0.0000 | 0.0000 | 0.0208 | 0.0000 |
| 19 | 3.12 | 0.1490 | 0.4560 | 0.6050 | 0.0178 | 0.3020 | 0.3198 | 0.7030 | 0.4045 |
| 20 | 3.40 | 0.0978 | 0.3980 | 0.4958 | 0.0200 | 0.2573 | 0.2773 | 0.5090 | 0.3323 |
| 21 | 1.98 | 0.0610 | 0.1910 | 0.2520 | 0.0190 | 0.1455 | 0.1645 | 0.2930 | 0.2110 |
| 22 | 2.39 | 0.0800 | 0.1888 | 0.2688 | 0.0200 | 0.1338 | 0.1538 | 0.2878 | 0.2580 |
| 23 | 1.86 | 0.1560 | 0.2063 | 0.3623 | 0.0255 | 0.1633 | 0.1888 | 0.3818 | 0.2360 |
| 24 | 2.32 | 0.1520 | 0.1850 | 0.3370 | 0.0148 | 0.1905 | 0.2053 | 0.3983 | 0.2133 |
| 25 | 1.05 | 0.2140 | 0.1855 | 0.3995 | 0.0110 | 0.1200 | 0.1310 | 0.4440 | 0.1770 |
| 26 | 1.50 | 0.1513 | 0.2635 | 0.4148 | 0.0470 | 0.0788 | 0.1258 | 0.4625 | 0.1610 |
| 27 | 0.98 | 0.0120 | 0.1670 | 0.1790 | 0.0000 | 0.0000 | 0.0000 | 0.2100 | 0.0000 |
| 28 | 1.16 | 0.0223 | 0.2390 | 0.2613 | 0.0000 | 0.0000 | 0.0000 | 0.2415 | 0.0000 |
| 29 | 0.93 | 0.2210 | 0.5133 | 0.7343 | 0.0190 | 0.0000 | 0.0190 | 0.9100 | 0.0188 |
| 30 | 1.28 | 0.2308 | 0.6270 | 0.8578 | 0.0203 | 0.0245 | 0.0448 | 0.8800 | 0.0120 |

Accuracy of analytical data: No error greater than ±5 per cent.

# EXPERIMENT 4

## The effects of growing *Lycopersicum esculentum* (tomato) in culture solutions containing different concentrations of iron and manganese and different iron to manganese concentration ratios

*Purpose and procedure.* In order to determine how far the findings of Somers and Shive (1942) for soy bean (see page 27) held in the case of *Lycopersicum esculentum*, 18 single plant cultures of this species were grown from June 1, 1951, to July 31, 1951 according to the general procedure described for experiments 2 and 3 (see page 79). The nine different treatments of iron and manganese (see Table XII, page 96) were supplied in the culture solution in the form of the salts ferrous sulphate ($FeSO_4.7H_2O$) and manganese sulphate ($MnSO_4.4H_2O$). These cultures comprised experiment 4.

The leaves of the plants used in this experiment were analyzed, and the amounts of sap soluble and insoluble iron and manganese were estimated (see page 67).

Data. The data resulting from experiment 4 are given in Tables XII and XIII (pages 96 and 97). Although the quantitative data are not identical, they furnish, from a different species, evidence in support of the first six observations derived from the results of experiment 2. The data indicate that healthy plants of *Lycopersicum esculentum* were produced when the iron to manganese concentration ratio in the culture solution lay within the range 0.5 to 5.0 inclusive; and that this species, for normal growth, free from the symptoms of manganese deficiency, required a smaller minimum of total manganese in the leaves than did *Phaseolus vulgaris*. Iron to manganese concentration ratios in the culture solution below the range apparently effective for health resulted in iron deficiency symptoms, whereas ratios above this range resulted in manganese deficiency symptoms. Figures 1 and 2 (pages 120 and 121) show graphically that, within the limits of the range of iron and manganese concentrations used in the culture solutions, the iron to manganese ratios were more important than the absolute concentrations of these metals in their effects upon the average dry weights of the plants. Figures 5 and 6 (pages 124 and 125) show graphically that the proportionate relationship between the concentrations of iron and manganese in the culture solutions consistently influenced their quantitative accumulation in the leaves of the experimental plants.

## TABLE XII

### Experiment 4

The effects of growing <u>Lycopersicum esculentum</u> in culture solutions containing different concentrations of iron and manganese and different iron to manganese concentration ratios

| Culture No. | P.p.m. in solution | | Ratio Fe : Mn in solution | Symptoms* | Time, days, required for symptoms to appear |
|---|---|---|---|---|---|
| | Fe | Mn | | | |
| 1 | 0.0 | 5.0 | ___ | -Fe | 4 |
| 2 | 0.0 | 5.0 | ___ | -Fe | 2 |
| 3 | 5.0 | 50.0 | 0.1 | -Fe | 3 |
| 4 | 5.0 | 50.0 | 0.1 | -Fe | 4 |
| 5 | 5.0 | 25.0 | 0.2 | -Fe | 5 |
| 6 | 5.0 | 25.0 | 0.2 | -Fe | 3 |
| 7 | 5.0 | 10.0 | 0.5 | N | |
| 8 | 5.0 | 10.0 | 0.5 | N | |
| 9 | 5.0 | 5.0 | 1.0 | N | |
| 10 | 5.0 | 5.0 | 1.0 | N | |
| 11 | 10.0 | 5.0 | 2.0 | N | |
| 12 | 10.0 | 5.0 | 2.0 | N | |
| 13 | 25.0 | 5.0 | 5.0 | N | |
| 14 | 25.0 | 5.0 | 5.0 | N | |
| 15 | 50.0 | 5.0 | 10.0 | -Mn | 29 |
| 16 | 50.0 | 5.0 | 10.0 | -Mn | 18 |
| 17 | 5.0 | 0.0 | ___ | -Mn | 12 |
| 18 | 5.0 | 0.0 | ___ | -Mn | 14 |

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*Symptoms indicated as N, normal; -Mn, manganese deficiency; -Fe, iron deficiency.

## TABLE XIII

### Experiment 4

Analyses of Lycopersicum esculentum plants grown in the culture experiments described in Table XII

| Culture No. | Average plant dry wt., gm. | Fe in dry leaf tissue, mg. / gm. | | | Mn in dry leaf tissue, mg. / gm. | | |
|---|---|---|---|---|---|---|---|
| | | Soluble | Insol. | Total | Soluble | Insol. | Total |
| 1 | 1.30 | 0.0000 | 0.0000 | 0.0000 | 0.1780 | 0.1720 | 0.3500 |
| 2 | 1.45 | 0.0000 | 0.0000 | 0.0000 | 0.2310 | 0.3140 | 0.5450 |
| 3 | 1.72 | 0.0153 | 0.0645 | 0.0798 | 0.2510 | 0.6640 | 0.9150 |
| 4 | 1.65 | 0.0218 | 0.0803 | 0.1048 | 0.1965 | 0.6885 | 0.8850 |
| 5 | 1.88 | 0.0435 | 0.1110 | 0.1545 | 0.1705 | 0.5945 | 0.7650 |
| 6 | 1.94 | 0.0190 | 0.0990 | 0.1180 | 0.1808 | 0.6290 | 0.8098 |
| 7 | 4.10 | 0.1190 | 0.3400 | 0.4590 | 0.1710 | 0.5640 | 0.7350 |
| 8 | 3.92 | 0.0650 | 0.2710 | 0.3360 | 0.1570 | 0.3800 | 0.5370 |
| 9 | 4.58 | 0.1093 | 0.3540 | 0.4633 | 0.1648 | 0.3288 | 0.4936 |
| 10 | 4.89 | 0.1105 | 0.3745 | 0.4850 | 0.1340 | 0.3738 | 0.5078 |
| 11 | 4.70 | 0.1508 | 0.3620 | 0.5128 | 0.0805 | 0.2915 | 0.3720 |
| 12 | 5.16 | 0.1465 | 0.4885 | 0.6350 | 0.1152 | 0.2865 | 0.4020 |
| 13 | 5.24 | 0.1530 | 0.5170 | 0.6700 | 0.0320 | 0.0988 | 0.1308 |
| 14 | 5.78 | 0.1630 | 0.5620 | 0.7250 | 0.0325 | 0.0948 | 0.1273 |
| 15 | 3.72 | 0.1655 | 0.6245 | 0.7900 | 0.0200 | 0.0643 | 0.0843 |
| 16 | 3.58 | 0.1910 | 0.6090 | 0.8000 | 0.0210 | 0.0795 | 0.0995 |
| 17 | 3.05 | 0.0735 | 0.2365 | 0.3100 | 0.0000 | 0.0000 | 0.0000 |
| 18 | 2.75 | 0.0798 | 0.2910 | 0.3708 | 0.0000 | 0.0000 | 0.0000 |

Accuracy of analytical data: No error greater than ±5 per cent.

# EXPERIMENTS 5 AND 6

## The effects of growing Phaseolus vulgaris var. humilis (dwarf bean) in culture solutions containing different concentrations of iron, manganese, and calcium, and different iron to manganese concentration ratios

Purpose. These experiments were undertaken in order to determine the effects of growing *Phaseolus vulgaris* var. humilis in culture solutions which, in one series of cultures, contained calcium in a relatively low concentration, and, in another series, in a relatively high concentration.

### Experiment 5

Procedure. Twenty single plant cultures of *Phaseolus vulgaris* var. humilis were grown from May 25, 1951 to June 24, 1951 according to the general procedure described for experiments 2 and 3 (page 79) with the exception that culture solutions B and C (see page 53), which differed only in calcium content, were used instead of culture solution A.

Ten plants which comprised a low calcium series were grown in culture solution B, containing a calcium concentration of 42 p.p.m., and ten plants which comprised a high calcium series were grown in culture solution C, containing a calcium concentration of 143 p.p.m. Each series of cultures was supplied, in duplicate, with five different concentration ratios of iron to manganese in the form of the ferrous ($FeSO_4.7H_2O$) and manganous ($MnSO_4.4H_2O$) salts in the culture solution.

A series of analyses were carried out on the leaves of the experimental plants, and the quantities of sap soluble and insoluble iron and manganese were estimated.

Data. The data from experiment 5, in Tables XIV and XV (pages 102 and 103) show that the total quantities of iron and manganese were depressed in the leaves of Phaseolus vulgaris var. humilis when the concentrations of these metals remained constant, while the concentration of calcium was increased from 42 to 143 p.p.m. in the culture solution. This result is demonstrated clearly in Figures 7 and 8 (pages 126 and 127). It will be noted that the effect of calcium level on iron content was very definitely greater than the effect on manganese content.

With 42 p.p.m. of calcium in the culture solution no manganese deficiency symptoms appeared in the plants, and healthy plants occurred within a range of iron to manganese concentration ratios of 0.5 to 5.0 inclusive. With 143 p.p.m. of calcium in the culture solution, an iron to manganese ratio of 2.0 resulted in healthy plants. Ratios lower than the one apparently effective for health resulted in iron deficiency symptoms in the plants, whereas those higher resulted in manganese deficiency symptoms. The results, therefore, were similar to those of the previous experiments.

The data suggest that an increase in the calcium content of the culture solution, by depressing the total amounts of iron and manganese in the leaves, below the minimum amounts necessary for plant health, narrowed the range of iron to manganese concentration ratios within which plant health occurred. The effect was greater with regard to iron than with manganese.

It will be observed that healthy plants occurring in high calcium cultures had somewhat higher average dry weights than healthy plants occurring in low calcium cultures.

The amounts of soluble iron and manganese in the leaves of the experimental plants appeared to reveal no significant information. With this exception, experiment 5 may be said to have furnished evidence in support of the first six observations derived from the results of experiment 2.

## TABLE XIV

### Experiment 5

The effects of growing Phaseolus vulgaris var. humilis in culture solutions containing different concentrations of iron, manganese and calcium, and different iron to manganese concentration ratios

| Culture No. | P.p.m. in solution Fe | P.p.m. in solution Mn | Ratio Fe : Mn in solution | Symptoms[x] | Time, days, required for symptoms to appear |
|---|---|---|---|---|---|
| Ca 42 p.p.m. | | | | | |
| 1 | 5 | 25 | 0.2 | -Fe | 14 |
| 2 | 5 | 25 | 0.2 | -Fe | 12 |
| 3 | 5 | 10 | 0.5 | -Fe | 19 |
| 4 | 5 | 10 | 0.5 | N | |
| 5 | 5 | 5 | 1.0 | N | |
| 6 | 5 | 5 | 1.0 | N | |
| 7 | 10 | 5 | 2.0 | N | |
| 8 | 10 | 5 | 2.0 | N | |
| 9 | 25 | 5 | 5.0 | N | |
| 10 | 25 | 5 | 5.0 | N | |
| Ca 143 p.p.m. | | | | | |
| 1 | 5 | 25 | 0.2 | -Fe | 5 |
| 2 | 5 | 25 | 0.2 | -Fe | 4 |
| 3 | 5 | 10 | 0.5 | -Fe | 6 |
| 4 | 5 | 10 | 0.5 | -Fe | 6 |
| 5 | 5 | 5 | 1.0 | -Fe | 9 |
| 6 | 5 | 5 | 1.0 | -Fe | 10 |
| 7 | 10 | 5 | 2.0 | N | |
| 8 | 10 | 5 | 2.0 | N | |
| 9 | 25 | 5 | 5.0 | -Mn | 9 |
| 10 | 25 | 5 | 5.0 | -Mn | 7 |

---

[x] Symptoms indicated as N, normal; -Mn, manganese deficiency; -Fe, iron deficiency.

## TABLE XV

### Experiment 5

Analyses of <u>Phaseolus vulgaris</u> var. humilis plants grown in the culture experiments described in Table XIV

| Culture No. | Average plant dry wt., gm. | Fe in dry leaf tissue, mg. / gm. | | | Mn in dry leaf tissue, mg. / gm. | | |
|---|---|---|---|---|---|---|---|
| | | Soluble | Insol. | Total | Soluble | Insol. | Total |
| Ca 42 p.p.m. | | | | | | | |
| 1 | 1.65 | 0.0590 | 0.0778 | 0.1368 | 0.1965 | 0.4015 | 0.5980 |
| 2 | 1.50 | 0.0568 | 0.1065 | 0.1633 | 0.2420 | 0.3760 | 0.6180 |
| 3 | 1.83 | 0.0875 | 0.1350 | 0.2225 | 0.1380 | 0.2653 | 0.3933 |
| 4 | 1.98 | 0.0810 | 0.1555 | 0.2365 | 0.1408 | 0.2050 | 0.3458 |
| 5 | 2.51 | 0.0930 | 0.1690 | 0.2620 | 0.1335 | 0.1375 | 0.2710 |
| 6 | 2.14 | 0.1010 | 0.1490 | 0.2500 | 0.1140 | 0.1430 | 0.2570 |
| 7 | 2.67 | 0.1233 | 0.2155 | 0.3388 | 0.1180 | 0.0835 | 0.2015 |
| 8 | 2.48 | 0.1570 | 0.1940 | 0.3510 | 0.0755 | 0.1495 | 0.2250 |
| 9 | 2.25 | 0.2140 | 0.4875 | 0.7015 | 0.0425 | 0.1320 | 0.1745 |
| 10 | 2.18 | 0.1423 | 0.4235 | 0.5658 | 0.0535 | 0.1025 | 0.1560 |
| Ca 143 p.p.m. | | | | | | | |
| 1 | 1.70 | 0.0540 | 0.0530 | 0.1070 | 0.1410 | 0.3190 | 0.4600 |
| 2 | 1.32 | 0.0208 | 0.0720 | 0.0928 | 0.1400 | 0.3180 | 0.4580 |
| 3 | 1.44 | 0.0360 | 0.1120 | 0.1480 | 0.1120 | 0.2085 | 0.3205 |
| 4 | 1.58 | 0.0620 | 0.0910 | 0.1530 | 0.0975 | 0.2438 | 0.3413 |
| 5 | 1.96 | 0.0510 | 0.1375 | 0.1885 | 0.0875 | 0.1445 | 0.2320 |
| 6 | 1.52 | 0.0848 | 0.1155 | 0.1993 | 0.0725 | 0.1628 | 0.2353 |
| 7 | 3.47 | 0.1028 | 0.1870 | 0.2898 | 0.0530 | 0.1335 | 0.1865 |
| 8 | 4.15 | 0.1203 | 0.1588 | 0.2791 | 0.0510 | 0.1405 | 0.1920 |
| 9 | 1.92 | 0.1745 | 0.2785 | 0.4530 | 0.0360 | 0.0843 | 0.1203 |
| 10 | 1.80 | 0.0918 | 0.3570 | 0.4488 | 0.0190 | 0.0965 | 0.1155 |

Accuracy of analytical data: No error greater than ±5 per cent.

## Experiment 6

**Procedure.** Experiment 6, conducted from July 1, 1951 to July 31, 1951, was essentially a repetition of experiment 5 with two differences in procedure; namely, (a) each culture contained three plants; (b) in addition to the analyses carried out on the leaves of the experimental plants in each culture, the stems and roots of the plants were analyzed as a combined fraction for each culture in order to determine their total contents of iron and manganese.

**Data.** The data resulting from experiment 6 are given in Tables XVI and XVII (pages 106 and 107). In this experiment the amounts of soluble iron and manganese in the leaves of the experimental plants appeared to reveal no significant information. In the roots and stems, the total amounts of iron and manganese bore a similar quantitative relationship to their concentrations in the culture solution as did the total amounts of these metals in the leaves, showing that the total quantity of one of the metals (iron or manganese) was depressed in the plant, as a whole, when its concentration in the culture solution remained constant while that of the other was increased. Again there is clear evidence that the depressing effect of calcium is definitely greater in the case of iron absorption than in the case of manganese absorption.

It will be observed that high calcium did not depress leaf manganese in culture 4. However, if the data for cultures with similar amounts of iron and manganese in the culture solution are averaged, it will be found that the results of experiment 5, relating to the calcium effect, are confirmed.

In all other respects the data obtained from experiment 6 paralleled and corroborated the data from experiment 5.

Certain results concerning root and stem analyses are expressed graphically in Figure 9 (page 128).

# TABLE XVI

## Experiment 6

The effects of growing <u>Phaseolus vulgaris</u> var. humilis
in culture solutions containing different
concentrations of iron, manganese and
calcium, and different iron to
manganese concentration ratios

| Culture No. | P.p.m. in solution | | Ratio Fe : Mn in solution | Symptoms* | Time, days, required for symptoms to appear |
|---|---|---|---|---|---|
| | Fe | Mn | | | |
| Ca 42 p.p.m. | | | | | |
| 1 | 5 | 25 | 0.2 | -Fe | 16 |
| 2 | 5 | 25 | 0.2 | -Fe | 14 |
| 3 | 5 | 10 | 0.5 | N | |
| 4 | 5 | 10 | 0.5 | N | |
| 5 | 5 | 5 | 1.0 | N | |
| 6 | 5 | 5 | 1.0 | N | |
| 7 | 10 | 5 | 2.0 | N | |
| 8 | 10 | 5 | 2.0 | N | |
| 9 | 25 | 5 | 5.0 | N | |
| 10 | 25 | 5 | 5.0 | N | |
| Ca 143 p.p.m. | | | | | |
| 1 | 5 | 25 | 0.2 | -Fe | 6 |
| 2 | 5 | 25 | 0.2 | -Fe | 5 |
| 3 | 5 | 10 | 0.5 | -Fe | 7 |
| 4 | 5 | 10 | 0.5 | -Fe | 9 |
| 5 | 5 | 5 | 1.0 | -Fe | 11 |
| 6 | 5 | 5 | 1.0 | -Fe | 9 |
| 7 | 10 | 5 | 2.0 | N | |
| 8 | 10 | 5 | 2.0 | N | |
| 9 | 25 | 5 | 5.0 | -Mn | 10 |
| 10 | 25 | 5 | 5.0 | -Mn | 12 |

* Symptoms indicated as N, normal; -Mn, manganese deficiency; -Fe, iron deficiency.

## TABLE XVII

### Experiment 6

Analyses of <u>Phaseolus vulgaris</u> var. humilis plants grown in the culture experiments described in Table XVI

| Culture No. | Average plant dry wt., gm. | Fe in dry leaf tissue, mg. / gm. | | | Mn in dry leaf tissue, mg. / gm. | | | Fe and Mn in dry roots and stems mg. / gm. | |
|---|---|---|---|---|---|---|---|---|---|
| | | Soluble | Insol. | Total | Soluble | Insol. | Total | Fe | Mn |
| Ca 42 p.p.m. | | | | | | | | | |
| 1 | 1.37 | 0.0460 | 0.1250 | 0.1710 | 0.2160 | 0.5790 | 0.7950 | 0.1080 | 0.8600 |
| 2 | 1.61 | 0.9535 | 0.1055 | 0.1590 | 0.2080 | 0.5270 | 0.7350 | 0.1600 | 0.7650 |
| 3 | 1.95 | 0.0720 | 0.1650 | 0.2370 | 0.1198 | 0.3090 | 0.4288 | 0.2400 | 0.4340 |
| 4 | 1.86 | 0.0760 | 0.1718 | 0.2478 | 0.1750 | 0.2305 | | 0.2485 | 0.4470 |
| 5 | 1.90 | 0.0980 | 0.1550 | 0.2530 | 0.1285 | 0.1515 | 0.2800 | 0.2633 | 0.3115 |
| 6 | 2.30 | 0.0880 | 0.1875 | 0.2775 | 0.0913 | 0.2040 | 0.2953 | 0.3030 | 0.3360 |
| 7 | 2.31 | 0.1400 | 0.2633 | 0.4033 | 0.1100 | 0.1390 | 0.2490 | 0.4065 | 0.2603 |
| 8 | 2.80 | 0.1630 | 0.2345 | 0.3975 | 0.0575 | 0.1740 | 0.2315 | 0.4235 | 0.2470 |
| 9 | 2.45 | 0.1700 | 0.4070 | 0.5770 | 0.0630 | 0.0815 | 0.1445 | 0.6248 | 0. |
| 10 | 2.05 | 0.1830 | 0.4390 | 0.6220 | 0.0690 | 0.1118 | 0.1808 | 0.6318 | 0.1828 |
| Ca 143 p.p.m. | | | | | | | | | |
| 1 | 1.49 | 0.0190 | 0.1018 | 0.1208 | 0.1310 | 0.3600 | 0.4910 | 0.1618 | 0.5805 |
| 2 | 1.28 | 0.0375 | 0.0935 | 0.1310 | 0.1283 | 0.3833 | 0.5118 | 0.1540 | 0.5920 |
| 3 | 1.77 | 0.0410 | 0.1190 | 0.1600 | 0.1180 | 0.2365 | 0.3545 | 0.2098 | 0.4100 |
| 4 | 1.60 | 0.0470 | 0.1233 | 0.1703 | 0.1005 | 0.3170 | 0.4175 | 0.1810 | 0.4608 |
| 5 | 1.69 | 0.0600 | 0.1538 | 0.2138 | 0.0785 | 0.1643 | 0.2428 | 0.2475 | 0.2010 |
| 6 | 1.82 | 0.0570 | 0.1455 | 0.2025 | 0.0848 | 0.1520 | 0.2360 | 0.2630 | 0.2765 |
| 7 | 3.67 | 0.1280 | 0.2010 | 0.3290 | 0.0470 | 0.1430 | 0.1900 | 0.3410 | 0.2293 |
| 8 | 3.31 | 0.0913 | 0.2070 | 0.2983 | 0.0250 | 0.1348 | 0.1598 | 0.3730 | 0.1928 |
| 9 | 2.00 | 0.1905 | 0.2125 | 0.4030 | 0.0290 | 0.0900 | 0.1190 | 0.5170 | 0.1480 |
| 10 | 1.76 | 0.1520 | 0.3230 | 0.4750 | 0.0330 | 0.0918 | 0.1248 | 0.5655 | 0.1560 |

Accuracy of analytical data: No error greater than ±5 per cent.

# EXPERIMENT 7

## The effects of growing Allium cepa (onion) in single salt solutions containing different concentrations of iron or manganese, and in double salt solutions containing different concentrations of iron and manganese

Purpose. During the course of the previous experiments it was observed that increases in the concentrations of iron and manganese in the culture solutions resulted in an increased absorption of these metals by the experimental plants. The increases in the amounts absorbed, however, were definitely less than the increases in the concentrations of these metals in the culture solutions (see page 83). In order, if possible, to arrive at some explanation of this, it was decided to make studies of absorption from single and double salt solutions over brief periods of time. These salts were used because preliminary trials with Phaseolus vulgaris in culture solution A, in mason jars, in a shaker, made it clear that solution analysis could not accurately determine the extremely minute amounts of iron and manganese absorbed from a complete nutrient solution over brief intervals of time. Allium cepa was selected for further studies with single and double salt solutions because of the ease with which a large absorbing area of root system could be obtained.

Procedure. Bulbs of Allium cepa were grown in culture solution A, containing humate iron and manganese in the concentrations 0.5 p.p.m. and 0.25 p.p.m. respectively, for one month, prior to the carrying out of an absorption experiment, in order to promote root development. They were returned to the culture solution for a period between each absorption experiment in order to maintain them in a satisfactory state of health.

Studies were made of the absorption, by Allium cepa, of the divalent ferrous and manganous ions, $Fe^{++}$ and $Mn^{++}$, during 24 hour periods, from single and double salt solutions containing various concentrations of ferrous sulphate ($FeSO_4.7H_2O$) and, or manganese sulphate ($MnSO_4.4H_2O$). The control solutions contained no plants. During the absorption experiments the plants were supported with their roots immersed in 500 ml. of the above mentioned solution in distilled water in Pyrex Berzelius beakers and agitated continuously in a shaker immersed in a water bath thermostatically maintained at 20 °C. In addition, some experiments were carried out with the shaker not in operation. In order not to add new ions, the pH of the solutions was not adjusted. In each series of runs the plants, after having been in a solution of one concentration for 24 hours, were rinsed in distilled water and immediately placed in a solution of a second concentration. Analyses of the solutions were made by spectrophotometric methods after each treatment in order to determine the amounts of iron and, or manganese absorbed by the plants during the experimental period.

Data. The data from this experiment are given in Tables XVIII and XIX, XX and XXI (pages 112, 113, 114 and 115). The results are summarized as follows:

(1) *Allium cepa*, per unit of time, absorbed more iron or manganese from continuously shaken than from unshaken single salt solutions.

(2) The data given in Tables XIX and XX and graphically represented in Figure 10 (page 129)

strongly suggest that Allium cepa, under the conditions of the experiment, absorbed the divalent ferrous ($Fe^{++}$) or manganous ($Mn^{++}$) ion from a single salt solution at a rate which was independent of the concentration of the cation in the culture solution; i.e., the number of ions absorbed per unit of time was the same regardless of their concentration in the culture solution.

(3) It was found that the rate of absorption of one of the metals (iron or manganese) by Allium cepa from double salt solutions was depressed when its concentration in the culture solution remained constant while that of the other was increased. This is graphically represented in Figure 11 (page 130).

## TABLE XVIII

### Experiment 7

The quantities of iron or manganese absorbed in twenty-four hour periods by <u>Allium cepa</u> plants growing in unshaken and in continuously shaken solutions of ferrour sulphate or manganese sulphate at a constant temperature of 20° C.

| Solution | Concn. of Fe or Mn in solution, p.p.m. | Fe or Mn absorbed in 24 hr., mg. | | | | | |
|---|---|---|---|---|---|---|---|
| | | Plant A | | Plant B | | Control | |
| | | Shaken | Unshaken | Shaken | Unshaken | Shaken | Unshaken |
| $FeSO_4.7H_2O$ (absorption of Fe) | 20 | 0.95 | 0.80 | 0.70 | 0.60 | | |
| | 5 | 0.90 | 0.64 | 0.65 | 0.50 | | |
| | 10 | 0.92 | 0.50 | 0.68 | 0.36 | | |
| $MnSO_4.4H_2O$ (absorption of Mn) | 20 | 1.20 | 1.10 | 0.80 | 0.65 | | |
| | 5 | 1.45 | 0.98 | 0.76 | 0.55 | | |
| | 10 | 1.25 | 0.74 | 0.85 | 0.48 | | |

Accuracy of analytical data: No error greater than ±5 per cent.

TABLE XIX

Experiment 7

The quantities of iron or manganese absorbed in twenty-four hour periods by <u>Allium cepa</u> plants growing in continuously shaken solutions of ferrous sulphate or manganese sulphate at a constant temperature of 20° C.

| Solution | Concn. of Fe or Mn in solution, p.p.m. | Fe or Mn absorbed in 24 hr., mg. | | | | | |
|---|---|---|---|---|---|---|---|
| | | Plant 1 | Plant 2 | Plant 3 | Plant 4 | Control | Control |
| $FeSO_4.7H_2O$ (absorption of Fe) | 20.0 | 0.80 | 0.90 | 0.65 | 0.55 | loss, 0.1 | |
| | 5.0 | 0.80 | 1.00 | 0.50 | 0.50 | | |
| | 10.0 | 0.76 | 0.94 | 0.62 | 0.54 | | |
| | 20.0 | 0.82 | 0.96 | 0.58 | 0.56 | | |
| | 5.0 | 0.95 | 0.92 | 0.60 | 0.58 | | |
| | 10.0 | 0.85 | 0.88 | 0.65 | 0.60 | | |
| $MnSO_4.4H_2O$ (absorption of Mn) | 20.0 | 0.94 | 1.00 | 0.72 | 0.62 | | |
| | 5.0 | 0.92 | 0.98 | 0.68 | 0.55 | | |
| | 10.0 | 1.00 | 1.10 | 0.74 | 0.60 | | |
| | 20.0 | 0.96 | 0.96 | 0.76 | 0.58 | | |
| | 5.0 | 0.95 | 1.30 | 0.70 | 0.65 | | |
| | 10.0 | 0.98 | 1.20 | 0.73 | 0.70 | | |

Accuracy of analytical data: No error greater than ±5 per cent.

# TABLE XX

## Experiment 7

The quantities of iron or manganese absorbed in twenty-four hour periods by *Allium cepa* plants (shown in Table XIX) growing in continuously shaken solutions of ferrous sulphate, or manganese sulphate, at a constant temperature of 20° C., following an interval of fourteen days growth in a complete nutrient solution

| Solution | Concn. of Fe or Mn in solution, p.p.m. | Fe or Mn absorbed in 24 hr., mg. | | | | | |
|---|---|---|---|---|---|---|---|
| | | Plant 1 | Plant 2 | Plant 3 | Plant 4 | Control | Control |
| $FeSO_4.7H_2O$ (absorption of Fe) | 20.0 | 1.15 | 1.42 | 0.82 | 0.70 | | |
| | 5.0 | 0.98 | 1.48 | 0.92 | 0.68 | | |
| | 10.0 | 1.25 | 1.28 | 0.82 | 0.66 | | |
| | 20.0 | 0.97 | 1.60 | 0.95 | 0.69 | | |
| | 5.0 | 1.12 | 1.52 | 0.86 | 0.71 | | |
| | 10.0 | 1.34 | 1.50 | 0.90 | 0.72 | | |
| $MnSO_4. 4H_2O$ (absorption of Mn) | 20.0 | 1.25 | 1.52 | 0.90 | 0.74 | | |
| | 5.0 | 1.42 | 1.54 | 0.86 | 0.78 | | |
| | 10.0 | 1.40 | 1.50 | 0.92 | 0.75 | | |
| | 20.0 | 1.35 | 1.45 | 1.00 | 0.72 | | |
| | 5.0 | 1.60 | 1.55 | 0.95 | 0.70 | | |
| | 10.0 | 1.30 | 1.50 | 0.96 | 0.86 | | |

Accuracy of analytical data: No error greater than ±5 per cent.

## TABLE XXI

### Experiment 7

The quantities of iron and manganese absorbed in twenty-four hours by *Allium cepa* plants growing in continuously shaken double salt solutions of ferrous sulphate and manganese sulphate at a constant temperature of 20° C.

| Concentration of Fe and Mn in solution, p.p.m. | | Fe and Mn absorbed in 24 hrs., mg. | | | | | |
|---|---|---|---|---|---|---|---|
| | | Plant A | | Plant B | | Control | |
| Fe | Mn | Fe | Mn | Fe | Mn | Fe | Mn |
| 5 | 25 | 0.15 | 0.67 | 0.10 | 0.53 | | |
| 5 | 10 | 0.20 | 0.55 | 0.23 | 0.46 | | |
| 5 | 5 | 0.31 | 0.43 | 0.28 | 0.33 | | |
| 10 | 5 | 0.44 | 0.35 | 0.40 | 0.30 | | |
| 25 | 5 | 0.60 | 0.18 | 0.48 | 0.17 | | |

Accuracy of analytical data: No error greater than ±5 per cent.

# EXPERIMENT 8

## The effects of growing *Phaseolus vulgaris* var. humilis in culture solutions in which different concentrations of titanium, nickel or cobalt were substituted for iron or manganese

Purpose. Somers and Shive (1942) held that cobalt replaced manganese in the nutrition of soy bean, and Sideris (1930) reported that titanium replaced iron in the nutrition of pineapple. Experiment 8 was undertaken, primarily, to determine how far these findings held in the case of *Phaseolus vulgaris* var. humilis; and to test, with nickel, the oxidation reduction hypothesis of Somers and Shive (see page 28). In connection with this, the position of nickel in the periodic table, next to cobalt, suggested that, on the basis of this theory, it should be possible to substitute it for manganese in its postulated role as an iron oxidizer.

Procedure. A number of ten day old bean seedlings, grown on paraffined filter paper floats and supplied only with distilled water, and a number of twenty day old seedlings, grown in culture solution A containing 0.5 p.p.m. of chelated iron, but no manganese, were transferred to the water-culture vessels, three plants to each vessel. The latter seedlings showed symptoms of manganese deficiency. Each vessel contained 500 ml. of culture solution A together with the treatment salts supplied as sulphates. The pH of the culture solution was adjusted to pH 4.5 at the beginning of each run, the solutions being renewed at intervals of 24 hours. They were aerated for 8 hours daily.

Data. The data relating to this experiment are given in Table XXII (page 119). It was found that none of the substitution metals could be used to replace either iron or manganese in the nutrition of the experimental plants. Titanium and nickel, in the concentrations used, were extremely toxic to *Phaseolus vulgaris*. On the fourth day of treatment with titanium, and on the tenth day with nickel, the leaves of the plants were withered and showed large areas of necrosis, also the stems were completely collapsed. Cobalt did not alleviate the symptoms of manganese deficiency. On the other hand it produced no indications of toxicity.

## TABLE XXII

### Experiment 8

The effects of growing <u>Phaseolus vulgaris</u> var. humilis in culture solutions containing different concentrations of titanium or nickel or cobalt substituted for iron or manganese

| Number of plants | Condition of plants at beginning of expt. | P.p.m. in solution | | | | | Symptoms[x] end of expt. | Duration of expt., days |
|---|---|---|---|---|---|---|---|---|
| | | Fe | Mn | Ti | Ni | Co | | |
| 3 | N | 0.00 | 4.00 | 6.00 | 0.00 | 0.00 | Ti toxicity | 15 |
| 3 | N | 0.00 | 4.00 | 0.00 | 6.00 | 0.00 | Ni toxicity | 15 |
| 3 | N | 0.00 | 4.00 | 0.00 | 0.00 | 6.00 | -Fe | 15 |
| 3 | N | 6.00 | 4.00 | 0.00 | 0.00 | 0.00 | N | 15 |
| 3 | N | 6.00 | 0.00 | 0.00 | 0.00 | 4.00 | -Mn | 15 |
| 3 | N | 6.00 | 0.00 | 0.00 | 4.00 | 0.00 | Ni toxicity | 15 |
| 3 | N | 6.00 | 0.00 | 4.00 | 0.00 | 0.00 | Ti toxicity | 15 |
| 3 | N | 6.00 | 0.00 | 0.00 | 0.00 | 4.00 | -Mn | 30 |
| 3 | N | 0.50 | 0.00 | 0.00 | 0.00 | 0.25 | -Mn | 30 |
| 3 | N | 6.00 | 4.00 | 0.00 | 0.00 | 0.00 | N | 30 |
| 3 | N | 0.50 | 0.25 | 0.00 | 0.00 | 0.00 | N | 30 |
| 3 | -Mn | 6.00 | 0.00 | 0.00 | 0.00 | 4.00 | -Mn | 30 |
| 3 | -Mn | 0.50 | 0.00 | 0.00 | 0.00 | 0.25 | -Mn | 30 |

[x] Symptoms indicated as N, normal; -Mn, manganese deficiency; -Fe, iron deficiency.

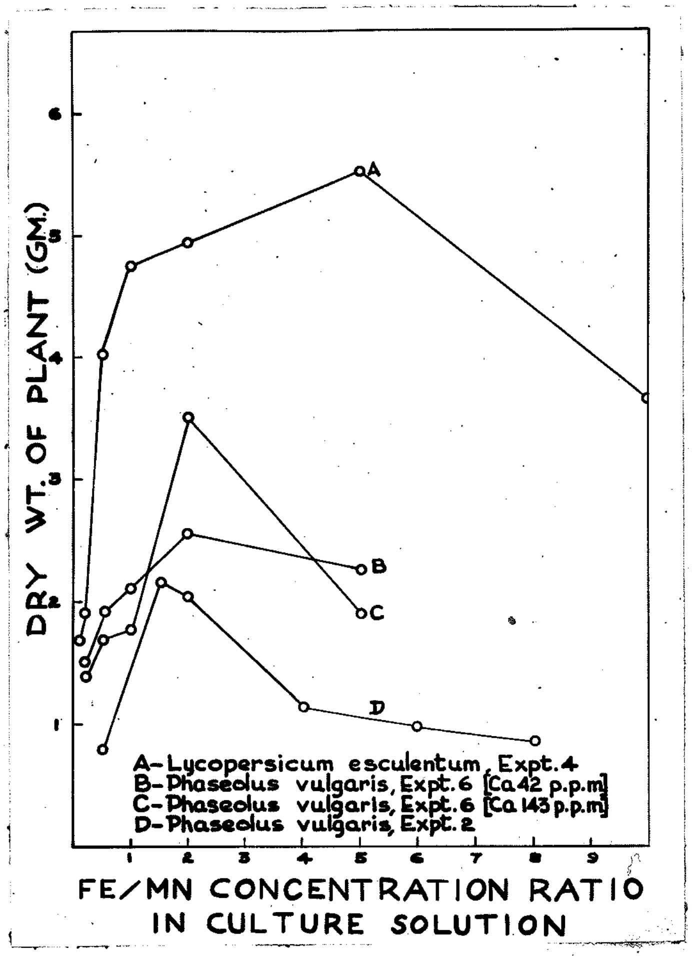

FIGURE 1. The average weights in grams of the experimental plants plotted against the iron to manganese concentration ratios in the culture solutions. The data are averaged for cultures containing similar amounts of iron and manganese in the culture solutions.

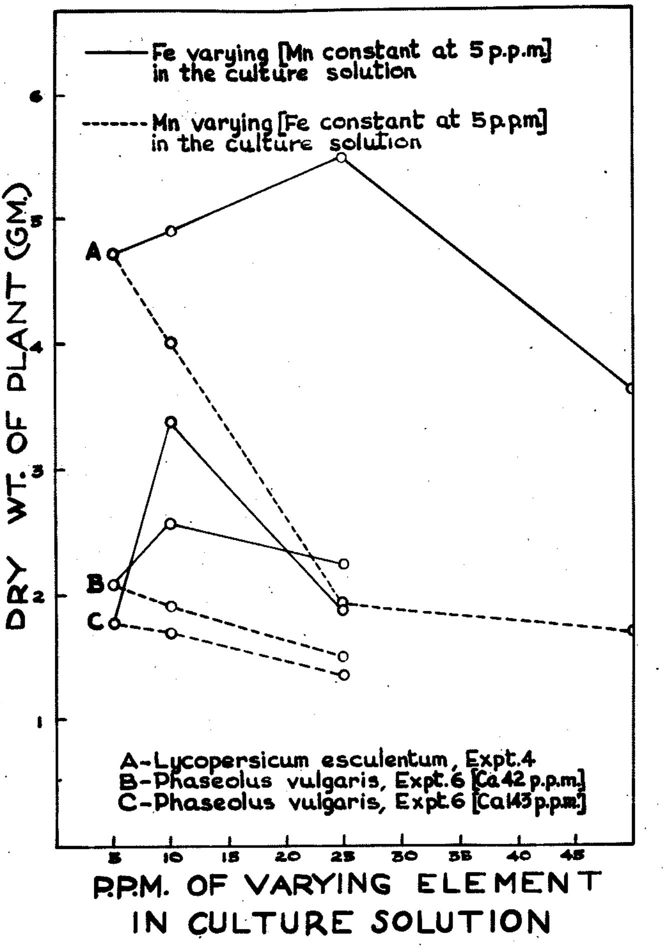

FIGURE 2. The relation of growth responses to the relative proportions and concentrations of iron and manganese in the culture solutions. The data are averaged for cultures containing similar amounts of iron and manganese in the culture solutions.

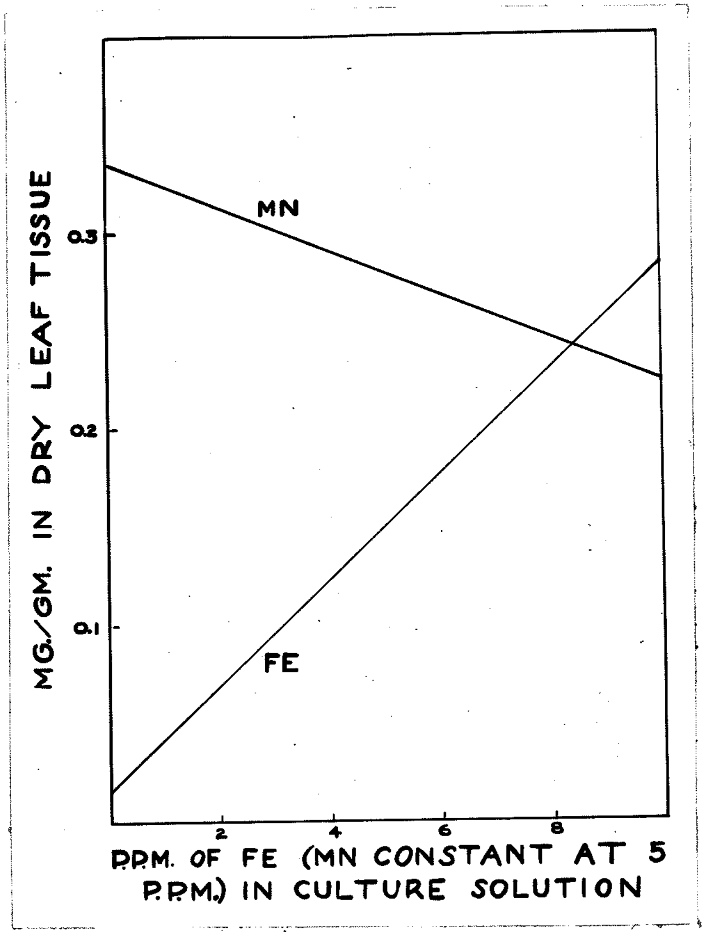

FIGURE 3. A graphical representation of the competition (or antagonism) between iron and manganese in their accumulation in the leaves of Phaseolus vulgaris var. humilis in experiment 2.

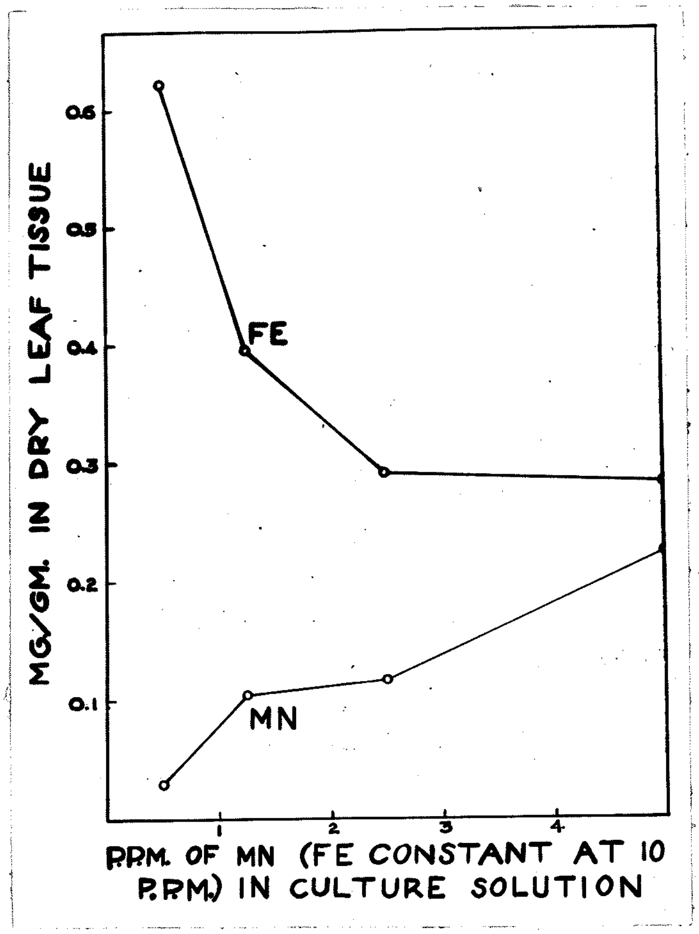

FIGURE 4. A graphical representation of the competition (or antagonism) between iron and manganese in their accumulation in the leaves of <u>Phaseolus vulgaris</u> var. humilis in experiment 2.

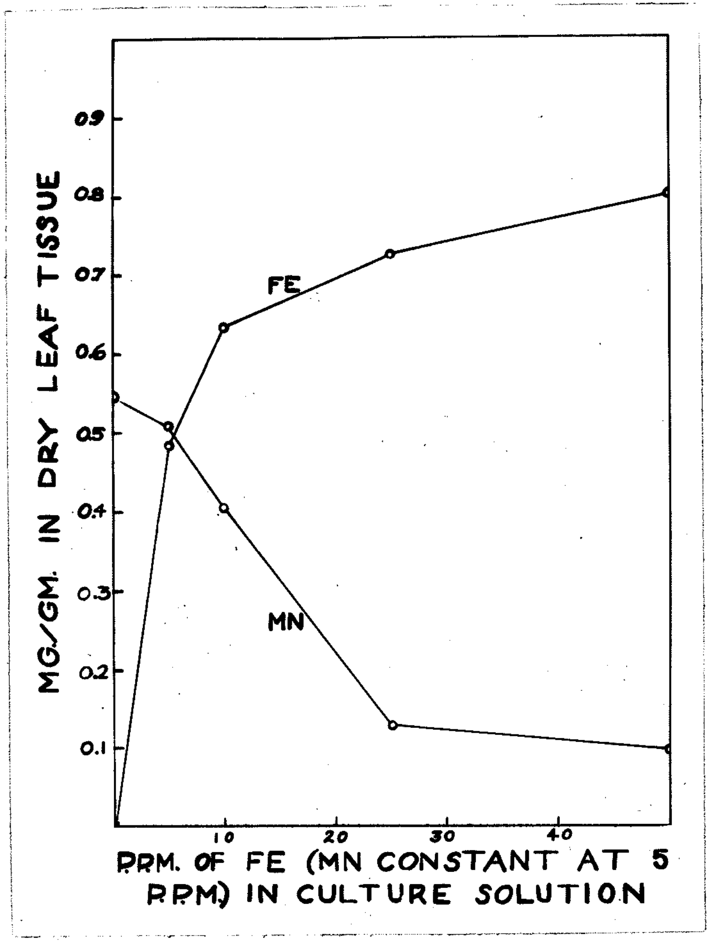

FIGURE 5. A graphical representation of the competition (or antagonism) between iron and manganese in their accumulation in the leaves of <u>Lycopersicum esculentum</u> in experiment 4. The data are for cultures 2, 10, 12, 14 and 16.

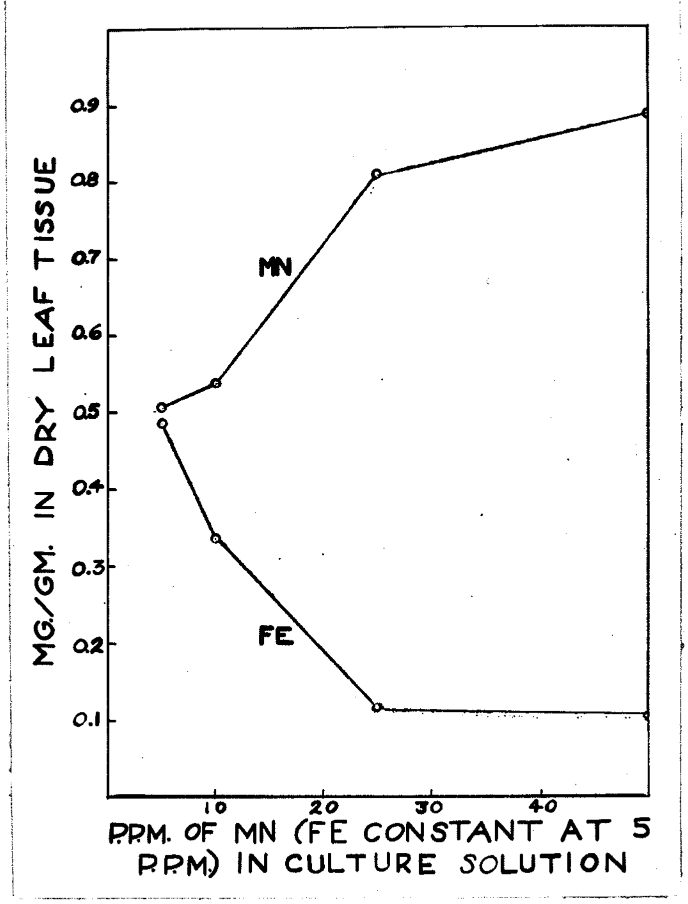

FIGURE 6. A graphical representation of the competition (or antagonism) between iron and manganese in their accumulation in the leaves of Lycopersicum esculentum in experiment 4. The data are for cultures 4, 6, 8 and 10.

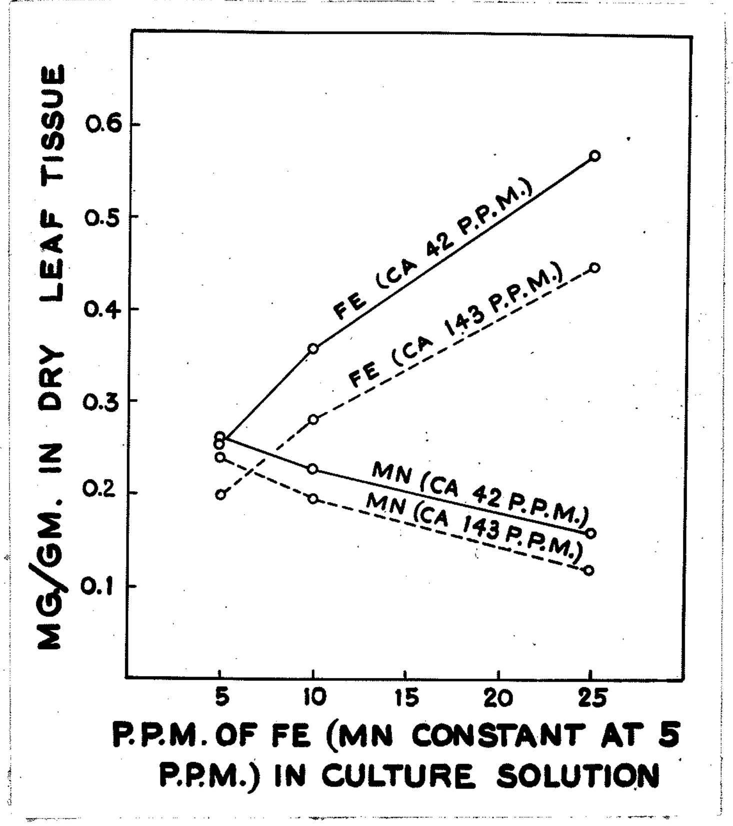

FIGURE 7. A graphical representation of the competition (or antagonism) between iron and manganese in their accumulation in the leaves of Phaseolus vulgaris var. humilis in experiment 5, and of the decreases in these accumulations which resulted from an increase in the calcium content of the culture solution. The data are for cultures 6, 8 and 10.

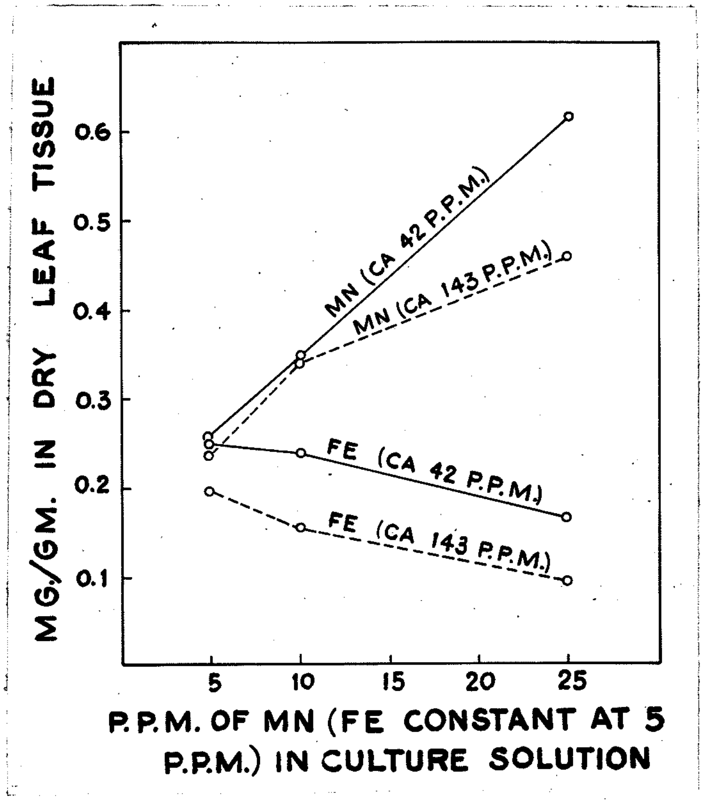

FIGURE 8. A graphical representation of the competition (or antagonism) between iron and manganese in their accumulation in the leaves of Phaseolus vulgaris var. humilis in experiment 5, and of the decreases in these accumulations which resulted from an increase in the calcium content of the culture solution. The data are for cultures 2, 4 and 6.

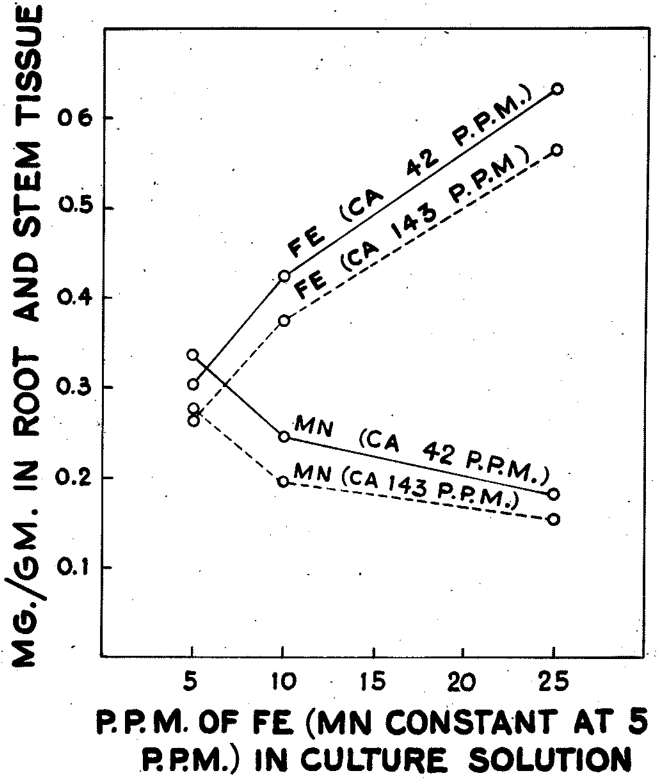

FIGURE 9. A graphical representation of the competition (or antagonism) between iron and manganese in their accumulation in the roots and stems of Phaseolus vulgaris var. humilis in experiment 6, and of the decreases in these accumulations which resulted from an increase in the calcium content of the culture solution. The data are for cultures 6, 8, and 10, and are for the results of analyses upon dry plant material.

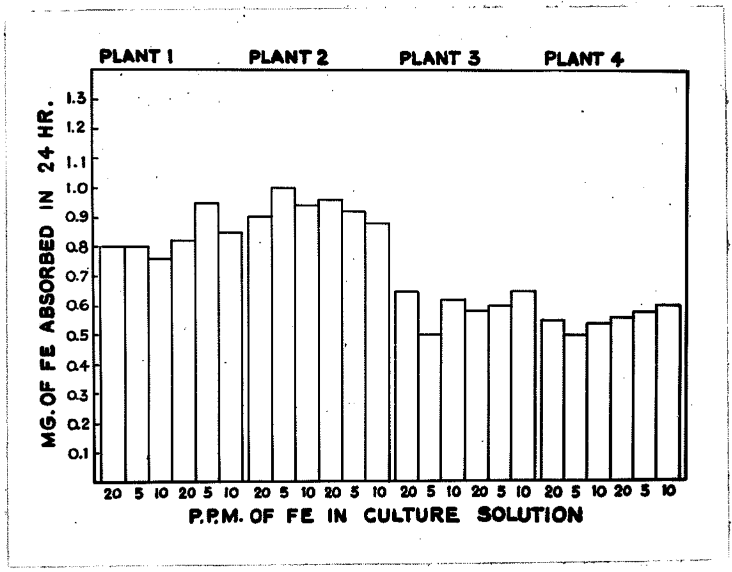

FIGURE 10. The absorption of iron by Allium cepa plants from single salt solutions containing different concentrations of iron.

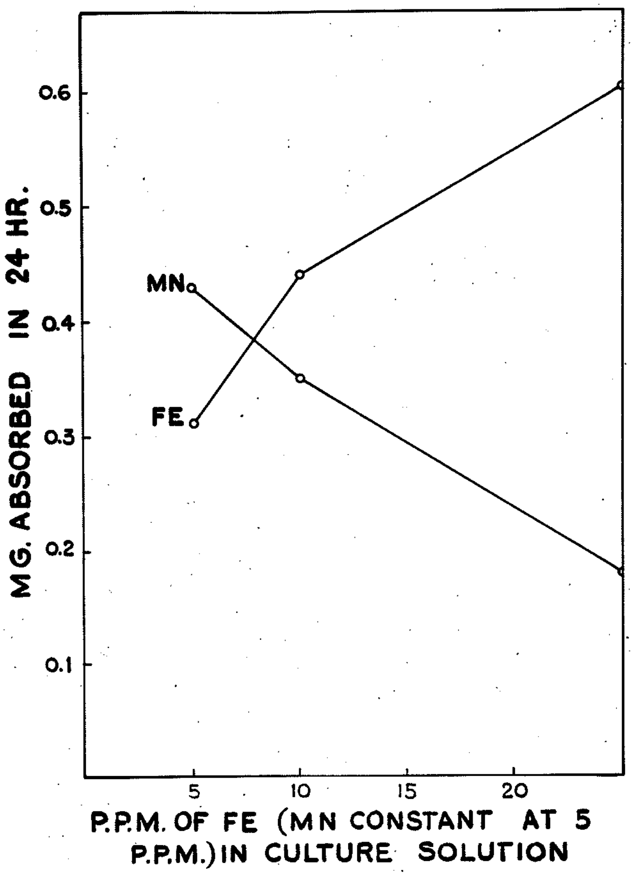

FIGURE 11. A graphical representation of the competition (or antagonism) between iron and manganese in their absorption by <u>Allium cepa</u> in experiment 7 (see data for plant A, Table XXI).

# V. GENERAL DISCUSSIONS AND CONCLUSIONS

Data derived from water-culture experiments 2 to 6 inclusive indicate that healthy plants of *Phaseolus vulgaris* var. humilis and *Lycopersicum esculentum*, free from the symptoms of iron and manganese deficiencies, occurred in cultures with wide variations in both the iron and manganese concentrations in the culture solutions (see Tables VII, X, XII, XIV and XVI, pages 84, 91, 96, 102 and 106); but the data suggest that healthy plants of these species occurred only when the total amount of each of these metals in the leaves of the plants exceeded a certain minimum quantity which may be stated in terms of mg. per gm. of dry material (see Tables VIII, XI, XIII, XV and XVII, pages 85, 92, 97, 103 and 107).

With respect, therefore, to the total quantities of iron and manganese in their leaves, it would appear that these species have minimal requirements which must be satisfied before they will grow normally in solution culture, and remain free from the symptoms of iron and manganese deficiencies. In regard to iron in the leaves of plants, these findings appear to be in agreement with those of Jacobson (1945), for pear, tobacco and corn.

Jacobson stated that chlorophyll formation can occur only when the total iron content of the leaf exceeds a certain minimum level which is determined by the species and the growth conditions.

Plants of Lycopersicum esculentum (see Table XIII, page 97) were free from manganese deficiency symptoms when the total manganese in the leaves was 0.1273 mg. per gm. of dry material, whereas Phaseolus vulgaris (see Table XI, page 92) exhibited the symptoms of manganese deficiency when its leaf content of manganese was 0.1310 mg. per gm. of dry material. Thus, of the two species, Lycopersicum esculentum, for normal growth, appeared to require the smaller minimum amount of manganese in its leaves.

Although the data from experiments 2 and 4 seem to suggest that Phaseolus vulgaris and Lycopersicum esculentum have minimal requirements for soluble iron and manganese in their leaves, as indicated by analyses of acetone extracts (see page 68 and Tables IX and XIII, pages 86 and 97), the data from experiments 3, 5 and 6 (see Tables XI, XV and XVII, pages 92, 103 and 107) do not consistently support this suggestion. In contrast with the findings of Somers and Shive (1942), who carried out analyses of expressed sap, the ratios of the quantities of the soluble iron to the soluble manganese in the leaves of the experimental plants did not correspond to their concentration ratios in the culture solutions (see page 27). It is possible that these results may be in disagreement with those of Somers and Shive because the analyses were carried out on acetone extractions rather than on expressed sap. It has already been pointed out (see page 67) that Sideris and Young (1949) stated that the acetone extraction method was a satisfactory one for the determination of soluble iron and manganese. The writer, however, did not carry out any work to test the validity of this view.

Experiments 3 and 6 (see pages 92 and 107, Tables XI and XVII) did not afford any consistent evidence to indicate that any condition of the experimental plants was related to the total amounts of iron and manganese in the roots and stems. This is probably to be expected in view of the fact that these structures have functions predominately related to conduction rather than to metabolism.

The data derived from a series of analyses carried out on the experimental plants (see Tables VIII, XI, XIII, XV and XVII, pages 85, 92, 97, 103 and 107 and Figures 3 to 9 inclusive, pages 122 to 128 inclusive) and on the culture solutions (see Table XXI, page 115 and Figure II, page 130) suggest that iron and manganese are mutually competitive or antagonistic (see pages 23 and 24) in their absorption from culture solutions by Phaseolus vulgaris, Lycopersicum esculentum and Allium cepa. In the leaves of Lycopersicum esculentum and Phaseolus vulgaris, and in the combined roots and stems of the latter, the total quantity of iron or of manganese was depressed when its concentration in the culture solution remained constant while the concentration of the other metal (iron or manganese) in the culture solution was increased. The total quantity of the latter, in the plant, was increased. The quantity of iron or of manganese absorbed per unit of time by Allium cepa from a double salt solution of ferrous sulphate ($FeSO_4 . 7H_2O$) and manganese sulphate ($MnSO_4 . 4H_2O$) was depressed when its concentration in the solution remained constant while the concentration of the other metal (iron or manganese) in the solution was increased.

The quantity of the latter, absorbed by the plant, was increased.

Similar findings are described by Bennett (1945) for Lycopersicum esculentum, Erkama (1947) for Pisum sativum, and Morris and Pierre (1947) for Lespedeza species. If the data of Somers and Shive (1942) are examined carefully, it will be seen that their findings, as regards manganese, in the case of soy bean, are also in agreement with those described above.

The data derived from experiments 2 to 6 inclusive show that healthy experimental plants of Phaseolus vulgaris and Lycopersicum esculentum, free from the symptoms of iron and manganese deficiencies, occurred only when the ratio of the iron concentration to the manganese concentration in the culture solution lay within a certain range of ratio values, regardless of the total concentration of iron or of manganese in the culture solution. When the experimental plants were grown in culture solution A (see page 50) this range was 1.5 to 3.0 for Phaseolus vulgaris (see Tables VII and X, pages 84 and 91) and 0.5 to 5.0 for Lycopersicum esculentum (see Table XII, page 96). For Phaseolus vulgaris in culture solution B (see page 52) the range was 0.5 to 5.0. In culture solution C (see page 52) Phaseolus vulgaris plants grew normally only at one ratio; namely 2 : 1 (see Tables XIV and XVI, pages 102 and 106).

With respect to the concentrations of its cations, culture solution C differed from culture solution B only in its calcium content, which was greater (see page 54). Figures 7, 8 and 9 (see pages 126, 127 and 128) show that when the calcium content of the culture solution was increased from 42 to 143 p.p.m., the total amounts of iron and of manganese were depressed in the tissues of the experimental plants. In addition to bringing about a reduction in the absorption of iron and manganese by the experimental plants, increased calcium concentration in the culture solution appears to have caused a narrowing of the range of the iron to manganese concentration ratios (in the culture solution) within which the plants were able to produce normal green leaves and maintain healthy growth. A possible connection between these calcium effects and the occurrence of lime-induced chlorosis in the field may be discerned.

The range of the ratios of the iron concentration to the manganese concentration (in the culture solution) within which the experimental plants of Phaseolus vulgaris and Lycopersicum esculentum were able to produce normal green leaves, appeared to be determined by the minimal requirements of the species with respect to the total amounts of iron and manganese in the leaves, and by their relative proportions and that of calcium in the culture solution.

The ratio effect was found to be inoperative when Phaseolus vulgaris was grown in culture solutions in which the concentrations of iron and manganese did not exceed 0.05 p.p.m. and 0.1 p.p.m. respectively (see Table X, page 91). The symptoms of iron and manganese deficiency symptoms developed simultaneously in individual plants. This, incidentally, proved conclusively that a deficiency of one of these metals did not correspond to a toxicity of the other, as suggested by Somers and Shive (1942). The work of Olsen (1950) furnishes further data in relation to this question. He found that the relative amounts of all of the nutrient elements in the culture solution influenced the rate at which a particular one was absorbed by rye and kale.

In cultures other than those in which the symptoms of iron and manganese deficiencies occurred simultaneously in individual plants, the growth responses of the experimental plants, as manifested by their average dry weights for each culture, were closely related to the iron to manganese concentration ratios in the culture solutions (see Figures 1 and 2, pages 120 and 121). These values decreased sharply with successive departures from the optimum iron to manganese concentration ratios in the culture solutions.

It was found in experiment 7 that Allium cepa growing in single salt solutions, absorbed more iron or manganese per unit of time from shaken than from unshaken solutions at a constant temperature of 20° C. (see Table XVIII, page 112). It was also demonstrated that the rate at which this species absorbed the divalent ferrous ($Fe^{++}$) or manganous ($Mn^{++}$) ion was independent of the concentration of the ion in the culture solution (see Tables XIX and XX, pages 113 and 114, and Figure 10, page 129). This may help towards an explanation of results brought out by experiments 2 and 3. In these experiments, when the concentrations of iron and manganese in the culture solution were doubled while the ratio of the iron concentration to the manganese concentration was kept constant, the total quantities of these metals found by analyses in the leaves of the experimental plants showed increases which were proportionately less than the increases in their concentrations in the substrate.

Experiment 8 showed that neither titanium, nickel nor cobalt could be satisfactorily substituted for either iron or manganese in the nutrition of Phaseolus vulgaris, Table XXII, page 119. In fact, the first two metals, in the concentrations employed, were toxic to the species. With respect to cobalt, the results obtained were contrary to those of Somers and Shive (1942) for soy bean (see page 28). Experiment 8 provided no evidence to support the theory of Somers and Shive (1942) in regard to the effects of manganese and cobalt on oxidation-reduction reactions in which the equilibrium between ferrous and ferric compounds within the plant tissues is involved (see pages 27 and 28), and an iron deficiency chlorosis is supposed to develop as a result of ferrous iron being oxidized to the inactive ferric state in the leaves of plants.

The data from experiment 3, showing that deficiency symptoms of iron and manganese developed simultaneously in plants of Phaseolus vulgaris in culture solutions of very low iron and manganese content, are in agreement with the results of Hewitt (1948a) for oats. On the other hand they do not support the belief of Somers and Shive (1942) that a deficiency of manganese is caused by the presence of an excess of reduced, or ferrous iron, within the leaves of plants, which is the same thing as manganese deficiency.

It seems necessary to be cautious in interpreting the effects of manganese or other metals in terms of their relationships with oxidation-reduction reactions in which the equilibrium between ferrous and ferric compounds is involved. Hewitt (1948a) demonstrated experimentally, in sand culture investigations, at Long Ashton, that symptoms of iron deficiency can be induced in sugar beet by the addition of divalent ions such as those of copper, manganese, cobalt and zinc, but that, in the case of the first three ions named, the severity of the induced chlorosis is not in the order of the oxidation potentials of the metals involved. He drew attention to the fact that zinc does not undergo the valency change associated with simple oxidation-reduction reactions. Hewitt thought that many metals may exist in plants as complex ions, and that the oxidation-reduction values of reactions involving these ions may differ greatly from those involving the simple ions.

On the whole, one seems to be led to the conclusion that neither the results described by Hewitt, nor those obtained during the present experiments, can be accounted for in terms of the oxidation-reduction potentials of reactions involving simple ions.

## VI. SUMMARY

Water-culture experiments were conducted with Phaseolus vulgaris var. humilis and Lycopersicum esculentum grown in culture solutions containing different concentrations of iron, manganese, and calcium, and different iron to manganese concentration ratios. The amounts of iron and manganese absorbed from the external solution, by the plants, were determined by a series of analyses carried out on the oven dried plant tissues. A quantitative differentiation was made between the insoluble and soluble fractions of iron and manganese in the leaf tissues, the latter fraction being extractable by acetone and assumed to approximate the proportion of these elements which were in solution within the cells. A spectrophotometer was used to estimate the iron by means of the o-phenanthroline method, and the manganese by means of the periodate method (permanganate method).

The absorption of iron and manganese by Allium cepa (onion) from single and double salt solutions, maintained at a constant temperature of 20°C., and continuously shaken, was examined by making quantitative spectrophotometric determinations of the iron and, or manganese remaining in the external solutions after the roots of the experimental plants had been immersed in them for specific periods of time.

In the light of the data furnished by the above described experiments, the results may be summarized as follows:

(1) The data indicated a definite interrelation between iron and manganese in plant nutrition.

(2) The data suggested that Phaseolus vulgaris var. humilis and Lycopersicum esculentum, in culture solution, exhibit normal growth, free from the symptoms of iron and manganese deficiencies, when the total content of each of these metals in the leaves of the plants exceeds a certain minimum level.

(3) It was found that the total quantity of iron or of manganese in the roots and stems of Phaseolus vulgaris var. humilis, and in the leaves of both this species and Lycopersicum esculentum, was depressed when its concentration in the culture solution remained constant while that of the other metal (iron or manganese) was increased. Solution analyses indicated that the rate of absorption of one of these

metals by Allium cepa was depressed when its concentration remained constant while that of the other was increased in the culture solution. It is suggested that iron and manganese ions of similar valence are mutually competitive or antagonistic in their absorption by the three species named above (see page 23).

(4) An induced deficiency of iron or manganese occurred when the ratio between their concentrations in the culture solution was varied so that the accumulation of either metal, by the experimental plant (Phaseolus or Lycopersicum) was depressed below the minimum amount required in the leaves for health.

(5) When Phaseolus vulgaris var. humilis was grown in culture solutions in which the concentrations of iron and manganese were not in excess of 0.05 p.p.m. and 0.1 p.p.m. respectively, the ratio effect was found to be inoperative, for the symptoms of iron and manganese deficiencies developed simultaneously in individual plants, thus showing that a deficiency of one of these metals does not correspond to a toxicity of the other.

(6) The data suggested that the absorption of iron and the absorption of manganese by Phaseolus

vulgaris var. humilis were depressed when the calcium content of the culture solution was increased from 42 to 143 p.p.m.

(7) None of the metals titanium, nickel or cobalt could be used as a substitute for iron or manganese in the nutrition of Phaseolus vulgaris var. humilis.

(8) The data indicated that the number of divalent ferrous ($Fe^{++}$) ions or manganous ($Mn^{++}$) ions absorbed per unit of time by Allium cepa plants, from a single salt solution at a constant temperature of 20°C., and continuously shaken, was constant and independent of the concentration of the ions in the culture solution.

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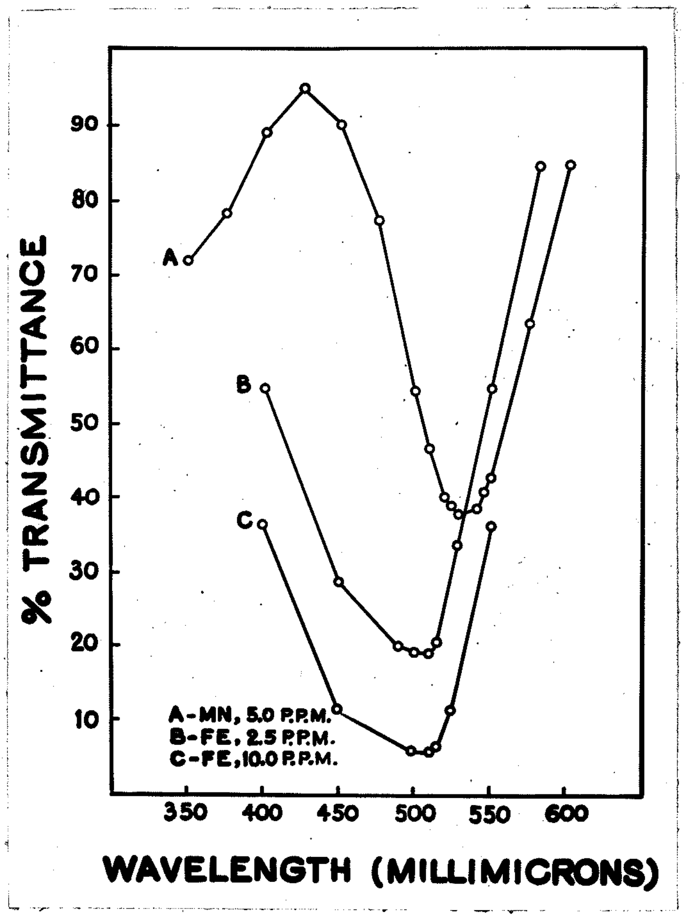
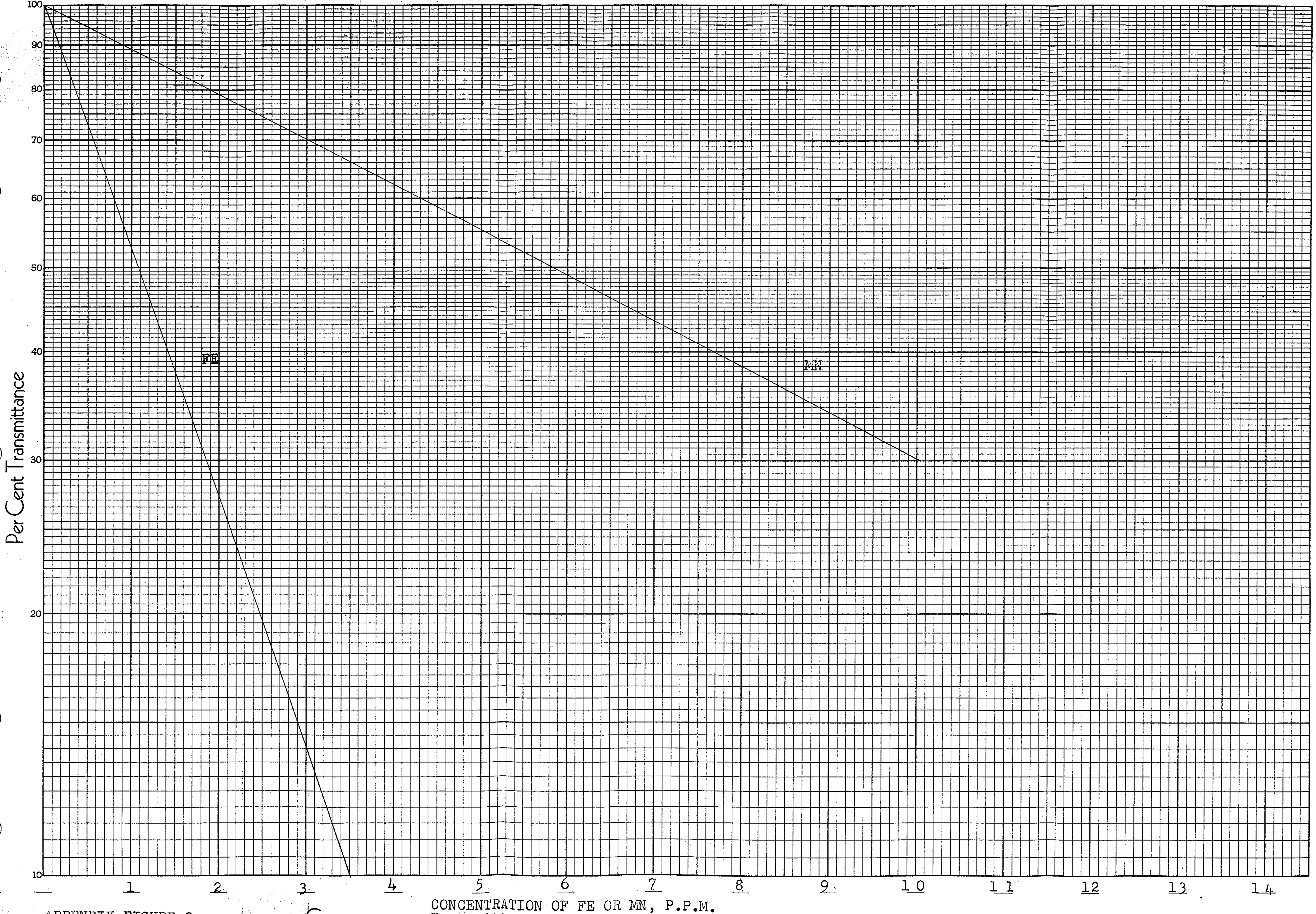
# APPENDICES

# APPENDIX I



APPENDIX FIGURE 1. Spectral-transmittance curves for iron by the o - phenanthroline method, and manganese by the permanganate method. The wave lengths at which the considered constituents absorbed most strongly are evident at the lowest points of the curves. These wave lengths were used in succeeding analyses.

Analyst ______ Date ______ File No. ______ Method : Iron by the o-Phenanthroline method (λ, 510 MU); manganese by the permanganate method (λ, 530 MU).



Coleman Instruments, Inc. Maywood, Ill.
14-322 Printed in U.S.A.

APPENDIX FIGURE 2

Concentration -Transmittance Graph

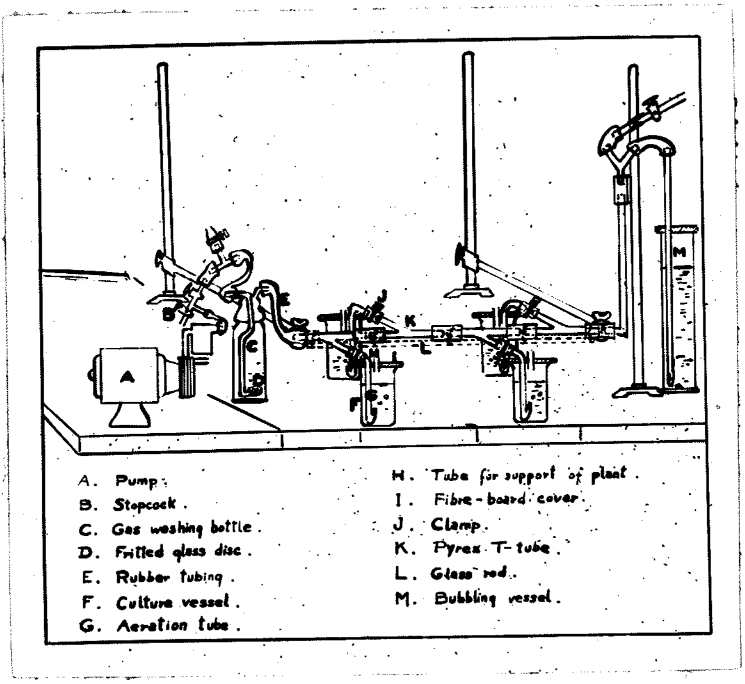
MODEL 14 COLEMAN SPECTROPHOTOMETER Serial No. ______

Reference ______

Remarks These analytical calibration curves were prepared by measuring the transmittance of several samples of known iron or manganese content, and were used when quantitatively estimating the iron and/or manganese in solutions containing them in unknown amounts.

Cell Length mm ______

Wave Length mμ ______



APPENDIX FIGURE 3. Diagram of water-culture installation showing apparatus used to aerate the culture solutions.

PLATE 1a. Coleman Universal spectrophotometer, model 14.

PLATE 1b. Shaker designed by Dr. W. Leach.

PLATE 1a

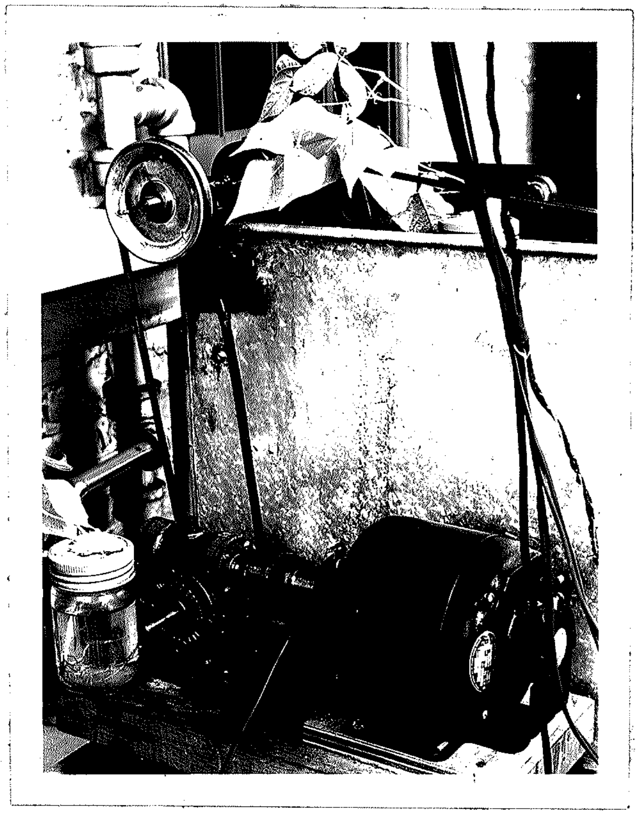


PLATE 1b

PLATE 2a. Phaseolus vulgaris var. humilis. A plant growing in Pyrex water - culture vessel (600 ml. Berzelius beaker).

PLATE 2b. Arrangement of apparatus in water-culture experiment with Phaseolus vulgaris var. humilis. The supplementary lighting system is shown clearly.

PLATE 2a

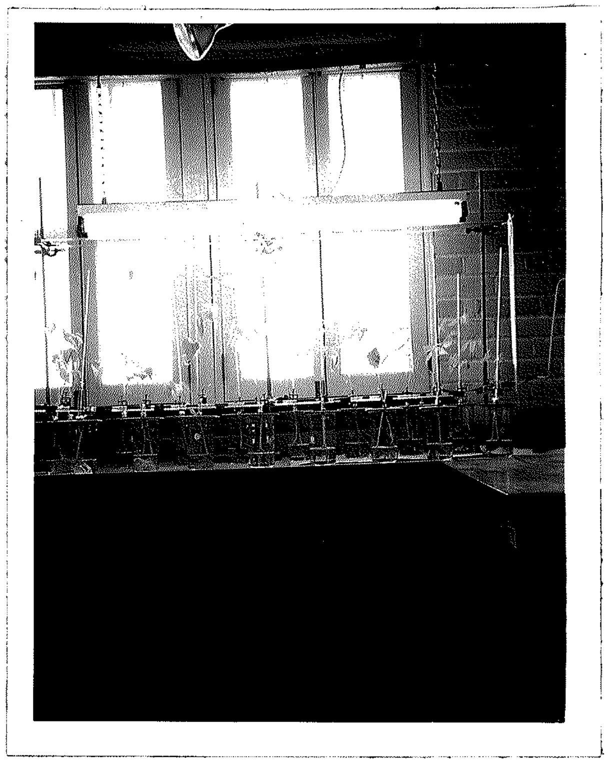


PLATE 2b

PLATE 3a. Arrangement of apparatus in water-culture experiment with _Phaseolus vulgaris_ var. humilis. Left; pressure pump and gas washing bottle. Right; bubbling vessel.

PLATE 3b. Water-culture experiment with _Phaseolus vulgaris_ var. humilis.

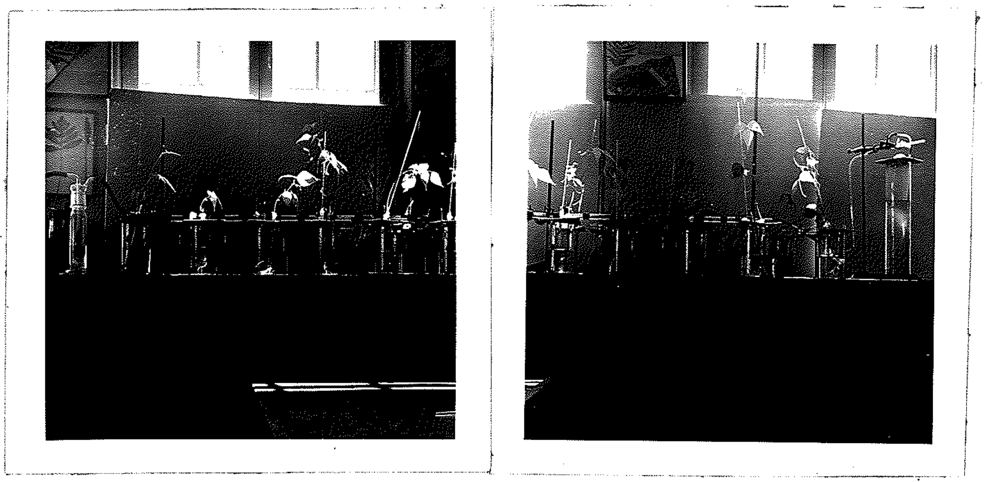


PLATE 3a

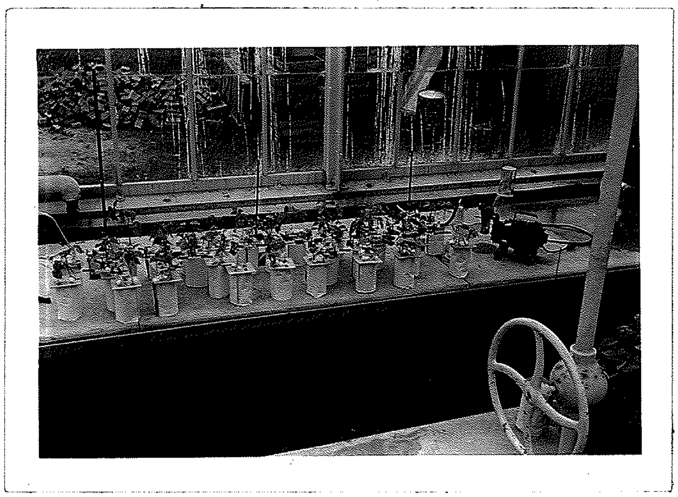


PLATE 3b

PLATE 4a. Phaseolus vulgaris var. humilis. Leaves of plants grown in water culture. Left; iron deficiency, indicated by yellow leaves. Centre; manganese deficiency, indicated by intervenal chlorosis and scattered areas of necrosis. Right; normal, green leaves of plant grown in a complete culture solution including iron and manganese.

PLATE 4b. Phaseolus vulgaris var. humilis. Plants grown in water culture. Left and centre; toxicity of nickel (6 p.p.m.) and titanium (6 p.p.m.) indicated by collapse of cells in stem tissues and wilting, withering and necrosis of leaves. Right; manganese was replaced in the culture solution by 0.25 p.p.m. of cobalt. The symptoms are those of manganese deficiency.

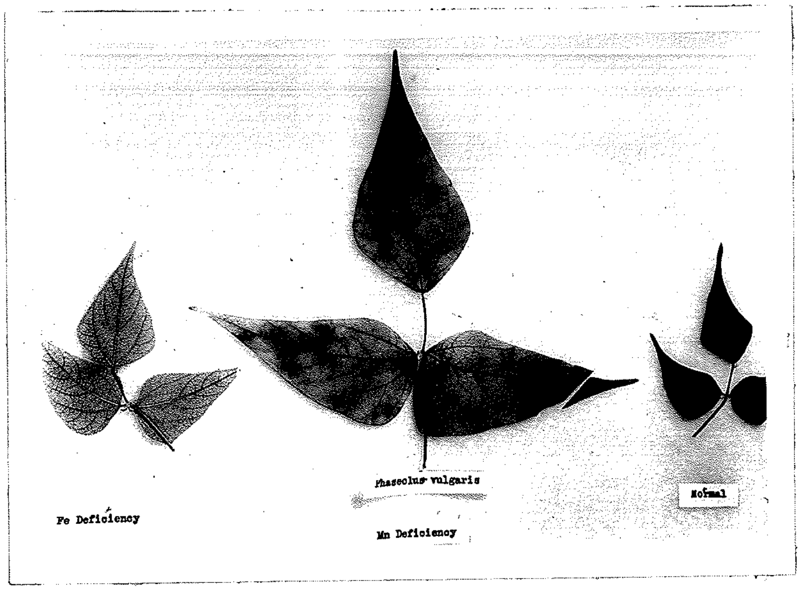


PLATE 4a

PLATE 4b

PLATE 5a. Phaseolus vulgaris var. humilis.
Normal seedling grown in a complete culture solution.

PLATE 5b. Phaseolus vulgaris var. humilis
The leaf symptoms indicate severe manganese deficiency.

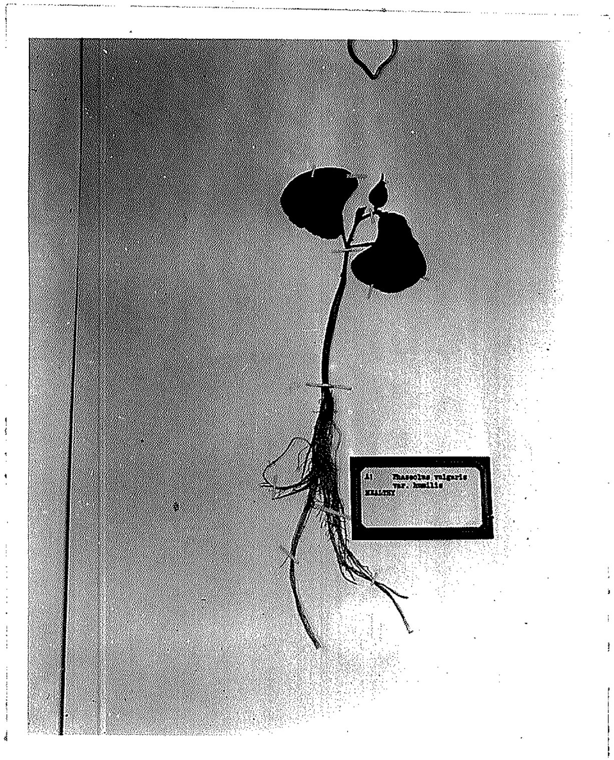


PLATE 5a

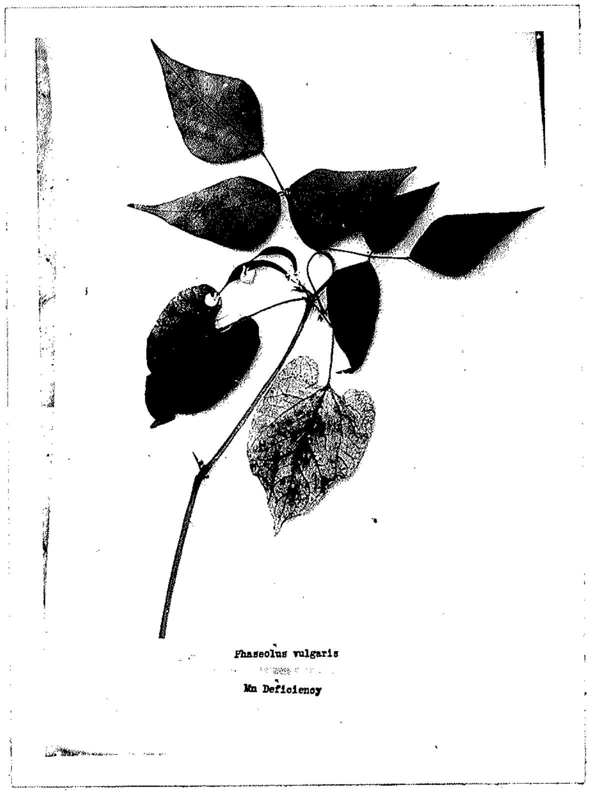


PLATE 5b

PLATE 6a. <u>Phaseolus vulgaris</u> var. humilis Plant grown in culture solution containing 0.002 p.p.m. of iron and 0.001 p.p.m. of manganese. The lower leaves enhibit manganese deficiency symptoms. The upper leaves show iron deficiency symptoms.

PLATE 6b. <u>Phaseolus vulgaris</u> var. humilis. Plant grown in culture solution containing 0.05 p.p.m. of iron and 0.025 p.p.m. of manganese. The deficiency symptoms are similar to those shown in Plate 6a.

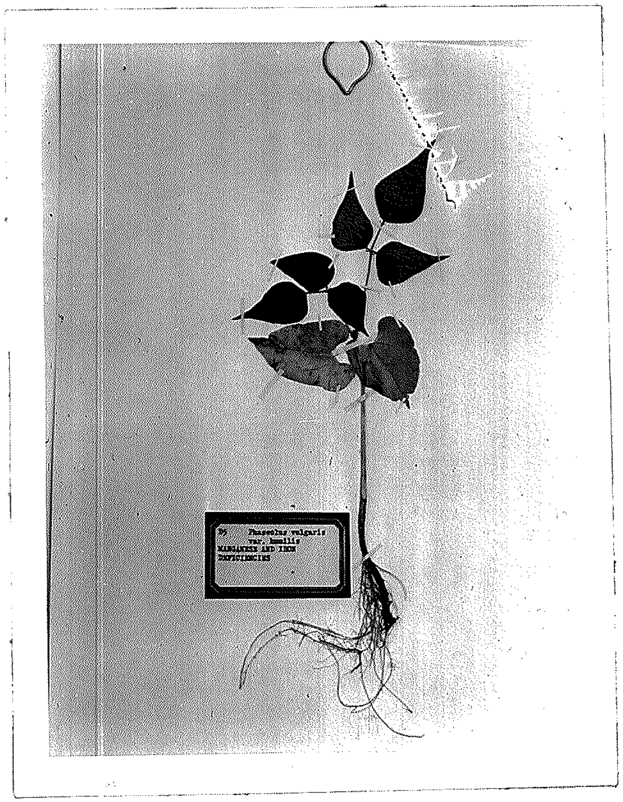


PLATE 6a

PLATE 6b

PLATE 7a. Leaves of Pisum sativum grown in water culture. Iron deficiency symptoms (left) are easily distinguishable from those of manganese deficiency (right).

PLATE 7b. Leaves of Lycopersicum esculentum plants grown in water culture. The completely yellow leaves, resulting from iron deficiency, differ greatly from the mottled, intervenal leaf pattern produced by manganese deficiency.

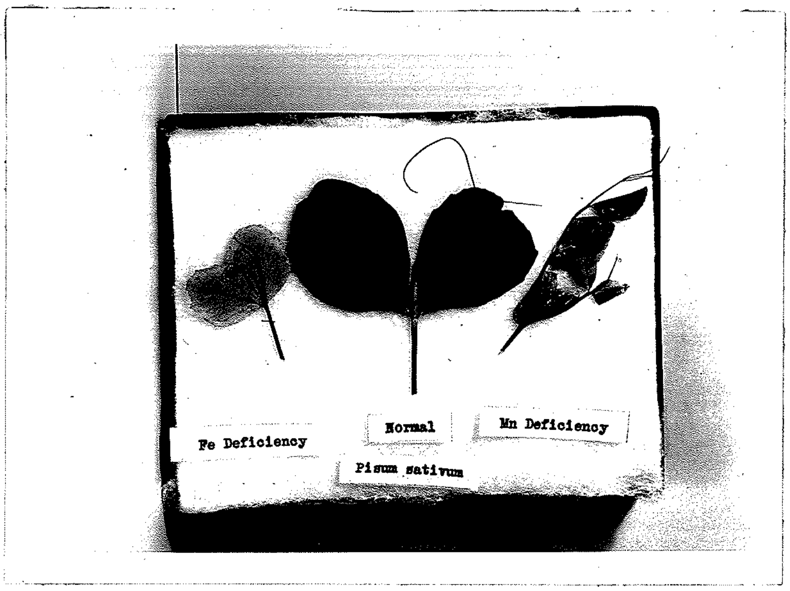


PLATE 7a

PLATE 7b

PLATE 8a. Phaseolus vulgaris var. humilis. Appearance of plants after 30 days growth in culture solutions containing iron and manganese in the concentrations indicated. Left; iron deficiency symptoms. Centre; normal plant. Right; manganese deficiency symptoms.

PLATE 8b. Lycopersicum esculentum. Appearance of plants after 10 days growth in culture solutions, containing iron and manganese in the concentrations indicated. The poor growth responses of the plants on the left are clearly the result of iron deficiency. Symptoms of manganese deficiency are not readily disconcernible in the plants on the right.

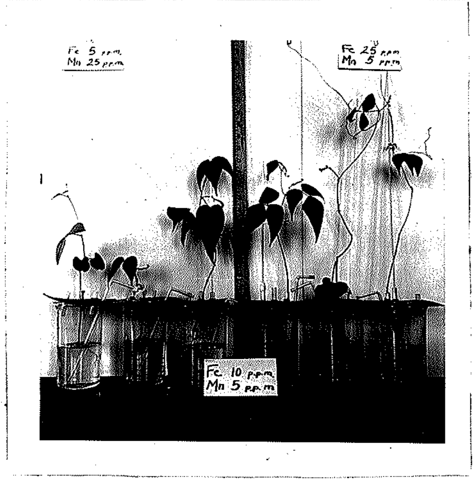


PLATE 8a

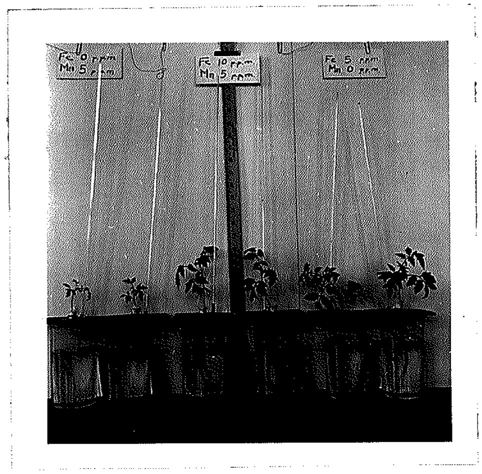


PLATE 8b

PLATE 9a. Lycopersicum esculentum. These are the plants shown in Plate 8b after 30 days growth in culture solutions containing iron and manganese in the concentrations indicated. Iron deficiency symptoms (left) and manganese deficiency symptoms (right) are readily apparent.

PLATE 9b. Symptoms of iron and manganese deficiencies shown by leaves of Zea mays plants grown in water culture.

PLATE 9a

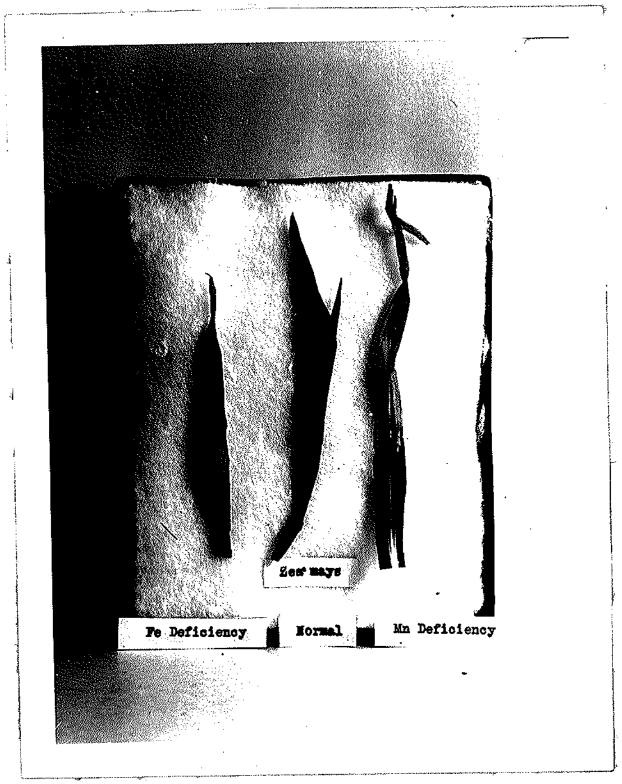


PLATE 9b

# APPENDIX II

# MANGANESE AND IRON DEFICIENCIES IN OATS

BY C. D. TAPER

## ABSTRACT

The effects on experimental plants of Avena sativa (common oat) resulting from the omission of iron, or manganese, from an otherwise complete culture solution were investigated by means of the water-culture technique.

The omission of iron from the culture solution, in all instances, produced iron deficiency symptoms in the experimental plants.

The omission of manganese from the cultures failed to produce deficiency symptoms in the experimental plants when they were grown at pH 4.5 in culture solutions which contained 5 p.p.m. of iron in the form of the inorganic salt, ferrous sulphate ($Fe SO_4 . 7 H_2O$).

The omission of manganese from the cultures, in all cases, produced manganese deficiency symptoms

in the experimental plants when they were grown at pH 6.5 in culture solutions which contained 5 p.p.m. of iron in the form of ferrous sulphate or the organic substance, humate iron.

Manganese deficiency symptoms and the symptoms of the grey speck of oats appeared to be identical.

## INTRODUCTION

The experiments described in this paper furnish strong evidence that grey speck disease of oats may be attributed to manganese deficiency. The data suggest that the development of manganese deficiency in oats grown in water culture is related to the pH of the culture solution. No direct attempt was made to investigate grey speck disease of oats per se. The primary intent in conducting these experiments was to produce the symptoms of manganese and iron deficiencies in plants of the monocotyledonous species <u>Avena sativa</u> (common oat) under experimental conditions so that the symptoms could be studied directly. The work was commenced October 1, 1950, in the laboratory, in the Department of Botany, The University of Manitoba, as a desirable adjunct to certain carefully controlled solution culture experiments during the course of which readily recognizable symptoms of manganese and iron deficiencies were produced in the dicotyledonous species *Phaseolus vulgaris* var. humilis, *Zea mays*, *Pisum sativum*, and *Lycopersicum esculentum*.

## MATERIALS AND METHODS

### Experimental Plants

The seeds of *Avena sativa* used in the present investigation were a sample of the variety "Tama" from plot 4, experiment F - 144, a field experiment conducted by Dr. W.A.F. Hagborg, of the Dominion Laboratory of Plant Pathology, Winnipeg, during the year 1949. These oats were harvested from plants exhibiting grey speck lesions to an extent designated as 80 per cent.

### Distilled Water

The distilled water used throughout the investigation was prepared in an electrically heated Barnstead water still lined with block tin.

Examinations made by means of a model 14 Coleman Universal spectrophotometer showed that this water contained no measurable quantity of iron or manganese after evaporating a volume of 2 liters down to 25 ml.

## Composition of the Culture Solution

In Table I are given the respective amounts of 0.5 M stock solutions of the chemicals (Baker's analyzed chemicals, chemically pure grade) which were diluted with distilled water in the preparation of the culture solution. Manganese and iron were removed from these stock solutions in the manner described by Stout and Arnon (1939). This purification method involves the adsorption of iron and manganese by calcium carbonate and calcium phosphate. Iron and manganese are co-precipitated with the calcium compounds after the solutions have been autoclaved at slightly alkaline reactions. Spectrophotometric analyses indicated that no detectable quantities of manganese or iron remained in the solutions. The culture solution employed was a modification of one in which Olsen (1950) grew rye. Monobasic potassium phosphate ($KH_2PO_4$), which cannot be purified by the method of

## Table II

## Stock Solution of Trace Elements

| Compound | Gm. in 1 liter of distilled water |
|---|---|
| Boric acid ($H_3BO_3$) | 2.860 |
| Zinc sulphate ($ZnSO_4 . 7 H_2O$) | 0.220 |
| Copper sulphate ($CuSO_4 . 5 H_2O$) | 0.080 |
| Molybdenum oxide ($Mo O_3$) | 0.075 |

Manganese in the form of manganese sulphate ($MnSO_4 . 4 H_2O$), iron in the form of ferrous sulphate ($FeSO_4 . 7 H_2O$), or humate manganese and humate iron were added to the culture solutions in the concentrations indicated in connection with each experiment.

Iron and manganese as colloidal "humates" remain available to plants within the pH range 3 to 9. These humates were prepared in the manner described by Horner, Burke and Hoover (1934) by adding manganese sulphate and ferrous sulphate to potassium humate.

The pH of each culture solution was

adjusted with sulphuric acid and potassium hydroxide.

## Culture Methods

The oats were sterilized by a two minute immersion in a 0.1 per cent solution of mercuric chloride ($Hg\ Cl_2$), and germinated at 25° C. in petrie dishes in an incubator. The endosperm was removed from each seedling in order to eliminate this source of iron and manganese.

Seven day old seedlings which had been grown on the surface of acid-washed quartz sand, in a glass vessel, and supplied only with distilled water, were transferred to the water culture vessels (600 ml. Pyrex Berzelius beakers), four plants to each vessel. Each culture vessel contained 500 ml. of the culture solution, and was covered with black paper to exclude light, in order to discourage the growth of algae. The plants were supported by wrapping with non-absorbent cotton and inserting them in Pyrex glass tubes 1 cm. in diameter and 4 cm. in length (see Plate 1). The tubes were pushed through circular openings in the square, paraffined, fibreboard covers. Additional support was provided by passing the plants through loops of paraffined, white, linen thread tied to glass rods 1 mm. in diameter.

The solutions were aerated for eight hours daily by means of an electric pressure pump. The air passed through distilled water in a 250 ml. gas washing bottle and, after passing through a main tube formed by the horizontal arms of Pyrex T-tubes, entered the solutions through Pyrex aeration tubes connected by rubber pressure tubing to the vertical arms of the T-tubes. Screw clamps on the pressure tubing permitted the regulation of the air stream to the rate of one bubble per second into each beaker. A constant pressure was maintained in the system, which was just sufficient to bubble air through a column of water, 30 cm. in height, in a bubbling cylinder at the end of the main tube. Approximate regulation was achieved by means of a glass stopcock between the pump and the aeration system.

Sunlight was supplemented for eight hours daily with three 40 watt fluorescent lamps (one daylight and two white), which were suspended two feet above the plants.

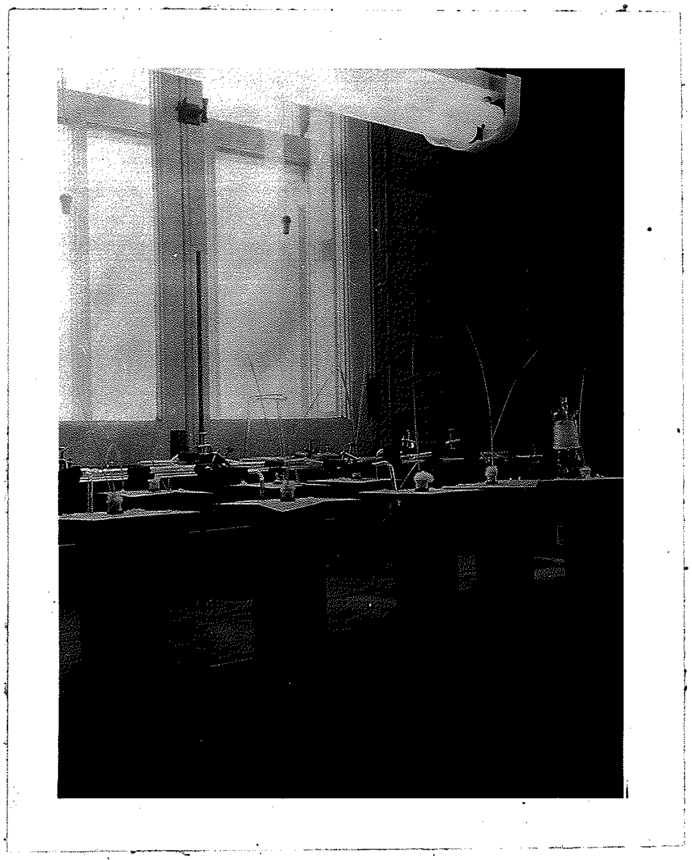


PLATE 1. Water-culture installation employed in growing Tama oats.

# EXPERIMENTAL RESULTS

## Experiment 1

The effects of growing Avena sativa in culture solutions at initial pH 4.5 and containing different concentrations of manganese and iron supplied in the form of the salts manganese sulphate ($MnSO_4 . 4 H_2O$) and ferrous sulphate ($FeSO_4 . 7 H_2O$).

Seven day old seedlings of Tama oats which had been grown on the surface of acid-washed quartz sand, and supplied only with distilled water, were transferred to the culture vessels, four plants to each vessel. Manganese and iron were supplied in the form of the salts manganese sulphate ($MnSO_4 . 4 H_2O$) and ferrous sulphate ($FeSO_4 . 7 H_2O$). In order to maintain the iron in solution as long as possible, the reaction of the culture solutions was adjusted to pH 4.5 at the beginning of each run; the solutions being renewed at intervals of 24 hours. Aeration and supplementary lighting were supplied for eight hours each day.

It will be noted from the data shown in Table III that the omission of manganese from the culture solutions failed to produce deficiency symptoms in the experimental plants.

The omission of iron from the culture solutions in all cases produced, in the experimental plants, pathological symptoms. These were diagnosed as iron deficiency symptoms by placing the plants in a culture solution containing 5 p.p.m. of iron (supplied as ferrous sulphate) and 2 p.p.m. of manganese (supplied as manganese sulphate) for one week following the termination of the experiment. The symptoms ceased to appear in the new growth; and when there was no extensive necrosis, the older leaves became green.

Iron deficiency symptoms in oats. The symptoms of iron deficiency appeared first in the new leaves of the experimental plants. A chlorosis gradually developed which took the form of continuous intervenal, yellow stripes (see Plate 2). Many leaves became entirely bleached within ten days after the symptoms first appeared. In these instances the tips

## TABLE III

The effects of growing Avena sativa in culture solutions at pH 4.5 and containing inorganic manganese and, or iron

| Culture used | No. of plants | p.p.m. in solution Fe | p.p.m. in solution Mn | Ratio Fe : Mn in solution | Symptoms* | Time, days, required for symptoms to appear | Duration of expt., days |
|---|---|---|---|---|---|---|---|
| 1 | 4 | 5 | 2 | 2.5 | N | | 30 |
| 2 | 4 | 5 | 2 | 2.5 | N | | 30 |
| 3 | 4 | 5 | 0 | ___ | N | | 30 |
| 4 | 4 | 5 | 0 | ___ | N | | 30 |
| 5 | 4 | 0 | 5 | ___ | -Fe | 10 | 30 |
| 6 | 4 | 0 | 5 | ___ | -Fe | 9 | 30 |

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* Symptoms indicated as N, normal; -Mn, manganese deficiency; -Fe, iron deficiency.

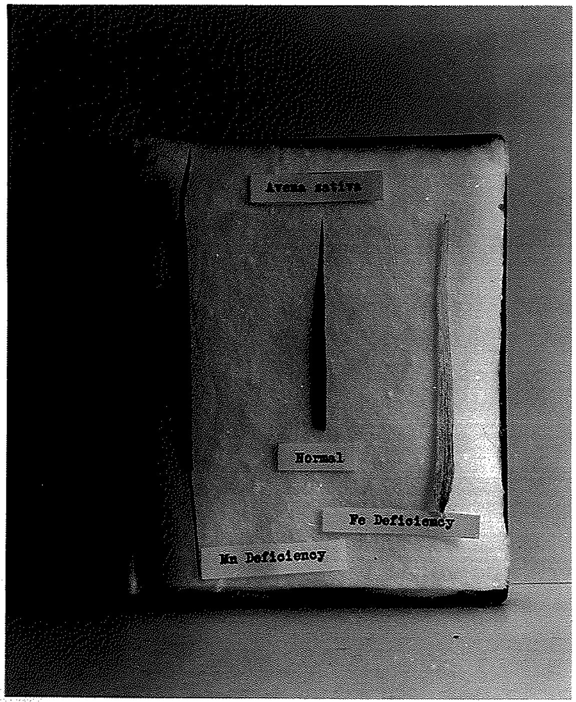

PLATE 2. Symptoms of mineral deficiencies shown by Tama oats.

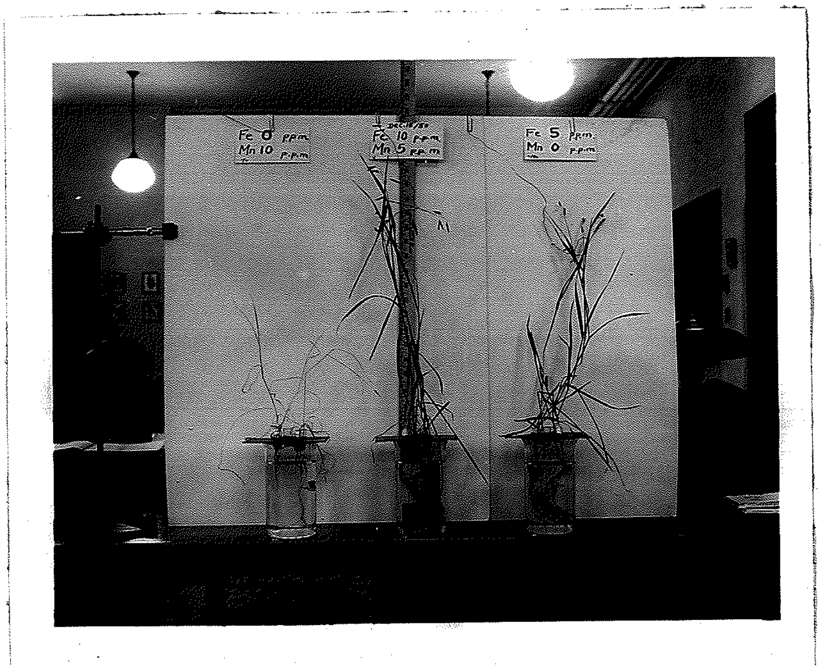

PLATE 3. The effects of mineral deficiencies upon the growth of Tama oats. Left; iron deficiency. Centre; growth in complete nutrient solution. Right; manganese deficiency.

of the leaves eventually died.

The growth of the plants, in general, was greatly retarded as compared with the normal plants in the other cultures. This is clearly indicated in Plate 3.

## Experiment 2

The effects of growing Avena sativa in culture solutions at pH 6.5 and containing different concentrations of manganese and iron supplied as humate manganese and humate iron.

The omission of manganese from the culture solutions having failed to produce deficiency symptoms in Tama oats when grown in culture solutions adjusted to pH 4.5, it was thought desirable to repeat the work with the reaction of the culture solutions adjusted to a higher level at the beginning of each run; namely, pH 6.5. Humate manganese and humate iron were used in order to prevent, as completely as possible, the precipitation of iron, which occurs at an increasing rate as the reaction of the culture solution is moved towards alkalinity. The solutions were renewed at intervals of seven days, because humate iron remains in solution within the pH range 3 to 9 for long periods before decomposition of the organic complex occurs.

Examinations made with a Beckman potentiometer disclosed that the reaction of the culture solution increased from pH 6.5 to pH 7.5 during the seven day run.

It will be noted from the data shown in Table IV that the omission of manganese or iron from the culture solutions, in all instances, produced deficiency symptoms in the experimental plants.

The pathological symptoms exhibited by plants growing in culture solutions from which manganese was omitted were typical of grey speck disease of oats. They were diagnosed as manganese deficiency symptoms by the procedure used in the case of iron deficiency symptoms in experiment 1 (see page xxxii). It was found that the symptoms of manganese deficiency already present could not be removed by adding manganese to the culture solution. The continued development of the symptoms, however, was arrested, and the new growth was green and healthy.

# TABLE IV

The effects of growing <u>Avena sativa</u> in culture solutions at pH 6.5 and containing organic manganese and, or iron

| Culture used | No. of plants | p.p.m. in solution Fe | p.p.m. in solution Mn | Ratio Fe : Mn in solution | Symptoms* | Time, days, required for symptoms to appear | Duration of expt., days |
|---|---|---|---|---|---|---|---|
| 1 | 4 | 5 | 2 | 2.5 | N | | 60 |
| 2 | 4 | 5 | 2 | 2.5 | N | | 60 |
| 3 | 4 | 5 | 0 | ___ | -Mn | 9 | 60 |
| 4 | 4 | 5 | 0 | ___ | -Mn | 40 | 60 |
| 5 | 4 | 0 | 5 | ___ | -Fe | 8 | 60 |
| 6 | 4 | 0 | 5 | ___ | -Fe | 11 | 60 |

* Symptoms indicated as N, normal; -Mn, manganese deficiency; -Fe, iron deficiency.

Manganese deficiency symptoms in oats. When they were grown at pH 6.5 in culture solutions containing humate iron, but no manganese, the experimental plants very slowly developed certain pathological symptoms. Nine days after the experiment was begun a plant, 6 inches in height, in culture 3, displayed small, grey, oval areas, near the margins, in the basal halves of two of the oldest or first leaves. The basal portions of these leaves became somewhat yellow in colour; but the grey specks did not increase in size until 31 days later, when the disease abruptly (within less than 48 hours) appeared, not upon first and second leaves, but upon all third leaves in both manganese free cultures. Within 24 hours the numerous grey specks enlarged considerably in size. After a day or two, some of the specks developed a water-soaked appearance, giving a blue-grey colour to the spots. This phase lasted for only two days. The water-soaked appearance disappeared, and the lesions enlarged to become grey or buff coloured streaks. The streaks tended to elongate to coalesce, and to turn yellow brown. The leaves eventually bent over near the distal ends of the seriously affected areas. The tips of the leaves remained green, but hung down limply. In some instances the brown areas twisted into the form of spirals. The disease continued to develop, and appeared gradually upon the later growth.

These are the typical symptoms of grey speck disease.

Oats affected with grey speck disease often have poorly developed root systems. In this instance the root systems of the affected plants were less extensive than those of the normal plants; but not markedly so.

Analyses were not carried out on the experimental plants because of the small amount of plant material available.

## Experiment 3

### The effects of growing Avena sativa in culture solutions of initial pH 6.5 and containing various concentrations of manganese and iron supplied in the form of the salts manganese sulphate ($MnSO_4 . 4 H_2O$) and ferrous sulphate ($FeSO_4 . 7 H_2O$).

In order to find how far the findings from

## TABLE V

The effects of growing *Avena sativa* in culture solutions at pH 6.5 and containing inorganic manganese and, or iron

| Culture used | No. of plants | p.p.m. in solution | | Ratio Fe : Mn in solution | Symptoms* | Time, days, required for symptoms to appear | Duration of expt., days |
|---|---|---|---|---|---|---|---|
| | | Fe | Mn | | | | |
| 1 | 4 | 5 | 2 | 2.5 | N | | 30 |
| 2 | 4 | 5 | 2 | 2.5 | N | | 30 |
| 3 | 4 | 5 | 0 | ___ | -Mn | 14 | 30 |
| 4 | 4 | 5 | 0 | ___ | -Mn | 16 | 30 |
| 5 | 4 | 0 | 5 | ___ | -Fe | 7 | 30 |
| 6 | 4 | 0 | 5 | ___ | -Fe | 8 | 30 |

* Symptoms indicated as N, normal; -Mn, manganese deficiency; -Fe, iron deficiency.

experiment 2 held when inorganic forms of manganese and iron were supplied to the culture solutions, experiment 1 was repeated exactly with the exception that the reaction of the culture solution was adjusted to pH 6.5 at the beginning of each run; the solutions being renewed at intervals of 24 hours.

Data relating to this experiment are given in Table V. It will be noted that the omission of manganese or iron from the culture solutions, in all cases produced manganese or iron deficiency symptoms.

## DISCUSSION

The experimental results here described seem to make it clear that the omission of iron from the culture solutions readily produces iron deficiency symptoms in Tama oats grown in water culture.

The results which occurred when Tama oats were grown in culture solutions from which manganese was omitted cannot be stated so simply. The symptoms of manganese deficiency (grey speck disease) were not produced in all cases.

The existence of an uncontrolled source of manganese, perhaps in the seed, might explain this irregular appearance of grey speck disease in the experimental plants. However, none of the evidence supported an explanation of this nature. It is contrary to the spectrophotometric evidence to suppose that the culture solution contained manganese following the process of purification.

There is another reason why one may question the validity of an explanation based upon the assumption that the culture solution retained undetected amounts of manganese which were sufficient to prevent the development of grey speck. In experiment 2 the culture solutions contained an organic material, potassium humate, which was capable of maintaining manganese in a chemical form which could be absorbed by the plants at the pH levels involved. Any manganese entering the solution was free to combine with the excess of potassium humate present (see Horner, Burke and Hoover (1934)). Nevertheless, in the cultures of experiment 2, the experimental plants developed manganese deficiency symptoms (grey speck disease).

The presence of organic matter, per se, in the culture solutions, could not have been a factor in the production of manganese deficiency symptoms, because these developed at pH 6.5 whether organic material was present or not.

The results of the experiments described suggest that the production of manganese deficiency symptoms (grey speck disease) in Tama oats was related to the pH of the culture solution. When manganese was omitted from the culture solution, the deficiency symptoms appeared when the pH of the solution was within the range 6.5 to 7.5; but the plants remained healthy when the pH of the solution was 4.5.

It is obvious that further experimental work into the cause of grey speck disease of oats needs to be carried out before definite conclusions can be arrived at. The writer understands that this is being done under the direction of Dr. W. Leach at the University of Manitoba.

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