The Relationship between Iron and Manganese Supply and Grey Speck Disease of Oats.

by

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INTRODUCTION

It is universally accepted that iron and manganese are essential elements for the nutrition of higher plants. On the other hand there is little or no definite knowledge of the physiological functions of these metals. Furthermore, it is only recently that attempts have been made to obtain data regarding the optimum requirements of plants for iron and manganese, and regarding the effects produced in plants by departure from these optimum requirements. It is, therefore, not yet possible to make any generalization regarding iron and manganese requirements as experiments have indicated that these vary from species to species and even between individuals of a single species grown under different conditions. A number of theories have been advanced to explain the function or functions of iron and manganese in plant metabolism but the evidence for or against these theories is so contraversial that no definite conclusions can be reached. The results of culture experiments, however, seem to suggest that the functions of iron and manganese are in some way linked together in the metabolic processes that go on within the plant. One of the earlier investigators who called attention to this point was Thatcher (63) in 1934. He held the view that iron and manganese were a pair of mutually coordinating catalysts for the oxidation-reduction reactions involving the removal

and addition of oxygen. In the past decade the investigations into the relationship between the trace elements, iron and manganese, and plant growth, have become fairly extensive.

Although the work which has been conducted into the connection between iron and manganese and plant growth is fairly extensive, there exists, at the present time a difference of opinion regarding this connection. Of recent years the theories of Shive and his co-workers (56, 59, 60) have attracted the attention of investigators in this field. According to these authors, the ratio of active (soluble) iron to active (soluble) manganese in the plant, for normal, healthy development, must lie within the limits, 2.5 to 1.5, if deficiency or toxicity symptoms are to be avoided. Somers and Shive (60), working with soy beans, found that if the ratio exceeded 2.5, iron toxicity or manganese deficiency symptoms resulted, and if the ratio was lower than 1.5, manganese toxicity or iron deficiency symptoms were produced. In experiments with oats they noticed that oat plants showed a greater tendency to develop grey speck symptoms when the iron concentration in the nutrient media was increased. In these experiments they found it impossible to separate iron toxicity and manganese deficiency symptoms. Hewitt (23), in more recent investigations, produced evidence which threw considerable doubt on the validity of the theories of Shive and his co-workers (56, 59, 60). In Hewitt's experiments

simultaneous symptoms of iron and manganese deficiencies appeared in the same leaves of oat plants when given low levels of both these elements. He found that if iron was near the minimum supply, manganese (in a normal or excess amount) could induce iron deficiency symptoms, therefore, iron deficiency and manganese toxicity symptoms were not one and the same thing.

There has been a great deal of research conducted into the cause of grey speck disease of oats. In spite of this there exists two different theories as to the actual cause of the disease. In theory the view is held that the disease is purely a nutritional one directly caused by a deficiency of manganese. The second is that a low supply of manganese renders the roots susceptible to attacks by micro-organisms and it is these micro-organisms which cause the appearance of grey speck symptoms. The former theory, supported by the majority of the investigators and the latter theory, held by Gerretsen (15, 16), are discussed at greater length on page 27

The purpose of the present investigation was two of old namely: (1) to examine the relationship between iron and manganese supply and grey speck disease of oats; and (2) to determine if possible, which of the two views mentioned above regarding the cause of the disease is the correct one.

REVIEW OF THE LITERATURE

The investigations conducted into the relationship between iron and manganese and plant growth, and into the cause of grey speck disease of oats, have been extensive but only some of the more important researches will be reviewed in the present paper.

I. The Relationship between Iron and Manganese and Plant Growth

The functions attributed to iron and manganese in plant metabolism rest almost entirely on a theoretical basis. Over a period of years, however, evidence has accumulated which suggests that the function of these elements in plant growth is to be found in their relationship to one another.

As early as 1849, the work of Salm-Horstmar (47) indicated that iron influenced the onset of manganese deficiency symptoms, especially if humus was present and that manganese in the presence of iron, or with very little iron, may have been the toxic agent bringing about iron chlorosis.

Pugliese (45) in 1913, grew various cereals in Knop's water culture solution to which were added varying amounts of ferrous sulfate and manganese salts. Manganese nitrate in concentrations greater than 0.05 grams per litre appeared to be detrimental in the absence of iron but was stimulating if iron were present as ferrous sulfate in concentrations between 0.07 and 0.15 grams per litre. He concluded, therefore, that in the

presence of iron, the plants could tolerate much larger doses of manganese, suggesting that iron antagonized the action of manganese.

In 1916, Tottingham and Beck (65) grew wheat in a Knop's solution minus ferric phosphate. They found that when iron, as ferric chloride, and manganese, as manganese chloride, were present in low concentrations the manganese depressed the stimulatory effect of iron, while at higher concentrations, the iron depressed the stimulatory effect of manganese.

Chapman (12), in 1931, noted that chloratic plants always recovered their normal color when sprayed with a 2 per cent solution of ferrous sulfate. When manganese was added with the iron or injected into the trees before spraying with iron, the leaves greened only in spots, suggesting a reduction in the solubility of iron by manganese in the leaf. Rippel (46), in 1923, made a similar observation. He noted that manganese in the form of soluble salts produced chlorosis in barley plants grown in water culture which was overcome or cured by increasing the iron supply. Since the iron content of green and chlorotic plants was the same, Rippel concluded that manganese did not interfere with the absorption of iron but with the activity of iron within the plant. In 1933, Haas (20), observing that walnut leaves affected with "yellows" contained a higher amount or iron and manganese than healthy ones, decided that the immediate availability of these elements and not their total

amounts was the determining factor in their utilization by plants.

Thatcher (63), in 1934, published a paper in which he suggested a classification of the chemical elements in relation to their functions in plant nutrition. In this classification he held the view that iron and manganese formed a pair of mutually coordinating catalysts for oxidation-reduction processes involving the addition and removal of oxygen.

Lundegardh (38), in 1939, found that the oxygen intake by wheat roots was increased by the addition of manganese chloride while ferric chloride or ferric citrate caused a reduction in oxygen intake. About the same time Burstrom (9), examining the assimilation of nitrate by whole wheat roots and wheat root pulp in the presence of small quantities of iron and manganese, came to the conclusion that without iron or manganese nitrogen assimilation did not take place. But in the presence of a low concentration of manganese, assimilation took place with both the whole root and the root pulp. Without added manganese, the addition of iron brought about only feeble nitrogen assimilation of whole wheat roots but not of wheat root pulp. According to Burstrom, this feeble nitrogen assimilation by whole wheat roots was due to the effect of iron on respiration and ion uptake. The observations of both Lundegardh and Burstrom emphasized the contrasting effects of manganese and iron.

Hopkins (28), in 1930, from observations on the growth of Chlorella, decided that manganese brought about the re-oxidation of iron after its reduction in the plant to the ferrous state. This suggested that the iron-manganese balance was important. He held the view that if the concentration of manganese in the plant was deficient, a too high proportion of ferrous iron resulted, and if manganese was in excess the reduction of ferric iron to the ferrous state was prevented and the ferric iron concentration was too high. In either condition, there was a disturbance in the oxidation-reduction processes of the cell involving iron.

Other investigators have found that the iron-manganese ratio was of more importance than the absolute concentration of manganese. In 1912, Bertrand (6, 7) observed that the ratio of manganese to iron and zinc determined the conidia development in Aspergillus niger, if the ratio of manganese to iron and zinc was too low the production of conidia was inhibited. Scharrer and Schropp (51) in 1934 found, using water culture methods, that the growth of maize roots was at a maximum when the iron-manganese ratio in the culture solution was 7/1.

More recent observations on the functions of iron and manganese in plant metabolism have been those of Shive (56), in 1941, Somers and Shive (60), in 1942, and Somers, Gilbert and Shive (59), in 1942. According to Shive and his associates the theoretical explanation of the roles of iron and manganese

in plant metabolism revolved around two points: (1) the active functional iron is in the ferrous condition, and (2) the oxidizing potential of manganese is higher than that of iron. Ferric iron absorbed by the plants is reduced within the cells to the ferrous state unless some counter-reactant prevents this reaction. Manganese, possessing the necessary chemical characteristics, was believed to be the counter-reactant preventing this reaction. If such a reactant is not present in the plant then small amounts of ferrous iron may become toxic causing symptoms of iron toxicity which were believed to be identical with those of manganese deficiency. In the presence of manganese, a strong oxidizing agent, the reduction of iron will be restrained, and if ferrous iron is present some at least will be oxidized to the ferric state and probably precipitated in the form of a ferric organophosphate. On the other hand, if the manganese concentration is too high, a chlorosis due to a shortage of active ferrous iron will result causing symptoms of iron deficiency which are identical to manganese toxicity.

In 1942, Somers and Shive (60), using soy beans, conducted water culture experiments into this iron-manganese relationship by varying the iron-manganese ratio in the culture solution. They analyzed the plants for soluble, insoluble and total iron and manganese. The soluble and insoluble fractions were separated by freezing the fresh material

and from this, in thawing, the juice was expressed under pressure. The juice expressed from the pressed cake was taken as containing the soluble iron and manganese.

As a result of these analyses, several pertinent facts were observed. It was found that the absorption rates of these elements were determined largely by their concentration in the substrate as the total iron and manganese content of the plants increased with increase in concentration of these elements in the nutrient media. However, the ratios between total iron and total manganese within the plant showed no definite relation to either normal plant growth nor to any pathological condition caused by a deficient or excess amount of iron or manganese. On the other hand, the ratios of the soluble fractions, especially those for the leaves, did show a narrow range of values which were definitely associated with normal growth and development; thus the ratio of active (soluble) iron to active (soluble) manganese within the tissues must lie between 1.5 to 2.5 or visible deficiency or toxicity symptoms would result. While the total quantities of these elements in the plants and in the substrate were comparitively unimportant, the quantity relation which one bore to the other was significant. The authors also observed that oat plants showed a greater tendency to exhibit symptoms of a grey speck when the iron concentration in the nutrient medium was increased.

Somers and Shive (60), in 1942, mentioned a series of

tests carried out with maize seedlings in which cobalt was substituted for manganese. The oxidation potential of cobalt is higher than that of manganese so it should, on the basis of the oxidation-reduction hypothesis, be an even more powerful counter-reactant than manganese. In the experiments this was found to be so, thus lending further support to their hypothesis.

Somers, Gilbert and Shive (59), in 1942, found that the respiration rate of soy bean in water culture was always definitely lower when the ratio between iron and manganese lay outside the range of 1.5 to 2.5 than when the ratio was within these limits.

Numerous investigators have since conducted further experiments in order to test the validity of the hypothesis of Shive and his co-workers (56,59,60). As a result there now exists considerable evidence that the hypothesis of Shive and his associates does not adequately explain the connection between iron and manganese in plant metabolism.

Arnon (2), in 1943, criticized the principle premise on which the hypothesis of Shive and his co-workers rested, namely that plants could only utilize ferrous iron. Arnon pointed out that the role of iron in oxidation-reduction systems, in which iron-porphyrin protein compounds such as the cytochromes or peroxidases take part, rests on the reversible oxidation-reduction of the iron, therefore, both the ferrous and the ferric form of iron must be regarded as

essential in plant metabolism; and it is also open to question whether the physiologically active iron-porphyrin enzymes would be included in the expressed sap or the press cake fraction of the iron determination.

Twyman (67), in 1951, carried out further investigations into the iron and manganese requirements of plants. In this study he made several different observations with regards to the supply of iron and manganese in relation to their apparent utilization in plants. With low supplies of both these elements, inadequate utilization of both iron and manganese was observed. The plants were always small and chlorotic and iron accumulated in their tissues. This supported the conclusion reached by other investigators that iron was inactivated within the plant. Due to the fact that large accumulations of inactive iron was associated with a low and intermittent supply of iron, Twyman suggested that a slow rate of entry of iron favoured inactivation. He put forward the idea that there may have been a primary or active iron acceptor responsible for transferring iron to active metabolic processes. However, if this primary or active iron acceptor did not react with iron it might have become irreversibly converted to a secondary product which formed a very stable compound with iron. The active iron acceptor in the absence of iron would likely be slowly and irreversibly converted to the inactive iron acceptor. Under conditions of low iron and adequate manganese supplies, he suggested that the plant

chlorosis was due to the manganese competing with the available iron for reaction with the active iron acceptors forming stable inactive metabolites with the resulting accumulation of both iron and manganese. With high manganese and adequate iron supplies, plants generally were chlorotic depending on the manganese concentration in the medium. The resulting chlorosis might have been caused by competition between the two elements for the active iron acceptors or to an antagonism between iron and manganese, the manganese in high concentration in the medium showing up the entry of iron, or to a direct toxic action of manganese. With low manganese and adequate iron supplies, manganese deficiency developed due to the small quantities of manganese accumulated in the tissues. Here iron antagonized the complete absorption of manganese from the media. All cases of manganese deficiency in oat and tomato plants were associated with high iron to manganese ratios in the medium and in the tissues. He observed with oats that as the iron-manganese ratio in the medium and in the tissues decreased it took longer for the first signs of grey speck to appear.

When the supplies of these elements were adequate and normal healthy plants were produced, the iron-manganese ratio in the medium appeared to have some effect on the yield. However, Twyman suggested that the optimum ratio was probably determined by factors other than nutritional such as temperature, day length, and light intensity. On the other hand, when the

supplies of iron and/or manganese were low, the absolute levels of iron and manganese supply were more important in determining the yield than the iron-manganese ratio.

Twyman observed that the symptoms of iron deficiency and manganese toxicity were practically indistinguishable in the early stages. He believed that the iron-manganese balance in the tissues and in the medium may have been pre-disposing factors in the development of iron deficiency, manganese deficiency, or manganese toxicity.

Hewitt (22,24) at Long Ashton carried out between 1944 and 1948 a series of extensive experiments on sugar beet, oat, tomato, potato and kale. In these experiments sand and water culture methods were used and at all times rigid precautions were taken in order to prevent contamination by iron and manganese. The effects of excess manganese, chromium, cobalt, nickel, copper, zinc and other metals on the iron status of these crops were observed. The most extensive experiments were carried out with sugar beet as it readily showed symptoms of iron deficiency and was remarkably tolerant to high concentrations of manganese.

The conclusions drawn by Shive and his associates (56,59),60) on the inter-relationships of iron and manganese required examination in relation to the results obtained by Hewitt (22). Other possible causes of metal induced iron deficiency also merited consideration on the basis of his findings. It has

been proved conclusively by Hewitt that iron and manganese exert independent effects in the metabolic processes, and that manganese toxicity is distinct from iron deficiency. Hewitt observed simultaneous symptoms of iron and manganese deficiency in the same leaves of oat plants when given low levels of both these elements. Observations made on several species between 1944 and 1948 showed that an excess of manganese resulted in characteristic symptoms which were different from those of iron deficiency. These symptoms of manganese toxicity might be produced without the accompanying chlorosis which even when present seldom resembled that produced by iron deficiency.

Hewitt (22) found that the absolute levels rather than the ratio of iron and manganese supplied, limited the growth of oat and sugar beet. If manganese was deficient increasing the iron supply was found to increase growth, but this was probably due to the trace of manganese carried by the ferric citrate, in spite of the fact that the ratio of iron to manganese was actually greater. However, the general incidence of chlorosis in sugar beet was definitely affected by both the level of iron and the iron-manganese ratio. He noted that if one element was in low concentration while the concentration of the other was increased, there was increasing severity of deficiency symptoms of the deficient metal. The nearer a metal was to the minimum supply the more easily it could be depressed thus, a Fe/Mn ratio of 0.5 with a near

borderline supply of iron could more quickly induce iron deficiency symptoms than a 0.2 ratio with higher iron. Unlike Shive and his associates (56, 59, 60), Hewitt (22) found no relation between substrate ratio, sap ratio or ratio of total iron and manganese in plants, absorption not being in the same ratio as that of the iron and manganese in the nutrient medium. Bennett (4), in 1946, made a somewhat similar observation. He showed that in tomato plants the ratio of iron to manganese in the tissues had no relation to the chlorosis produced and that manganese caused chlorosis by depressing the absorption of iron. This suggested the existence of a mutual antagonism between the two elements.

The visual results of Hewitt's (22, 23, 24) experiments showed that not only manganese but also several other metal ions may induce symptoms of iron deficiency. It was noted that many of the metals studied were far more active than manganese in causing iron deficiency symptoms. With sugar beet Hewitt gave the following arrangement of metallic ions according to the severity of their effects in inducing chlorosis:

Severe chlorosis -- Co^{++}, Cd^{++}, $CrO_4^{=}$, Cu^{++}, (Zn^{++})
Moderate chlorosis -- Zn^{++}, NO_3^{-}, Ni^{++}, Cr^{+++}, $MoO_4^{=}$
Slight chlorosis -- Ti^{+++}, Mn^{++}, Pb^{++}, $AsO_4^{=}$
Trace or nil -- Sn^{++}, Ce^{++++}, WO_4^{-}, Tl^{++}

The effect of extra molybdenum in combination with manganese was also examined. Hewitt observed that sugar beet

plants receiving manganese only were not chlorotic but those receiving extra molybdenum as well were severely chlorotic. However, recent work by Millikan (41) has demonstrated a manganese-molybdenum antagonism in water culture experiments with peas, cabbage and tomatoes.

The ability of the above metals to induce iron deficiency in tomato, potato, oat and kale, with a few exceptions, followed a similar order as that for sugar beet, but the different crops varied considerably in susceptibility. For example, it was observed that copper induced chlorosis in potato and tomato but not in oat or kale which are two plants showing strong susceptibility to the lack of iron.

Sugar beet plants receiving zinc developed symptoms resembling manganese deficiency simultaneously with those of iron deficiency. Determination of low soluble manganese in the tissues confirmed this. Nickel was found, by Hewitt (22, 23, 24) to induce manganese deficiency symptoms in potato and tomato. However, oat, a crop very susceptible to manganese deficiency, did not show these symptoms.

The suggestion by Shive and his associates (56,59,60) that the relative oxidation-reduction potentials of manganese and cobalt were responsible for inducing iron deficiency symptoms that required re-examination on the basis of the findings of Hewitt. In this connection it was shown that manganese and cobalt were not unique in their ability to induce chlorosis.

An important fact brought out by Hewitt was that zinc and cadmium which possessed the ability to cause chlorosis, did not undergo the valency change associated with simple oxidation-reduction reactions. The oxidation-reduction potentials of the simple ions as given by Latimer (32) showed no relation, to the results obtained by Hewitt. Hewitt further drew attention to the fact that the oxidation potential for iron is actually greater than that of some of the other metals such as Cu^{++}, Cr^{+++} and also Cd^{++}, therefore, on the hypothesis of Shive and his associates these metals should be incapable of oxidizing the postulated active ferrous iron. In criticizing this hypothesis Hewitt pointed out that as manganese and cobalt were given in the divalent form they must be first oxidized to the higher valency state by reactions in the plant.

On the other hand, according to Hewitt, (22,23,24) many of the metals studied would very likely be combined as complexes after absorption by the plant. The oxidation-reduction potentials of such complex ions might be very different from and bear no relation to those of the simple ions as given by Latimer (32). Hewitt suggested that the oxidation-reduction potential of these complex ions relative to the oxidation-reduction potential of iron, also in a complex form, might have presented a different situation. He pointed out that manganese or cobalt in a complex form would exist more readily in the trivalent rather than the divalent state. The sub-

sequent decomposition of these complexes would momentarily produce a powerful oxidizing potential which might possess the ability to maintain iron in the oxidized inactive state. The effects produced by zinc and cadmium, however, could not be explained on this basis. Hewitt concluded that hypotheses based on the oxidation-reduction potentials of simple ions are inadequate to explain the findings observed in his experiments and that any explanation based on the oxidation-reduction potentials must await more detailed knowledge of these metal compounds as well as of the exact nature of active iron in plants.

Other suggestions have been put forward to explain the production of symptoms of iron deficiency in plants. Hewitt (22,24) pointed out that chlorophyll production is not the only reaction involving iron as this element is required in catalase and cytochrome enzyme systems. He found that it was impossible to distinguish visually between effects caused either by interference in the specific function of iron in chlorophyll formation or by competition between this and other reactions for limited amounts of available iron, if the iron requirement in one of these other reactions increased by the presence of an excess of one of the toxic metals. He suggested that competition between the toxic metal ions and iron in enzyme systems involved in chlorophyll formation, or the activation of enzyme systems influencing

the ferrous-ferric iron equilibrium may also be important in reactions inducing symptoms of iron deficiency.

According to Hewitt, it was possible that some of the metals studied may have competed with iron simply by blocking a reaction leading to chlorophyll synthesis because of a similarity in their ionic radii, valency, and electron configuration. Protoporphyrin 9, which in 1948 was found by Granick to be a metabolic forerunner of chlorophyll, had the basic plan of the ring structure and similarities of the side chain patterns of chlorophyll but with iron instead of magnesium being attached to the four nitrogen atoms of the pyrrol ring. The basic structural units of the cytochromes, peroxidases, oxidases and chlorophyll were porphyrins. The findings of Granick (19), Taylor (62) and Gjessing and Sumner (18), indicated the conversion of porphyrins to chlorophyll and the replacement of the metal in the pyrrol ring by other metals. This suggested a hypothetical mechanism of plant chlorosis resulting from a low iron and high manganese content of the plant tissues which was put forward by Sideris and Young (57). They explained it on the basis of biochemical antagonism of homologous substances whereby manganese is substituted for iron in protoporphyrin 9. As a result this chlorophyll precursor is inactivated thus preventing the subsequent conversion to chlorophyll. Baudisch (3) explained the physiological activity of the metal atoms in the pyrrol ring as due to paramagnetic properties generated by the

arrangement of a positive ion (Fe, Mn, Cu, etc.) in the centre, surrounded by neutral or negative atoms (n) which tended to gain electrons by co-ordinating atoms, forming functional units in definite energy stages of electric or magnetic fields. Hence, any changes in the structure of the porphyrins, which may have resulted from replacement of iron by manganese or other metals, might either increase or decrease the activity of the prosthetic substance, whether it is an enzyme or chlorophyll, and thus alter the rhythmic operation of processes on which most cell functions are dependent.

The investigations of Oserkowsky (44) in 1933, Jacobsen (31) in 1945, Bennett (4) in 1946 and Twyman (67) in 1951, have all produced evidence for the existence of active and inactive forms of iron. Bennett (4) and Jacobsen (31) postulated that the active iron may be closely associated with a definite protein fraction in the leaves. Futhermore, some of the metals may cause inactivation by precipitation or denaturation of these proteins or by adsorption on them cause a resulting interference with their functions.

Bennett (4) divided iron and protein in pear leaves into two fractions active iron and residual iron and active protein and residual protein. The active iron and the active protein were found to be proportional to chlorophyll content. On this basis, Bennett assumed that active iron was associated with active protein and residual iron with residual protein. Because

protein is the most abundant substance in the protoplasm and is known to have a considerable binding capacity for iron, Bennett suggested that the development of chlorophyll appeared to depend on a sufficiently rapid rate of entry of iron so that not all of it was absorbed by the residual protein. According to this author, chlorosis is a disturbance of nitrogen metabolism as well as of iron metabolism and the two are intimately related. Thus, the influence of various nutritional factors would probably be exerted through their relation to nitrogen metabolism, particularly any effect they might have on the nature and amount of proteins produced.

It is interesting to note that quite a number of investigators have held the opinion that manganese is associated with nitrogen metabolism. In 1919, McHargue (40) observed that manganese was concerned with the assimilation of nitrogen. In 1942, Leeper (34) and in 1941, Whitehead and Olson (68) each have shown that nitrates accumulated in plants when the culture medium was low in manganese. Eversmann and Aberson (14) found that the incidence of grey speck generally coincided with the presence of nitrite ion in the roots, the application of manganese, by increasing the oxidase content of the roots had the effect of decreasing the nitrite content. In 1940, Burstrom (10) came to the conclusion that manganese directly catalysed nitrate assimilation.

Hewitt (22, 23) stated that at the present time it cannot

be inferred that the mechanism of induced chlorosis is the same for each metal. Evidence has shown that the problems of metal induced iron deficiency are complex, and hypotheses based on the relative oxidation-reduction potentials of the simple ions of the metals, which Hewitt studied, are inadequate to explain their activity and further, more than one mechanism may be involved. From Hewitt's experiments it has been shown that manganese is not unique in its ability to induce chlorosis, in fact it may in this respect, be much less active than other metals.

The observations, by Hewitt (22) of simultaneous symptoms of iron and manganese deficiencies in the same plants would appear to indicate independent functions for these two elements and this conclusion is supported by the visual distinction between manganese toxicity and induced iron deficiency. On the other hand, Shive (56) issued a warning against this tendency of isolating the functions of individual trace elements. While realizing that on the basis of existing data it was impossible to assign any one function to a given trace element, Shive believed each trace element to be a critical factor in every important physiological process involved in plant nutrition.

II. Grey Speck Disease of Oats

Grey speck disease of oats is widely distributed, occurring in different parts of Europe, including Britain, in America, and in Australia. The disease is characterized by the appearance

of greyish colored spots in the leaves. These small chlorotic areas occurring chiefly in the lower half of the leaf may gradually enlarge until they spread across the entire leaf. The symptoms usually appear when the plant has reached the third or fourth leaf stage. As the spot enlarges, the grey color gradually gives way to yellow, the yellow color finally extending over a considerable length of the leaf. At about the time the infected area has reached the full width of the leaf, the leaf droops leaving the yellow portion standing up. During all this time the tip of the leaf remains green but may eventually turn brown and die. Badly affected plants may be stunted and die early. In less severe cases flowers may be produced but little grain is formed. Root development tends to be poor.

In accordance with the widely held view that grey speck disease of oats is directly or indirectly the result of manganese deficiency the absence of visible symptoms of the disease during the early stages of growth is presumably due to a certain amount of manganese stored in the seed, this being sufficient to provide for the healthy appearance of the first one or two leaves of the seedling. But when this store is exhausted the future well being of the plant will then depend on the availability of manganese in the soil.

Several theories have been advanced to explain the conditions under which manganese in the soil is rendered unavailable

to the plant and to account for the characteristic symptoms of grey speck disease of oats. More recent investigations are those of Sherman and Harmer (53,54) in 1941 and 1942. To be available to plants, manganese must exist as exchangeable manganese, as a part of organic matter, or as inorganic, easily reducible manganese. It is readily oxidized to the manganic form thus factors affecting this manganous-manganic equilibrium are of utmost importance. According to Sherman and Harmer, this equilibrium is affected by relative acidity or alkalinity of the soil lime and phosphate content, aeration, temperature, clay content, and the presence of certain reducing and oxidizing compounds. They observed that on alkaline soils high in lime, this equilibrium would swing towards the manganic side thus rendering the manganese unavailable. Likewise any manganese applied in a soluble form would be quickly oxidized to the manganic form. They noted that grey speck is prevalent on such alkaline soils especially if the amount of organic matter present is high. Steenbjerg (61) in 1933, found that the amount of exchangeable or soluble manganese in the soil was regulated by the soil reaction. According to Steenbjerg, this soluble manganese was not only influenced by the conditions of oxidation-reduction in the soil but increased as the pH of the soil decreased. In 1935, Leeper (33) observed that grey speck disease was confined to soils of pH 6.7, or higher, occurring especially on heavily limed and sandy soils. In 1934, Gilbert (17) found

that the cause of manganese chlorosis was definitely linked with soil alkalinity and that the pH range within which the chlorosis occurred appeared to vary with the crop and the climate and not with the soil type. Lundegardh (37) in 1934, believed that the disease was caused by a disproportionate quantity of lime in the soil as compared with other minerals, thus upsetting the balance of the mineral content which resulted in an abnormal absorption of certain salts. Samuel and Piper (48, 49) in 1928 and 1929, maintained that if manganese was present in the highly ionized sulfate form, an excess of calcium ions did not necessarily render the manganese unavailable.

Microbial activity in the soil has been shown to affect the solubility of manganese. As early as 1904, Schorler (52) reported that some iron bacteria were capable of oxidizing manganese salts and precipitating manganese hydrate in their bodies. Around 1937, Gerretsen (15, 16) stated that the precipitation of insoluble manganic oxides in the soil was caused by specific microorganisms between pH 6.5 and 7.8. He found that these limits appeared to coincide with values obtained in the field where grey speck was more frequent. MacLachlan (39) in 1941, and Chaplin (11) in 1946, were able to isolate manganese-oxidizing bacteria and fungi thus attributing the deficiency of available manganese in the soil to microbiological activity. Furthermore, Chaplin pointed out that soil fungi and actinomycetes were able to oxidize manganese directly or indirectly

by lowering the oxidation-reduction potential of the medium. Söhngen (58) in 1914, suggested that the beneficial action of acid fertilizers, a well-known treatment in the control of manganese deficiency diseases, may not have been due so much to a change in the pH values of the soil as to the fact that, at lower values of soil reaction, insoluble manganese compounds such as manganic oxide were reduced to soluble forms by the inter-action of certain microorganisms. Gerretsen (15, 16) observed that in mixed bacterial cultures containing manganese sulfate, some of the bacterial colonies were found to contain manganic oxides presumably precipitated by the appropriate organism, but as soon as the reaction of the media became more acid these substances were dissolved, possibly by the metabolic products of other organisms in the mixed culture.

That grey speck disease of oats is in some way linked up with the presence or absence of manganese cannot be disputed but whether manganese is directly or merely indirectly concerned has not yet been conclusively established.

In 1928 and 1929, Samuel and Piper (48, 49) using Algerian oats conducted carefully controlled water culture experiments from which the smallest trace of manganese had been removed. They observed that in the absence of manganese, the experimental plants exhibited in every detail the characteristic symptoms of grey speck as produced in the field. They found that with 1 part of manganese in 10 million no grey speck symptoms appeared.

They calculated, that to satisfy the needs of the average oat plant, about 1 part of manganese in from one to five million parts of solution was the optimum for growth in water culture, one or two changes of the culture solution being made during growth until the plants reached fruition. Twyman (66) in 1943, also using water culture technique, produced the typical symptoms of grey speck in solutions containing no manganese. In 1942, Hodge, Hageman, McHargue, and Sherman (27) using sand culture methods, also produced symptoms of grey speck in the cultures receiving no manganese; the symptoms being identical with those produced on manganese deficient soils.

The relationship between iron and manganese in regard to the appearance of grey speck disease of oats has already been reviewed on pages 23 - 25. From this it may be seen that there is some evidence to show that a high iron to manganese ratio will cause the rapid appearance of grey speck symptoms.

Gerretsen (15,16) disputed the view that grey speck disease of oats is the direct effect of manganese deficiency. He drew attention to the fact that Lundegardh (36) in 1932, observed that the manganese content of healthy plants might be lower than the manganese content of diseased plants. In fact Lundegardh recorded values up to 420 p.p.m. of manganese in affected plants and down to 1 p.p.m. in healthy ones. This was definitely contrary to general experience. Samuel and Piper (48,49) found that approximately 14 p.p.m. was the minimum

amount of manganese likely to be present in healthy Algerian oats at the flowering stage.

Gerretsen (15, 16) found that when a soil which had yielded a crop showing symptoms of grey speck was sterilized with formalin, the following crop of oats grown on it were free from grey speck although the available manganese content was not changed. On reinfecting the sterilized soil with 10 per cent of the original soil, grey speck symptoms appeared on the oats grown in it and both the dry weight and manganese content of the affected plants were reduced. In similar experiments using sand and water culture methods the results were the same, except that the sand culture experiment the diseased plants had a higher manganese content than the healthy ones.

A poorly developed root system, with a number of decaying roots and early destruction of the root hairs, was one of the most striking features of manganese deficiency. According to Gerretsen (15, 16) the roots soon became decayed at the tips and under such conditions were rendered highly susceptible to bacterial infection. He concluded that it was necessary to differentiate between the direct physiological effect of manganese deficiency, which was an overall retardation of growth and the symptoms of grey speck disease of oats which were related to the presence of microorganisms, these microorganisms, infecting the roots thus causing the onset of the disease. He considered that the ability of the roots to resist parasitic

attack by microorganisms was dependent upon the manganese content of the plant, the amount of manganese present determining whether certain bacteria act as saprophytes or parasites. Gerretsen (15,16) maintained that if the roots were kept sterile, healthy plants were produced in the presence of a very small supply of manganese so that the manganese content of the plant was only 5 to 35 p.p.m. Nevertheless Samuel and Piper (48,49) found that the application of a germicide to the soil did not check the trouble.

Timonin (64) in recent investigations carried out with diseased (manganese deficient) and healthy soils, determined that a susceptible variety of oat harbored in its rhizosphere a denser population of manganese oxidizing, casein hydrolyzing, and dentrifying bacteria than the rhizosphere of a resistant variety when grown in the same soil and under identical conditions. Furthermore, the application of soil fumigants greatly reduced or completely eradicated the bacteria capable of oxidizing manganese. The plants grown on the treated soils were free from grey speck and showed a marked increase in yield of grain.

MacLachlan (39) in 1941, found that the cause of manganese deficiency in a soil on which grey speck developed was apparently biological. Bacteria were isolated which actively converted manganese sulfate to the oxide form. It seemed evident, therefore, that this soil contained an excess of

bacteria and possibly other microorganisms, capable of, or contributing to, manganese oxidation, reducing the level of available manganese below the minimum requirements for the normal development of oats. The typical symptoms were observed by MacLachlan but Gerretsen's (15, 16) explanation of the cause on the basis of root injury did not apply. A foliar application of manganese sulfate to the crop eliminated any evidence of chlorosis and prevented the further development of necrotic areas.

In a number of tests the ammonium content of a grey speck diseased plant was found to be two or three times that of a normal plant. Gerretsen (15, 16) thought this increase to be due not only to the ammonium produced by bacterial activity in the diseased root tips but also by protoplasmic autolysis followed by carbohydrate hunger which was caused by reduced photosynthesis. MacLachlan (39) decided from the results of his investigations, that if ammonia or other decomposition products were responsible for the necrosis it must have been produced by protoplasmic autolysis within the leaves alone.

Hedlund (21) in 1937, believed that the primary cause of grey speck lay in the shortage of sugar in the leaves, the result being an immediate lowering of the resistance of the plant.

Hiltner (25, 26) in 1924 and 1926, and Hopkins (29) in 1934, decided that the action of manganese was that of a stimulant

or a catalyst having an indirect effect on the photosynthetic activities of the plant.

Gerretsen (15, 16) stated that manganese played an important role in the oxidation-reduction process connected with photosynthesis. He found that in manganese deficient leaves carbon assimilation was reduced to approximately one half that of normal leaves, thus accounting for the diminished yield, the reduced root system and the lowered resistance of the roots to invading microorganisms.

In 1905 Bertrand (5) who first called attention to the importance of manganese in 1897, established the fact that the quantity of oxidases in the roots was increased in the presence of manganese, the effect being most evident in the early stages of growth. Unaware of the possible role of soil organisms in relation to grey speck disease, Eversmann and Aberson (14) in 1927, observed that the incidence of the disease generally coincided with the presence of the nitrite ion in the roots and perhaps also in the leaves, the application of manganese by increasing the quantity of oxidases in the roots, had the effect of decreasing the nitrite content.

From the foregoing it will be seen that there is general agreement on the idea that manganese deficiency is an important factor in causing grey speck. Whether this deficiency is the direct cause or whether it operates indirectly through the medium of its influence on bacterial infection as suggested by

Gerretsen does not seem to have been definitely established. More experimental data are needed if a definite conclusion is to be reached and it is for the furnishing of such data that the present investigation was carried out.

MATERIALS AND METHODS

Experimental Plants

The experimental plants used were *Avena sativa* var. *Tama* grown from seed provided by Dr. W.H. Hagborg of the Dominion Laboratory of Plant Pathology, Winnipeg.

This variety of oat was known to be susceptible to grey speck disease and the seed was obtained from Tama oat plants grown on experimental plots in Oak Bank, Manitoba in 1949. The plants received no treatment and showed severe symptoms of grey speck.

Analyses of a sample of this seed showed an iron content of 0.063 mg. per gram of dry material and a manganese content of 0.0023 mg. per gram of dry material.

Reagents and Methods of Purification

Owing to the nature of the investigation it was decided that the water culture technique was the one most likely to yield satisfactory results. Certain necessary precautions such as purification of the salts used, and the employment of distilled water of high purity had to be observed in order to remove any danger of contamination by iron and manganese.

Distilled water from an electrically operated Barstend still was used throughout the study both for making the culture solutions and for washing the glass apparatus. The distilled water was found by use of a Coleman potentiometer to have a constant pH of 6.5. Two litres of the distilled water was

evaporated down to 25 ml. It was then tested for iron and manganese by spectrophotometric methods, and was found to contain no detectable quantities of either iron or manganese.

Pyrex glassware, both for culture vessels and for other purposes, was used throughout the experiments.

The glass apparatus, such as pipettes, aerators, test tubes, volumetric flasks etc., was cleaned by treating with cleaning solution (potassium dichromate and concentrated sulfuric acid) for at least an hour followed by final rinsings with not less than six changes of distilled water.

B.D.H. AnalaR or Fisher T.P. reagents were used for making the culture solutions. In order to eliminate any possible traces of iron and manganese from the macro salts used in the preparation of the stock solutions, they were purified as described below.

The purification procedure used was that of Arnon and Stout (1). This method, because of its simplicity and efficiency, has become widely used in research dealing with the study of trace elements. It is an adsorption procedure involving the co-precipitation of heavy metals with calcium carbonate and calcium phosphate under alkaline conditions.

One or two litres of a M/2 stock solution of each of the macro elements was prepared. Calcium carbonate was then added to all the stock solutions, the amount added depending on the salt being purified. Dibasic potassium phosphate and

calcium nitrate were also added unless they were already present. The solutions were autoclaved for 90 minutes at 18 pounds pressure, allowed to settle for 24 hours, filtered and restored to volume with distilled water. Ammonium sulfate was heated for 45 minutes in an Arnold Steam Chamber instead of being autoclaved, other than that the procedure was the same as that for the other salts.

The purified salts were tested for iron and manganese by spectrographic and spectrophotometric methods. Twenty millilitres of each solution were mixed and evaporated to dryness. Spectrographic analysis of this mixture showed that all detectable traces of manganese had been removed but that some iron still remained in the nutrient solution. Spectrophotometric analysis showed that the amount of iron remaining in the stock solutions did not exceed 0.08 p.p.m. This amount was not significant when the dilution of the stock solutions in the preparation of the nutrient medium was taken into consideration. However, the solutions were purified once again with calcium carbonate, which procedure completely removed all traces of iron and manganese.

Salts of the micro elements were not purified. These salts were only present in trace amounts, 0.05 to 0.02 p.p.m., in the culture solution so that any impurity they might contain was negligible.

Another purification procedure which was attempted will be mentioned briefly. To a solution containing iron was added an adsorbing agent, amberlite. The whole was well

shaken and allowed to stand for several hours. At intervals during this time a few millilitres were decanted and iron tests carried out. However, this method did not prove satisfactory as the adsorbent, while removing some of the iron, left a measureable quantity of the metal still in the solution.

Culture Solutions

During the course of this study three different culture solutions were used. All the solutions were prepared from the purified stock solutions and made up to volume with distilled water. The culture solutions were designated by letters and these letters will be used throughout this account when referring to particular solutions.

Solution A

Salt	ML. M/2 Stock Per L. of Nutrient Solution
K_2HPO_4	4.6
$Ca(NO_3)_2 \cdot 4H_2O$	9.0
$MgSO_4 \cdot 7H_2O$	4.6
$(NH_4)_2SO_4$	1.4

Solution B

Salt	ML. M/2 Stock Per L. of Nutrient Solution
K_2HPO_4	1.16
K_2SO_4	1.63
$Ca(NO_3)_2 \cdot 4H_2O$	9.00
$MgSO_4 \cdot 7H_2O$	4.60
$(NH_4)_2SO_4$	1.40

Solution C

Salt	ML. M/2 Stock Per L. Nutrient Solution
$Ca(NO_3)_2 \cdot 4H_2O$	7.15
$MgSO_4 \cdot 7H_2O$	4.40
K_2HPO_4	1.67

Solution A was a modification of a solution designed by Shive and Robbins (55). Dibasic potassium phosphate replaced the monobasic salt, which could not be purified with calcium carbonate. The pH of the solution was found to change only very slightly when the plants were grown in it.

Solution B was a modification of a solution used by Somers and Shive (60) for soy bean. Again the dibasic potassium phosphate replaced the monobasic salt. The nitrogen concentration was increased by the addition of nitrogen in the form of ammonium sulfate. The solution changed slowly to alkalinity when the plants were grown in it.

Solution C was based on one used by Olsen (43) for rye. It was found to be the most satisfactory solution for oat plants. However, the solution changed pH (towards alkalinity) more rapidly than did other Solutions A or B.

Two stock solutions were prepared containing the micronutrients, Solution I containing boron, copper and zinc and Solution II containing molybdenum. One millilitre of Solution I and one millilitre of Solution II was added to each

litre of culture solution. This provided a concentration of 0.5 p.p.m. of boron, 0.05 p.p.m. of zinc, 0.02 p.p.m. of copper and 0.05 p.p.m. of molybdenum.

These two micronutrient solutions were prepared from AnalaR salts as follows:

Micronutrient Solution I

$ZnSO_4 \cdot 7H_2O$	0.220 grams
H_3BO_3	2.860 grams
$CuSO_4 \cdot 5H_2O$	0.080 grams
Distilled water	to 1,000. millilitres

Micronutrient Solution II

MoO_3	0.075 grams
Distilled water	to 1,000. millilitres

After the addition of the predetermined quantities of iron and manganese to the culture solution, an adjustment of pH was generally required. AnalaR nitric acid and AnalaR potassium hydroxide were used to make this necessary adjustment of pH.

The supply of iron and manganese was varied in the different experiments. Manganese was always supplied as manganese sulfate, $MnSO_4 \cdot 4H_2O$. Iron was supplied either as ferrous sulfate, $FeSO_4 \cdot 7H_2O$ or as iron humate.

Manganese sulfate was found to very soluble and stable within the pH range used in the culture solutions. Ferrous sulfate, on the other hand presented a major problem

as the iron would not remain soluble in the culture solution. Various procedures were attempted in order to keep the iron in solution when it was supplied as ferrous sulfate. At low iron concentrations (0.25 p.p.m.) and by maintaining a low pH (around pH 4) the iron tended to remain in a soluble form in the solution for several days. However, when the iron concentration was increased, although the pH was kept around 4, the iron was precipitated and nearly completely removed from the culture solution in a matter of a few hours. In one experiment when the culture solution was being changed, one of the beakers containing the solution to be discarded was set aside and tested for iron. A few millilitres were decanted from the top of the solution and analyzed; no iron was found to be present. The solution in the lower part of the beaker containing suspended precipitates was then tested and was found to contain substantial amounts of iron. Similarly, the fresh solution which contained a slight precipitate was also tested. This time the solution was filtered and the filtrate and precipitate tested. A positive test was noted in both cases but the concentration of iron in the precipitate greatly exceeded that in the filtrate. Upon further investigation it was discovered that the phosphate ion was responsible for the precipitation of the iron as ferric phosphate. Because the phosphate ion could not be omitted from the culture solution, the concentration of all the salts (macro

and micro) except for iron and manganese, was halved. In this way the mineral balance was not altered but the concentration of each element was lowered. Hence with a lowered phosphate concentration it was thought that there would be less tendency to precipitate the iron especially if the pH was around 4. More iron did remain in solution for a longer period of time but it was found to be eventually completely removed by precipitation. In another instance when iron was supplied in a single salt solution, the solution if left standing but covered, developed an oily looking layer on the top of the solution. The iron, which is readily oxidized, had been oxidized to the highly insoluble ferric oxide which could only be dissolved by treatment with concentrated nitric acid and concentrated sulfuric acid. Ferric chloride was then substituted for ferrous sulfate but the same difficulties were encountered. It was concluded, therefore, that the only way to maintain ferrous sulfate in a soluble form would be to use the continuous flow method whereby the solution is continually being changed and fresh iron being added. This, however, was not feasible with facilities available.

Because it was necessary to maintain high concentrations of iron in solution, organic humate iron was prepared. Humate iron has been found to be an active stimulator of bacterial growth and probably fungal growth as well. This was the main reason the use of an inorganic form of iron was in the

beginning considered desirable. In spite of the above-mentioned drawback to the use of humate iron, the necessity of employing a method which would maintain iron in solution suggested that it should be given a trial.

A synthetic potassium humate was prepared as outlined by Horner, Burke and Hoover (30). This method had its advantages as it was convenient to use and the humate was not precipitated by high concentrations of phosphate. In an alkaline solution the humate will readily adsorb iron. Iron presented in this colloidal form remained available to the plants for long periods and was only subject to precipitation by relatively high amounts of calcium.

The method used in the preparation of the synthetic humate is described below.

One litre of 30 per cent by volume sulfuric acid was brought to the boiling temperature. One hundred and twenty grams of B.D.H. sucrose were added to the acid solution and the mixture was then allowed to simmer for three hours at 115 degrees centigrade. The preparation was cooled and filtered, the liquid phase being discarded.

A 40 per cent potassium hydroxide solution was added to the solid phase until upon being partially dissolved the solution became alkaline to phenolphthalein. The preparation was then filtered, and the solid phase discarded after one or more washings with dilute alkali. The alkali extract and

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washings contained relatively pure potassium humate but were further purified by one more cycle of precipitating with sulfuric acid at pH 3-4, filtering and discarding the liquid phase, and redissolving the solid hydrogen humate by neutralization with 1-5 per cent potassium hydroxide to give a neutral solution. This solution was transferred to a 1,000 ml. volumetric flask and brought up to volume with distilled water and potassium hydroxide so that the solution remained slightly alkaline.

The concentration of humate in the final stock solution thus prepared was determined by precipitating the humate in a 5 ml. aliquot, filtering through a weighed fritted glass filter drying the precipitate at 100 degrees centigrade, then weighing the filter and precipitate. The total yield of potassium humate was found to be approximately 18 grams.

Iron humate was prepared by adding ferrous sulfate to the neutral solution of potassium humate. The potassium humate was capable of taking up 1.5 to 2.25 mg. of iron per millilitre. Because the potential acidity of the ferrous salt added to the humate solution lowered the pH, a precipitate of chiefly iron humate and some hydrogen humate was formed. However, the addition of a strong alkali brought the precipitate back into solution.

<u>Culture Methods</u>

The use of water culture technique, while having its

advantages in allowing rigidly controlled conditions, cannot fully replace the soil which is the normal environment of plants. Conditions operating in the soil may not be duplicated in sand or water culture methods. Nevertheless successful plant growth will result if various factors are carefully controlled. These factors are aeration of the solution, a balanced nutrient supply, and a controlled pH. Other external factors such as light, temperature, and atmospheric humidity may affect the experiments. However, under greenhouse conditions the control of these factors does not present a major problem.

Preliminary procedures such as germination of seeds and setting up of apparatus will be discussed under separate headings.

1. <u>Germination Procedures</u>

The oat seeds were germinated by one of three methods as follows:

(i) The seedlings were grown with their roots passing through one quarter inch holes in paraffined, ashless filter papers. These filter papers, to the lower sides of which small pieces of cork were attached, were floated on distilled water in glass dishes. Passing through the holes and resting under each seed was a narrow strip of ashless filter paper which acted as a wick thus keeping the seeds in contact with the water.

(ii) The seeds were germinated on sterile quartz sand kept moist with distilled water.

Before use the quartz sand was freed of iron and manganese by soaking in 10 per cent nitric acid for 14 days, washing under a hot water tap for 48 hours, soaking in distilled water for 48 hours and rinsing in distilled water. The rinsings with distilled water were repeated until the pH of the wash water was 6.5.

After receiving this initial purification, between usage the sand was soaked in 10 per cent nitric acid for 24 hours, then rinsed with distilled water.

This method was satisfactory for germination of the seeds. However, because of the limited amount of distilled water which was available and because of the large amount of distilled water required to free the sand from the nitric acid, this method was discarded.

(iii) The seeds were germinated in a glass dish on moist ashless filter paper. This method was found to be the most satisfactory of the three.

In all the methods the glass dish, containing the germinating seed, was partially covered with a glass plate until the seedlings were $1\frac{1}{2}$" in height.

2. The Culture Experiments

When the seedlings were approximately 6" in height, they were ready to be transferred to the nutrient solutions. In order to eliminate such sources of manganese and iron, the endosperms were removed from the seedlings before the

the experiments were set up.

The pyrex culture vessels (300 or 600 ml. Berzelius beakers) were covered with paraffined cardboard squares. The plants were supported by non-absorbent cotton in glass tubes $\frac{1}{2}$" in diameter and 1 to 2" long. These were pushed through bored holes in the cardboard squares and held in place by rubber bands. Additional support was required as the plants grew in size. In the earlier experiments this support was provided by pyrex glass tripods, 8" in height, which rested on the tops of the paraffined covers. In later experiments support was provided by enamelled copper wire loops which were hooked on to horizontal glass rods. The glass rods were supported above the plants by means of clamps and stands.

The culture solutions were kept up to volume in the beakers by the addition of distilled water.

To inhibit or prevent the growth of algae the beakers were covered with black paper.

A one-third h.p. electric pressure pump provided aeration of the nutrient solution. Air from the pump was passed through a fritted glass disk into a Pyrex bubbling vessel and was then filtered by water. Stands and clamps held in place a solid glass rod 4' long. To this was wired a tube formed by the horizontal arms of Pyrex T tubes linked by rubber. One end of this tube was attached to the Pyrex bubbling vessel by means of 3 mm. bore pressure tubing. The other end was

attached to a Y tube. One arm of the Y tube carried the air into the upper end of a long, glass tube which permitted the air to enter near the bottom of a glass cylinder 2' in height, and containing water to a constant level of 1½'. This latter apparatus served for the adjustment of the pressure to a satisfactory level. Bubbler tubes connected to the horizontally arranged series of T tubes and passing to the bottom of the culture vessels served to keep the latter aerated. Screw clamps on the rubber tubes connecting the aeration tubes to the T tubes served to adjust the aeration supplies to the individual culture vessels. The general arrangement is shown in Figure 1.

Such factors as, aeration of the nutrient solution, a balanced nutrient supply, and a controlled pH could be maintained. However, external factors such as light, temperature and humidity, which under greenhouse conditions can be controlled, presented a major problem in this work. In the majority of experiments, the oat plants were grown in the Potting Room on the top floor of the Science Building. At all times the atmosphere was very dry and nothing would be done to alleviate this condition. Different methods were attempted but the only one which would have proved successful was not feasible. This method entailed keeping the cement floor of the room continually wet.

Temperature was also a problem during the winter months especially due to the fact that the heat was turned off in the building over the weekend. This was partially overcome by use

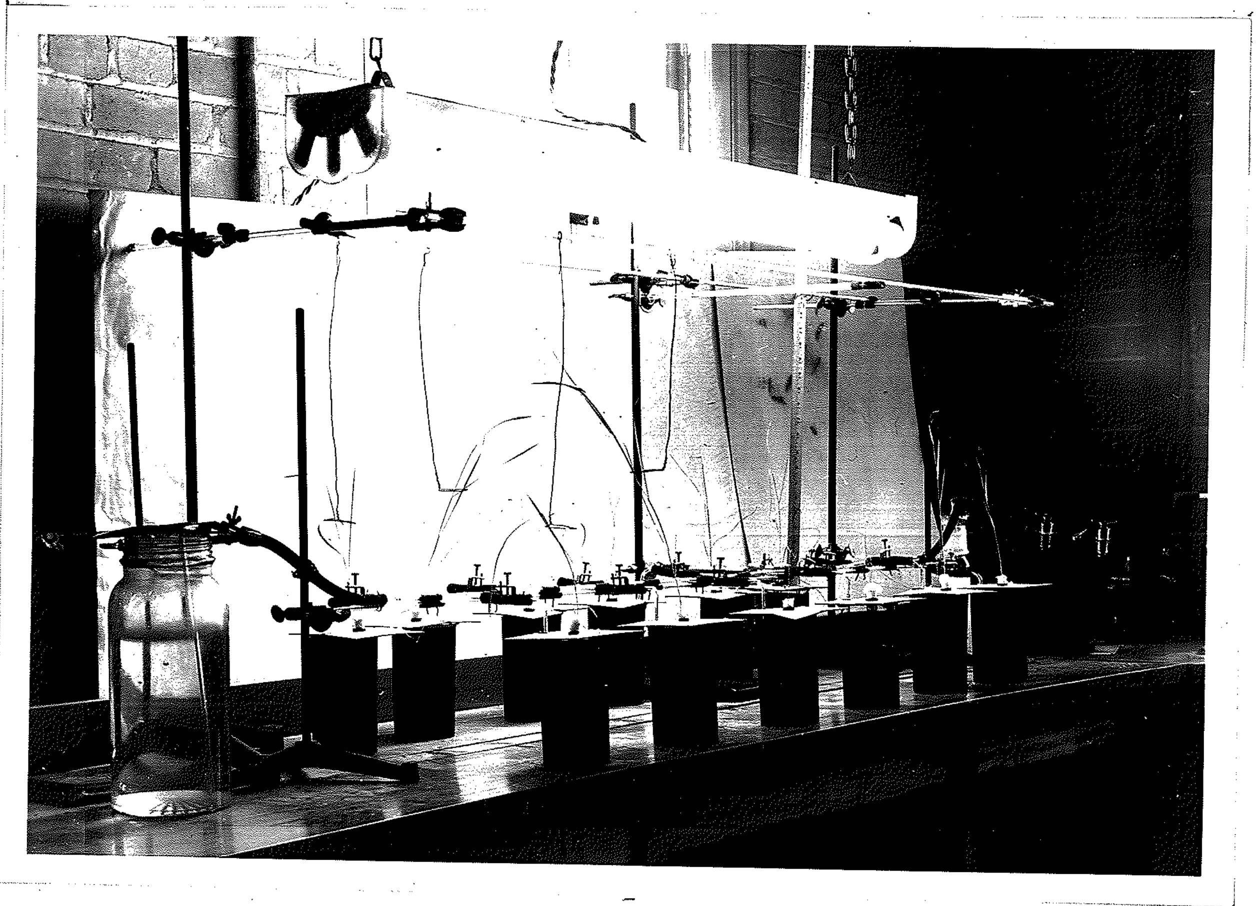

FIGURE 1.

THE GENERAL ARRANGEMENT FOR THE SETTING UP OF THE WATER CULTURE EXPERIMENTS

of infra red lamps. However, this provided continuous heat, the room frequently became far too warm. As a result the heat combined with the dry atmosphere definitely did not provide optimum conditions for healthy plant growth.

The windows of the experimental room faced north which was far from ideal in the winter. Supplementary lighting was provided by three 40 watt fluorescent lamps, one of which was daylight and the other two white. These were suspended above the plants by chains of adjustable length.

During the summer months of 1950 the plants were grown in a laboratory along a south facing window. Here no artificial lighting was necessary.

Analytical Methods

Various micro analytical methods were tried for the quantitative estimation of iron and manganese in plant material. These involved the use of both the spectrophotometer and the quartz spectrograph.

The Spectrophotometric Method of Analysis

The use of the spectrophotometric methods in analytical chemistry is based on the possibility of converting the constituent to be determined into a substance whose solution is strongly colored. Such a solution shows differential absorption of light of different wave lengths. The measurement is made first with a reference and then with the colored experimental

sample interposed in the light beam, the ratio of the two intensity measurement being a measure of the transmittance of the sample at the wave length of the test. It is customary to assign a transmittance of 100 per cent to the reference (reagent solution plus distilled water or distilled water alone) as this represents zero concentration of the constituent, the concentration of which is to be determined.

The first step in the method is the construction of the Spectral-Transmittance (S-T) curve. The purpose of this curve is to determine that wave length at which the constituent to be measured has minimum transmittance. The S-T curve was made by measuring the transmittance of solutions of iron and manganese in which the appropriate colors had been developed, at a series of wave lengths within the range of the spectrophotometer, using the corresponding colorless references. The data were then plotted on a graph with transmittance, expressed as per cent as ordinate, and with wave length expressed as millimicrons as abscissa. The S-T curves for iron and manganese were thus obtained. From these curves the minimum transmittance for iron occurred at 510 millimicrons and for manganese at 530 millimicrons. To illustrate the type of curve produced the S-T curve for manganese is shown in Figure 2.

The usual method of using the spectrophotometer involves the construction of a Concentration-Transmittance (C-T) Calibration curve for the constituent being determined. For

this purpose solutions of iron and manganese of known concentration were taken and treated in exactly the same manner as the sample solutions for the development of colors and measurements of the transmittance at the optimum wave length were made. The transmittance of each colored sample of known concentration (C) was determined, plotted on semi-log paper and a straight line drawn intersecting this point and the point (C=0, T=100 per cent). Thus C-T calibration curves were obtained for both iron and manganese. As the relationship between concentration and transmittance follows Beer's law the curve theoretically is a straight line. The curves were checked several times by preparing iron and manganese solutions of known concentrations, reading the transmittance then noting if these points fell on or very close to the C-T calibration curves. The C-T calibration curves for iron and manganese are shown in Figure 3.

These C-T curves for iron and manganese were used for the determination of these metals throughout the course of this research.

The methods mentioned above for obtaining the S-T curve and the C-T calibration curve are described in detail in the <u>Operation Directions for the Model 14 Coleman Universal Spectrophotometer</u>, which was the instrument used, and also by Sandell (50).

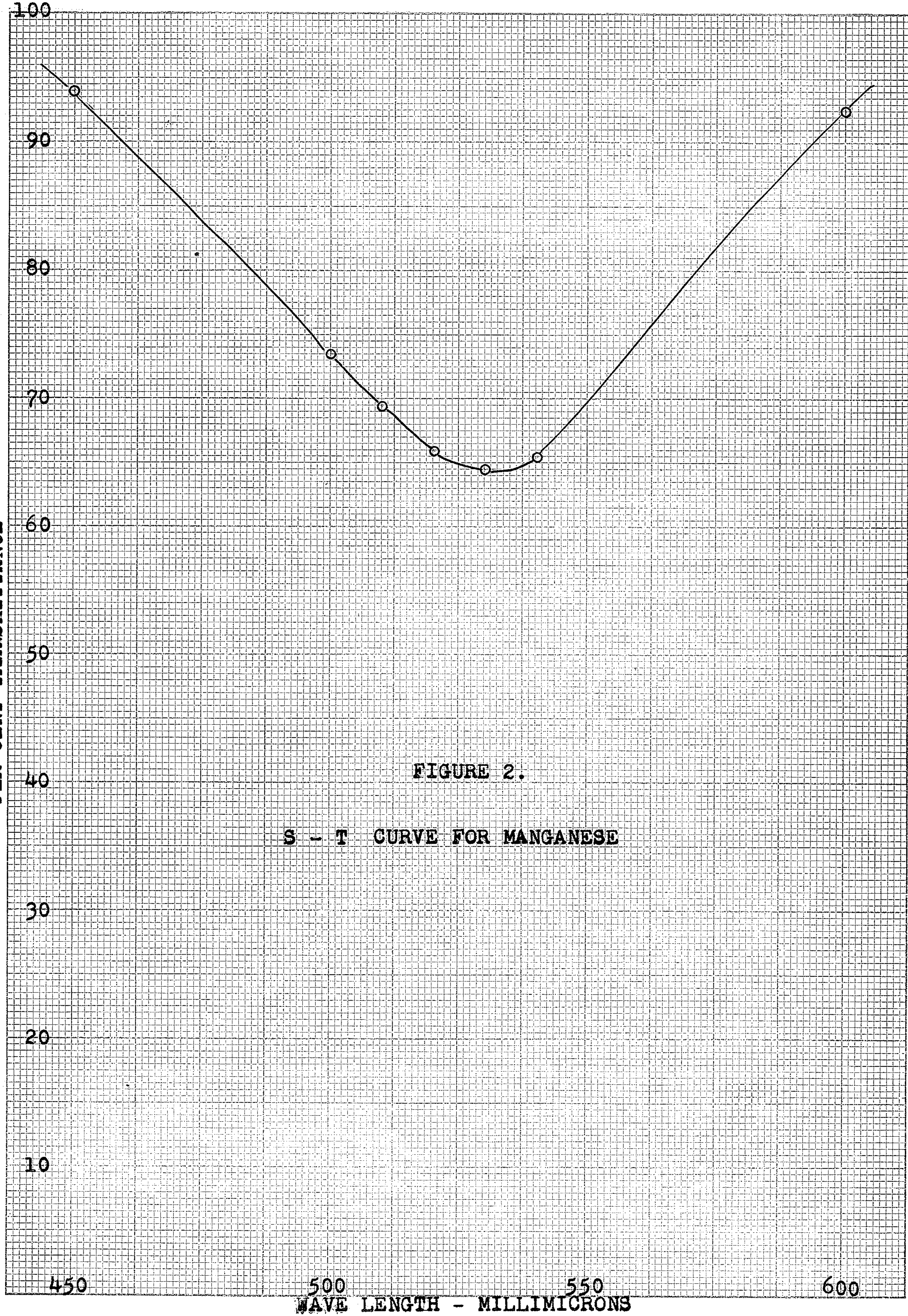

FIGURE 2.

S - T CURVE FOR MANGANESE

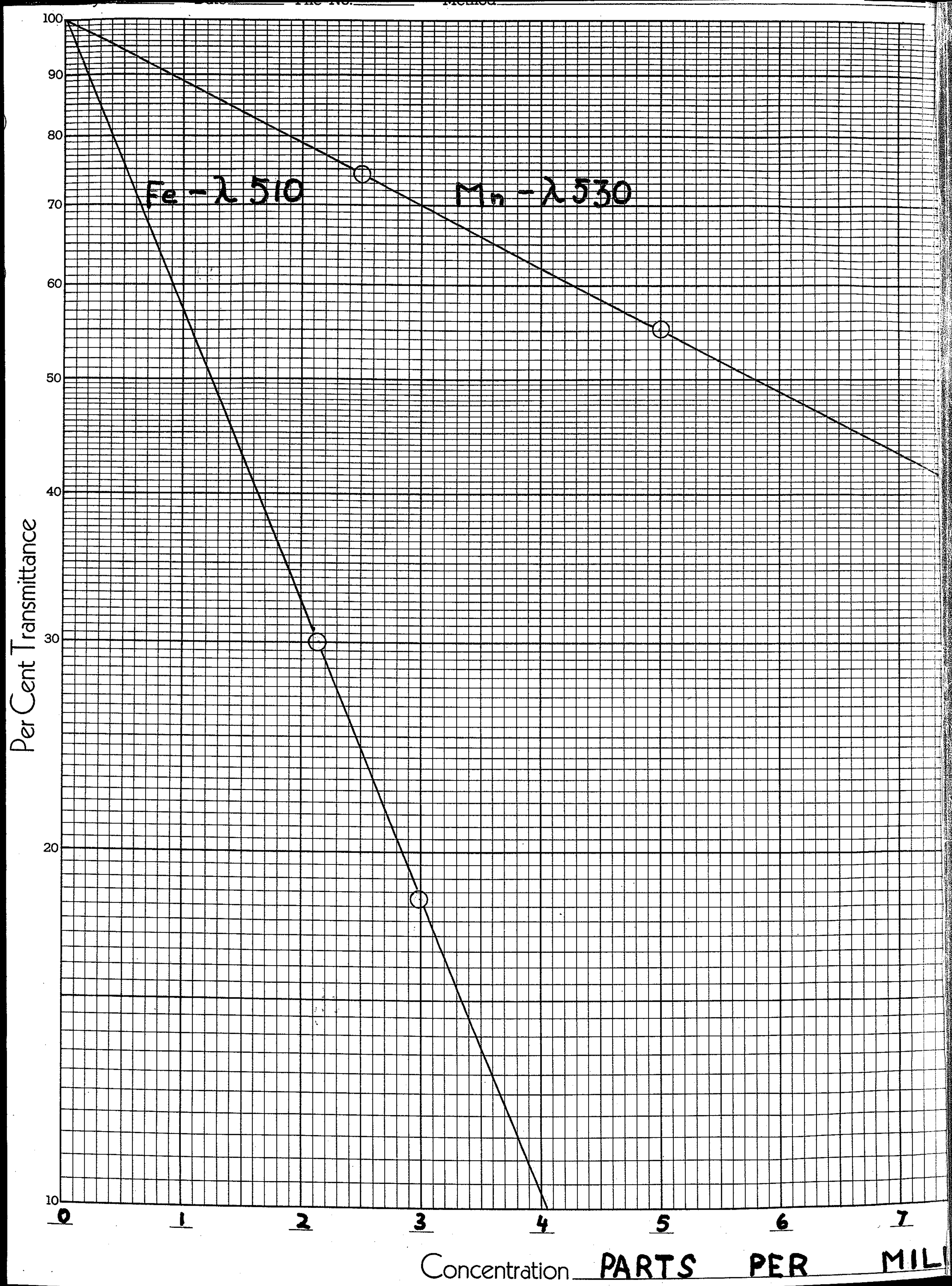

FIGURE 3

C-T CALIBRATION CURVES FOR IRON AND MANGANESE

In all analyses the same cuvettes (that is the optical test tubes employed, for containing the sample when their transmittance is being measured) were used respectively for the colored sample and the reference solution. This was done to obviate any error that might arise through slight variations in the dimensions of the cuvettes. The error of this analytical method when all precautions are taken has been shown to be as low as ± 1 per cent.

As already stated, the spectrophotometric method is based on the conversion of the constituent to be determined into a compound whose solution is colored and the intensity of the color depends upon the concentration of the constituent in the solution. The procedures used for the determination of iron and manganese were those described by Sandell (50). The method for the determination of iron was based on the formation of an orange red complex $(C_{12}H_8N_2)_3Fe^{++}$ between o-phenonthroline and ferrous iron; ferric iron in the solution being reduced with hydroquinone. The color intensity is independent of the acidity within a pH range of pH 2 to pH 9. The colorimetric determination of manganese involved the oxidation of manganese by sodium periodate to permanganate in an acid solution.

It was found possible to estimate with accurracy 0.05 p.p.m. of iron in solution by this method and a concentration as low as 0.01 p.p.m. produced a color sufficiently intense to be readable, a concentration of manganese as low as 0.1

p.p.m. could be determined.

Prior to analyzing the plants, their dry weights were determined after drying in an electrically heated oven at 105 degrees centigrade for 24 hours.

A modification of Sandell's (50) wet oxidation method of analyses was first tried, the procedure used being as follows. Samples of the dried plant material (0.5 to 1 gram samples) were transferred to 250 ml. Erlenmeyer flasks which were closed by placing small glass funnels in their necks. The plant material was moistened with distilled water and 5 ml. of concentrated nitric acid and 2 ml. of concentrated sulfuric acid added. Digestion was carried out by placing the flasks on an electric hot plate under a fume hood. Concentrated nitric acid, a few drops at a time, was added during the course of the digestion until on evaporation to fumes of sulfuric acid a practically colorless solution was obtained. The solution was then cooled, 2 to 3 ml. of distilled water added, and again evaporated until white fumes were given off. It was then filtered, the precipitate on the filter paper washed with 3N nitric acid, and brought up to volume with distilled water in a 25 ml. volumetric flask.

The volume of 1:1 ammonium hydroxide required to neutralize the acid in a 5 ml. aliquot of the solution was determined. Another 10 ml. aliquot was transferred to a 25 ml. volumetric flask and the correct volume of 1:1 ammonium hydroxide required

to neutralize the acid present was added. The solution was again diluted to 25 ml. with distilled water.

In the early stages of this research the following procedure was followed in the estimation of iron. To a 10 ml. aliquot of the above-mentioned neutralized solution was added first, 1 ml. of a hydroquinone solution of the following composition: 6.25 ml. of 1 M acetic acid, 3.75 ml. of 1 M sodium acetate, 0.5 grams of hydroquinone and diluted with distilled water to 50 ml.; followed by 1 ml. of a 0.5 per cent o-phenanthroline solution.

According to Sandell (50) the transmittance may then be determined after one hour but in the present investigation it was found that the full color was not developed in this time so the solutions were allowed to stand overnight in corked test tubes.

The manganese was estimated on the remaining 10 ml. portion of the solution. To this was added 0.05 to 0.1 gram of sodium periodate. The solution was heated for 1 to 2 hours on the hot plate under a fume hood, distilled water being added at intervals to prevent evaporation to dryness. After this time the pink permanganate color developed, however, as the solution cooled the intensity of the color faded and the remaining color completely disappeared as soon as distilled water was added to bring the solution up to volume. It could not be said that, because of the faintness of the color, the

dilution merely rendered the solution to weak to weak to read the transmittance as when the water was added initially the color remained but disappeared within a few seconds.

After a series of trials it was found that the acid content in the solution was too high for the minute traces of manganese present in the plant material. While the nitric acid content could be reduced by evaporation on the hot plate, the sulfuric acid was very difficult to remove by evaporation, not only was it difficult but also time consuming. Furthermore, it had to be continually watched as there was a tendency to splutter as the volume became small.

In a paper by Clark (13), in 1933, attention was called to the fact that the acid content should be kept around or below 5 per cent of the volume when determining small concentrations of manganese. However, an acid content of not less than 2 per cent of the volume is necessary in order to prevent the precipitation of iodates or periodates of manganese. Phosphoric acid prevents this precipitation as well as decolorizing ferric iron by complex formation. Clark obtained satisfactory results using phosphoric acid and sodium periodate only, in determining small quantities of manganese in solution.

However, as the organic matter had to be destroyed before the analysis could be attempted and because wet digestion could not be carried out without adding both nitric acid and sulfuric acid, it was decided to use the ashing method. With this method

not only could the acidity be controlled but also the use of sulfuric acid be eliminated.

After careful consideration of the problem it was decided to try a modification of the method of Lindner and Harley (35). According to these authors the plant material was ashed at 450 degrees centigrade in a muffle furnace and the ash dissolved in 5 ml. of 6N nitric acid, heated for an hour to oxidize the ferrous iron, then filtered if necessary. On trying out the method, however, it was found that 450 degrees centigrade was not sufficiently high a temperature to ensure the complete oxidation of the carbonaceous material. The temperature of the furnace was therefore raised to 550-590 degrees centigrade after which no further trouble was experienced.

Previous to ashing the dried plant material was ground to the desired fineness in a synthetic sapphire mortar. The ground material, 0.5 to 1 gram, was transferred to a silica crucible and ashed in the muffle furnace overnight at 550-590 degrees centigrade. To the weighed ash was added a few drops of distilled water and 5 ml. of 6N nitric acid. This was digested for one hour, filtered and the filter paper washed with 3N nitric acid and distilled water, and diluted to 50 ml. in a volumetric flask.

The manganese determination was conducted on a 35 ml. portion. To this was added 1 ml. of 85 per cent phosphoric acid and 0.05 to 0.1 grams of sodium periodate. No other acid

was added except, of course, the nitric acid already present. Before the color appeared the solution had to be evaporated down to a certain volume. As the solution was kept just below the boiling point, it was not possible to determine whether the sudden appearance of the color at a certain volume was due to a reduction in the dilution factor, or, to the gradual evaporation of the nitric acid and consequent reduction of the acid content. The latter idea seemed to be the more logical one. However, if after the color appeared the solution was left for only an additional thirty minutes on the hot plate, there was a very definite fading of the color on cooling. Several methods were attempted to check this fading. Satisfactory results were finally obtained by keeping the solution just below boiling point for 2 hours after the initial appearance of the color and adding boiled distilled water from time to time to compensate for evaporation. The solution was finally made up to volume in a 25 ml. volumetric flask before reading the transmittance.

While the ashing method eliminated many of the difficulties encountered in the wet oxidation method for manganese determination, new difficulties were encountered in connection with iron determination. Although the pH range over which the iron determinations may be made lies between pH 2 and pH 9, it was found that more satisfactory results were obtained if the pH of the solution was kept at pH 5.5 or slightly lower. In

attempting to adjust the pH of a 5 ml. aliquot of the solution with ammonium hydroxide (1 part of ammonium hydroxide to 2 parts of distilled water) it was found that the pH would not gradually change but with one drop of ammonium hydroxide it would suddenly change from pH 2 to pH 7. Several methods were attempted to overcome this. Adjusting the pH with a .001 N ammonium hydroxide solution proved more successful but because of the large volume of ammonium hydroxide solution required, the method was discarded. The method which was adopted was very tedious but proved satisfactory. This entailed the addition of an acetic acid-sodium acetate buffer to the portion of the solution on which the iron determinations were to be made. To a 10 ml. aliquot of the plant ash solution were added 1 ml. of 1 M acetic acid and 1 ml. of 1 M sodium acetate. To this was then added double the volume of ammonium hydroxide required to neutralize a 5 ml. aliquot of the solution to which had been added 0.5 ml. of 1 M acetic acid and 0.5 ml. of 1 M sodium acetate. The neutralized solution was then diluted to 25 ml. in a volumetric flask. The iron determination was conducted on a 10 ml. aliquot of this neutralized solution in the way already described ~~on page~~ .

In all analytical work either B.D.H. AnalaR or Fisher T.P. reagents were used.

The Spectrographic Method of Analysis

It is well known that for many types of work the spectro-

graphic method has been proved to be a very sensitive one for the quantitative determination of metals when in low concentration. An attempt was therefore made to apply the spectrographic procedure to the quantitative analysis of plant material for iron and manganese.

In this work a Hilger medium quartz spectrograph was used.

In exciting spectra by means of the arc a substance can be made to emit radiation, this radiation being limited to certain wave lengths which are characteristic of the elements in the substance. The light thus produced is passed through a quartz prism which resolves it into its components of different wave lengths. A spectrum thus results, the radiations possessing the longest wave lengths being at one end of the spectrum and those having the shortest wave lengths at the other. The spectrum is made to fall on a photographic plate, and the plate, on development, constitutes a photographic record of the spectrum. From the position and density of the lines in the resulting photographic record of the spectrum, the elements present in the experimental material and, under certain conditions, their concentrations, may be determined.

In the DC arc method the material to be analyzed, usually in the form of ash, was carried in the bored tip of the lower of a pair of purified graphite electrodes, and burned before the spectrograph slit.

A modification of the "Cathode Layer Arc" method described by Mitchell (42) was used in the present work. In this method the sample was carried in the negative electrode thus giving greater sensitivity owing to the concentration of positively charged atoms of the metals under investigation at its tip. A screen placed between the electrodes and the slit allowed only light produced in the vicinity of the cathode to enter the spectrograph.

Because the arc is so variable it has been found impossible to rely upon similar concentrations of a particular element always giving the same intensity of radiation. Therefore, in quantitative estimation of any element by means of the arc spectrum, it is necessary to use an internal standard. This is achieved by introducing, along with the substance to be analyzed, a known amount of some other element which yields a suitable spectral line in the same region of the spectrum as the line to be measured. It is necessary to select by trial internal standard lines and analysis lines which show similar variations under the normal fluctuations that may occur in the arc during the burning process and that lie reasonably close together.

The actual procedure followed in quantitative analysis is first of all to prepare a working curve. From this curve the intensity measurements of analysis lines in the spectrogram of the plant ash sample can be converted into actual concen-

trations of the element under investigation in the sample.

To construct this working curve it is necessary to make a series of spectrograms, using as material a synthetic plant ash containing known concentrations of the element or elements under investigation and also containing the internal standard.

Various methods were used in preparing the standards, and in adding the standards to the synthetic plant ash. Different internal standards were tested in order to find a suitable one. However, due to the fact that the spectrographic method was not adopted for plant analysis in this work, only the procedure involved in making the working curve which presented the most likely possibilities will be mentioned.

Firstly a synthetic plant ash was made up containing only the major elements present in actual plant ash in approximately the same proportion as they occur in an average ash sample. The composition of this mixture was as follows:-

Synthetic Plant Ash

Salt	Grams
$CaSiO_3$	3
$K_4P_2O_7$	6
Na_2SO_4	4
Al_2O_3	2
$Mg_3(PO_4)_2 \cdot 4H_2O$	1

This gave an approximate percentage composition of calcium, 6 per cent; silicon, 4 per cent; potassium, 44 per cent;

phosphorus, 20 per cent; sodium, 9 per cent; sulfur, 6 per cent; aluminum, 5 per cent; and magnesium, 4 per cent.

These were thoroughly mixed and ground in an agate mortar.

Bismuth was found to be the most satisfactory element to use as the internal standard. A known concentration of this metal in the form of bismuth chloride was added to powdered graphite and thoroughly mixed. Equal quantities of the bismuth-graphite mixture mixed with equal quantities of synthetic plant ash gave a final concentration of 500 p.p.m. of bismuth.

Two stock solutions, one containing 1000 p.p.m. of iron and the other containing 1000 p.p.m. of manganese were prepared. By a series of dilutions the standard solutions containing 3.2 p.p.m., 16 p.p.m., 80 p.p.m., and 400 p.p.m. of iron were prepared from the stock solution. Similarly, standard solutions containing 1.6 p.p.m., 8 p.p.m., 40 p.p.m. and 200 p.p.m. of manganese were prepared.

Spectrophotometric tests carried out on the solutions containing 8 p.p.m. of manganese and 16 p.p.m. of iron indicated that the accuracy of the standard solutions could be relied upon. To four, 0.2 grams samples of synthetic plant ash weighed out in quartz crucibles were added equal weights of graphite containing the bismuth standard. To these, were added 0.2 ml. each of the appropriate iron and manganese solution. The mixtures were then stirred thoroughly with

thin glass rods which being dried on the hot plate. This mixing and drying not only prevented the accumulation of iron and manganese on the sides of the crucibles but also resulted in standards of uniform mixture. The drying was finished in an oven at 110 degrees centigrade.

After drying the samples were powdered with glass rods and mixed with an equal weight of graphite. Four bored graphites were then packed with the respective samples, arced and stepped spectrograms made using a seven step 1 - 2 ratio log sector, and a 220 volt, 10 ampere arc current.

The light transmission in three steps of each spectrogram was measured with a microphotometer, for selected lines of iron, manganese and bismuth, and also the light transmission of the clear plate in the vicinity of the lines. The lines finally slected were those used by Brody and Ewing (8); the iron line 3020.6A, the manganese line 2801.1A, and the bismuth line 2898.0A. Nevertheless, it should be mentioned that during the course of this aspect of the work, not only were a number of different excitation methods tried but also various iron, manganese and bismuth lines were tested before those mentioned above were selected as being the most satisfactory ones. The iron manganese and bismuth lines tested were as follows:

Iron	Manganese	Bismuth
2947.88A	2949.20A	2897.88A
2953.90A	2605.57A	2938.30A
2598.37A	2605.69A	3239.73A
2599.57A		2627.91A
2612.77A		
2588.57A		

For purposes of constructing a working curve

$$\text{Log } \frac{Io}{I} \quad \text{i.e. Log } \frac{\text{Transmission of clear plate}}{\text{Transmission of line}}$$ gives the

density of the line with Io = 50.

These densities are plotted against Log exposure to give plate characteristic curves for each line. In the present experiment four graphs were plotted, each graph corresponding to a known concentration of iron and manganese in the sample. The separation between the curves for the iron and manganese lines and that of the bismuth line at density 0.5 was determined in the four samples; the separation being positive if the iron or manganese curves lay to the right of the bismuth line and negative if the lines lay the left of the bismuth line. The curves for one sample obtained by plotting densities against Log exposure for the four samples are shown in Figure 4.

The separations between the curves for the iron and manganese lines and that of the bismuth line for a given density, plotted against Log concentration of iron and manganese in the standards gave the working curves for iron and

FIGURE 4.

SEPARATION CURVES FOR IRON AND MANGANESE AT

DENSITY 0.5 FOR SAMPLE - 4

IRON SEPARATION .... -19

MANGANESE SEPARATION -29

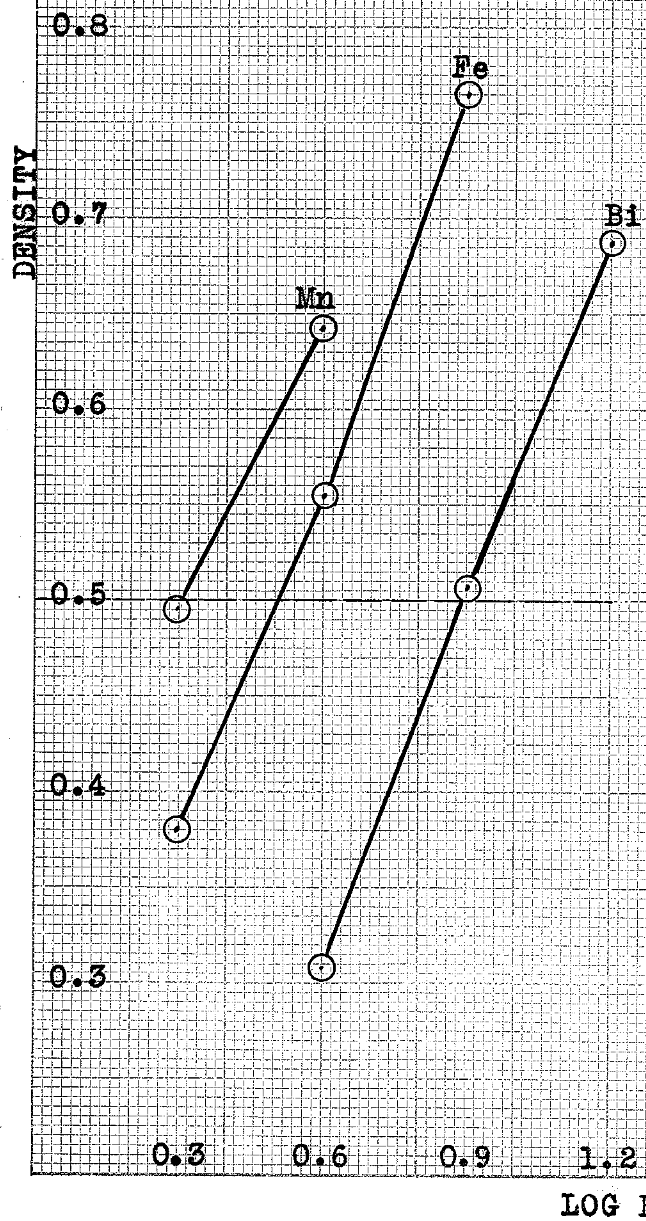

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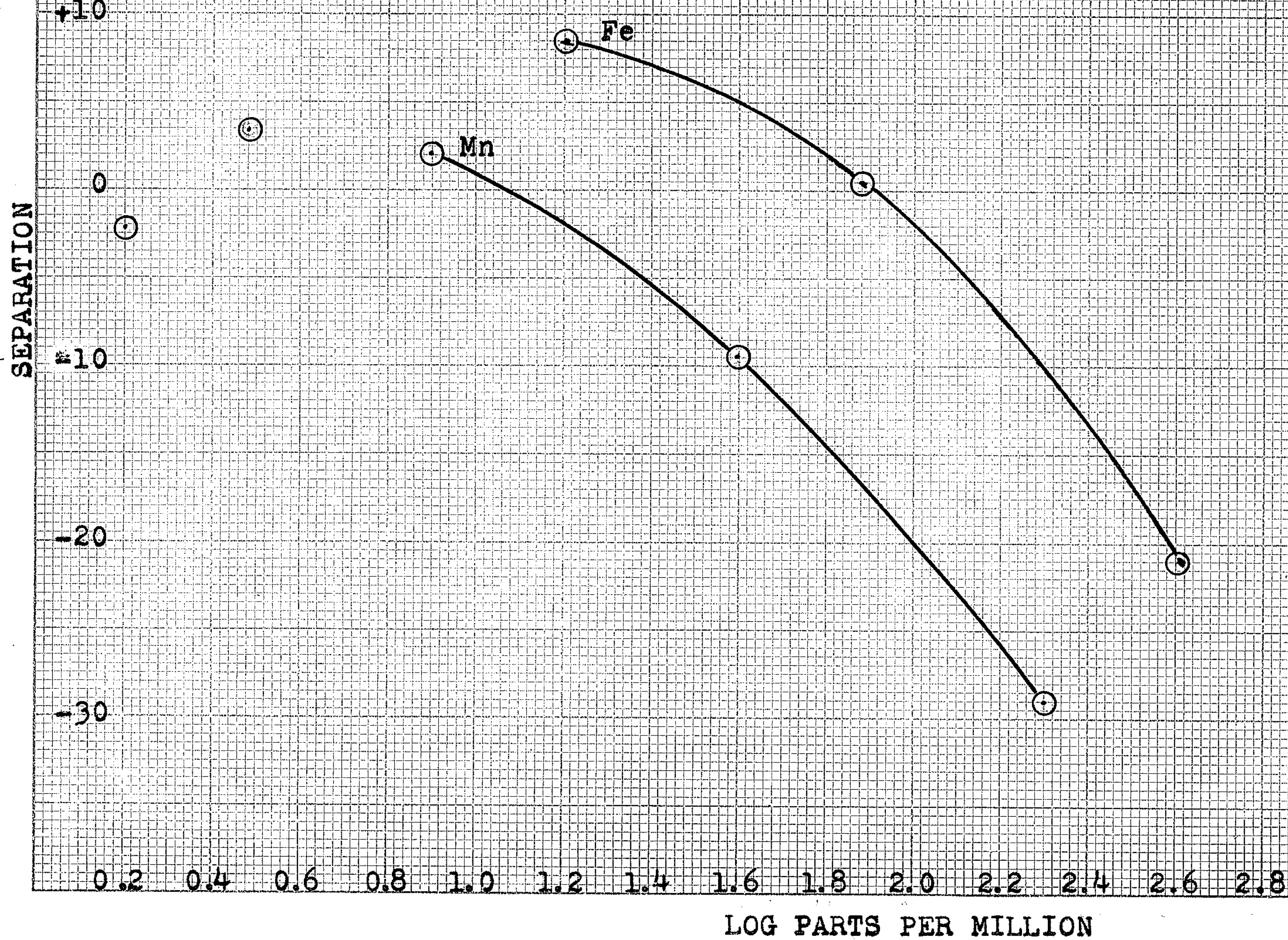
FIGURE 5.

WORKING CURVES FOR IRON AND MANGANESE DETERMINATIONS



manganese which are shown in Figure 5.

These working curves were plotted from the following data:

	P.P.M. Iron	Log.P.P.M. Iron	Separa-tion	P.P.M. Manga-nese	Log.P.P.M. Manganese	Separa-tion
Sample I	3.2	0.5051	+3.25	1.6	0.2041	-2
Sample II	16	1.2041	+8.5	8	0.9031	+2
Sample III	80	1.9031	+0.5	40	1.6021	-9.5
Sample IV	400	2.6021	-19.0	200	2.3010	-29.0

The positions of the points for Sample I (see Figure 5) would seem to indicate that concentrations of 3.2 p.p.m. of iron and 1.6 p.p.m. of manganese in the sample are below the satisfactory lower working limit of this particular method.

Once these working curves were obtained, the plant ash samples were to be substituted for the synthetic plant ash and treated in an identical manner to that described except for the addition of iron and manganese; the concentrations of these elements being read from the working curve.

One of the chief difficulties in the method was spluttering of the arc during burning but this was overcome with the synthetic plant ash by adding powdered graphite in the quantity stated. However, the spluttering of the arc was much more frequent and more serious with the plant ash than with the synthetic ash even though both were mixed with equal quantities of graphite. In order to overcome or alleviate this spluttering, it was

found necessary to mix the plant ash with a much greater quantity of graphite than was used when preparing the synthetic standards.

To use this method in quantitative work the plant ash must be treated in an identical manner as the synthetic ash, except for the addition of iron and manganese. Therefore, a new working curve would have had to be made. This would have entailed the preparation of a complete new set of standards. The preparation of a new set of standards and the carrying out of the whole procedure is a very time-consuming task. It is necessary to take every conceivable precaution against possible contamination by iron and/or manganese. But of more importance is the fact that the success of the results depends almost entirely upon the accuracy with which these standards are prepared.

Due to the fact that time was running short and that in the meantime the difficulties which were at first encountered in determining trace amounts of manganese by the spectrophotometric method had been overcome, it was, therefore, decided to abandon the spectrographic method and carry out the plant analyses with the spectrophotometer.

Nevertheless the spectrographic method for analysis outlined in the present work has definite possibilities and if it is fully developed and proved satisfactory, it will have decided advantages over any other analytical method in determining the concentration of small quantities of an

element or elements in biological material. Not only is it a sensitive method but so far it is the only method that can be used satisfactorily with small samples of material. One advantage that cannot be overlooked is that a photographic record of the results is obtained and these results may be checked. A further advantage is that a single spectrogram can be used for the determination of an indefinite number of metals provided suitable analysis and internal standard lines can be selected.

EXPERIMENTAL RESULTS

Experiment 1. - A preliminary investigation into the relationship between iron and manganese supply and grey speck disease of oats.

The main purpose of this experiment was to produce symptoms of grey speck disease in Tama oat plants growing in aerated culture solutions containing different iron-manganese ratios.

Seedlings of Tama oats were germinated in distilled water using method (i) previously described. (See page 43). When the seedlings were 4 to 5 " in height, they were transferred to the culture solutions containing different iron-manganese ratios as shown in Table I.

Solution A (see page 36) provided the macro elements. Iron was supplied as ferrous sulfate and manganese supplied as manganese sulfate. The pH of the nutrient solution was adjusted to pH 5.4.

During the first 8 weeks the solutions were not renewed. After this first period they were renewed every 2 weeks as tests indicated that iron and manganese were completely removed from the solutions at the end of 2 weeks.

In order to find out if the addition of a growth hormone would have any effect in causing the rapid appearance of grey speck symptoms, 10 p.p.m. of indole 3 acetic acid, was added

to the culture solutions containing the oat plants in group IV, Table I.

For the duration of this experiment the oat plants were grown in the Potting room.

The complete results of these culture experiments are shown in Table I.

From the data given in Table I it will be observed that in no case did the characteristic symptoms of grey speck appear.

No chlorosis symptoms developed in any of the plants during the period of the experiment. The plants in group I and group II were more healthy in appearance than those in group III. The healthiest plant of all was in group III in which no manganese had been supplied throughout the experimental period. In all cases there was browning and shrivelling of the leaves from the tips downwards.

The plants receiving 10 p.p.m. indole 3 acetic acid were definitely abnormal. The roots were short, thick and yellowish in colour and the plants were stunted and died early.

Because the plants removed the iron and manganese from the culture solutions rapidly, it was decided in experiment 2 to use higher concentrations of both elements in the culture solutions. It was hoped that by doing this, more sturdy and more healthy plants would result, as the general health and vigor of the plants in this experiment was definitely poor.

TABLE I - Experiment 1

THE EFFECT OF GROWING TAMA OATS IN CULTURE SOLUTIONS CONTAINING DIFFERENT Fe/Mn RATIOS

Duration of Experiment	Group No.	No. of plants in group	P.P.M. Fe in nut. soln.	P.P.M. Mn in nut. soln.	Root development	Shoot development	Specific deficiency symptoms
Nov.17,1949 to Feb.10,1950	I	3	0.5	0.25	fair	fairly good	no chlorosis browning and shrivelling from tips downwards
85 days	II	3	0.0	.25	fair	fair to good	"
	III	3	0.5	0.00	fair	poor to fair	"
	IV	3	0.5 + Indole 3 acetic acid	0.0	poor abnormal (short and thick)	very poor all dead by 71st day	"

Experiment 2. - (Similar to Experiment 1 but with an increased iron and manganese supply.)

By increasing the iron and manganese supply in this experiment it was hoped that sturdier and healthier plants would result than were produced in the previous experiment. Another purpose in increasing the supply of iron and manganese was to determine how far the findings of Twyman held for oat plants. He (67) found that oat plants more readily showed symptoms of grey speck when the iron-manganese ratio in the culture solution was increased. Thus, in complete absence of manganese, and when supplied with sufficient quantities of iron, the plants should rapidly develop grey speck symptoms.

Oat seedlings were germinated on quartz sand using method (ii) previously described on page 43. When the plants were 4 to 5" tall, the endosperms were removed. The seedlings were, then, transferred to aerated culture solutions containing different iron-manganese ratios as shown in Table II.

Solution B (see page 36) provided the macro elements. Iron was supplied as ferrous sulfate and manganese as manganese sulfate. The pH of the nutrient solution was adjusted to between pH 4.2 to 4.8, in order to keep the iron in solution. However, regardless of the pH, it was impossible to prevent the precipitation of the iron by the phosphate ion present in the solution.

During the course of the experiment the solutions were

TABLE II - Experiment 2

THE EFFECT OF GROWING TAMA OATS IN CULTURE SOLUTIONS CONTAINING DIFFERENT Fe/Mn RATIOS

Duration of Experiment	Group No.	No. of plants in group	P.P.M. Fe in nut. soln.	P.P.M. Mn in nut. soln.	Root develop-ment	Shoot develop-ment	Specific deficiency symptoms
March 1st to April 21st 1950	I	4	5.0	2.5	poor	fairly good -1 spik-let/plant .sturdy	browning and shrivelling of leaf tips
41 days	II	4	5.0	0.0	poor	-stunted -poor and feeble -flaccid	rapid browning and shrivelling of leaves from tip to base
	III	4	0.0	2.5	good	good some-what flaccid	leaves showed browning at tips but spreading downwards was very slow

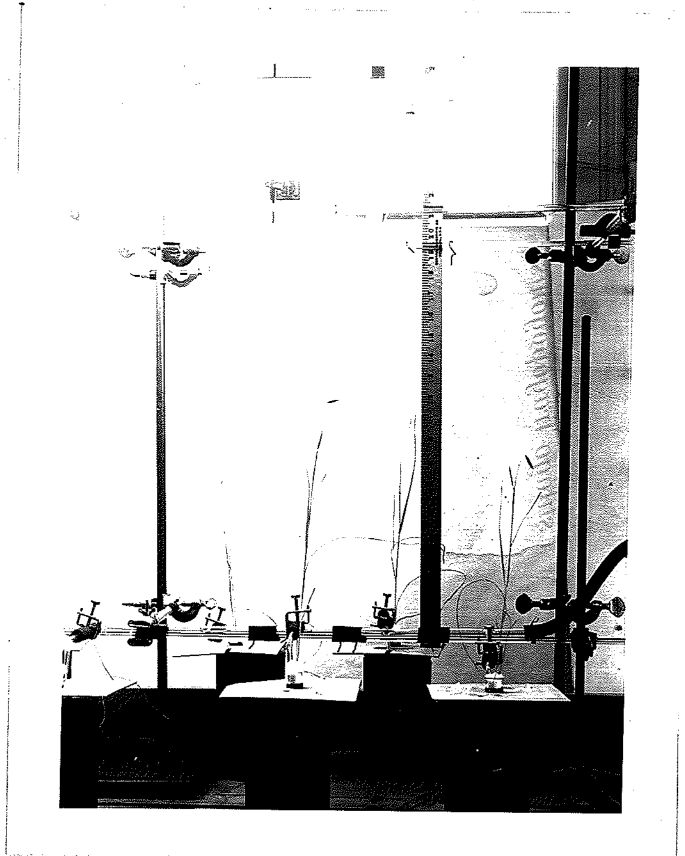


Figure 6.

THE EFFECT OF GROWING TAMA OATS IN
CULTURE SOLUTIONS RECEIVING BOTH IRON AND MANGANESE

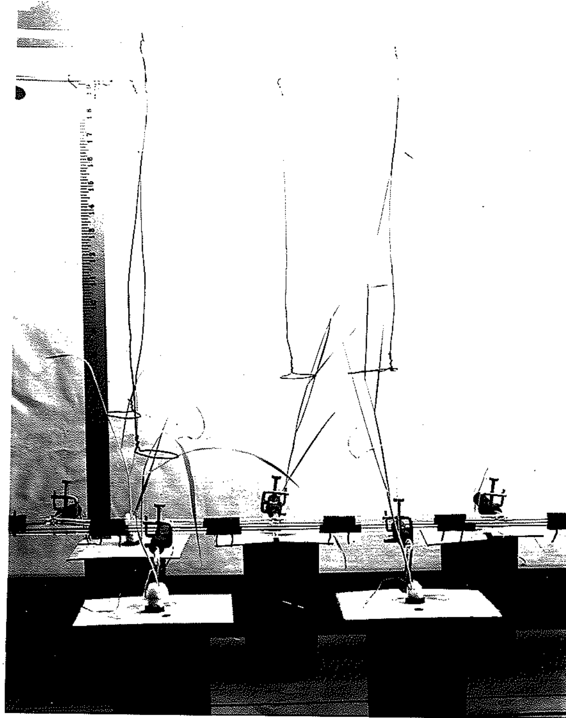


Figure 7.

THE EFFECT OF GROWING TAMA OAT PLANTS IN CULTURE SOLUTIONS SUPPLIED WITH MANGANESE ONLY

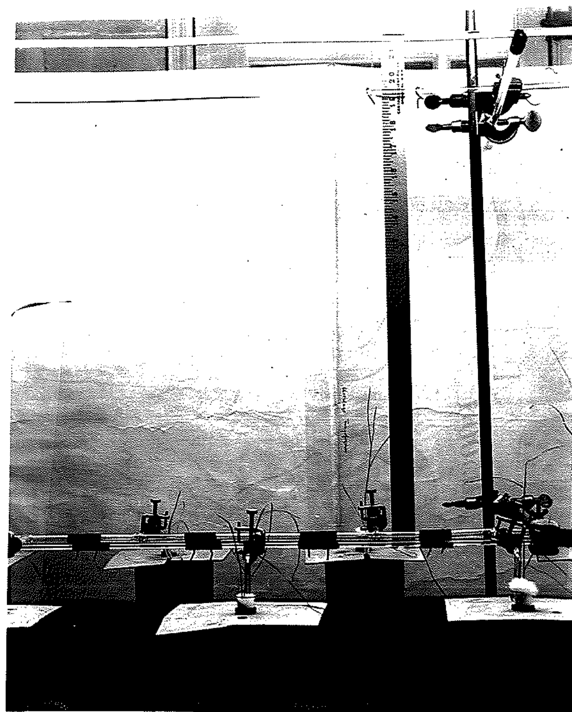


Figure 8.

THE EFFECT OF GROWING TAMA OAT PLANTS IN CULTURE SOLUTIONS SUPPLIED WITH IRON ONLY

changed every 2 weeks.

For the duration of the experiment the oat plants were grown in the Potting room.

The results of these culture experiments are shown in Table II. At the close of the experiment the photographs of the plants which are shown in Figures 6, 7, and 8 were taken.

From the data given in Table II, it will be seen that again no typical symptoms of grey speck disease developed in any of the plants. However, each group presented certain characteristic features.

The plants in group I were straight, sturdy, and uniform in size. Each plant had one spiklet. The leaf blades of these plants were neither as broad nor as green as the leaf blades of the plants in group III.

The plants in group II receiving iron only, were stunted, flaccid, feeble, and died early. The leaf blades were narrow and curled inwards.

In group I and II root development was poor and there was rapid browning and shrivelling of the leaves from the tip downwards.

The plants in group III were the largest and greenest. The leaf blades were broader than those of group I and II. Browning and shrivelling of the leaves from the tip downwards was not as rapid in this group as in group I and II.

Generally speaking more striking observational results

were obtained in this experiment than in experiment 1. This could have been due to several reasons namely; the endosperms were removed from the seedlings in this experiment; the plants were supplied with higher levels of both iron and manganese; and the time of the year in which the experiment was conducted was more favourable to growth.

While in this experiment healthier and sturdier plants were produced than in experiment 1, the findings of Twyman (67) were not confirmed as in no case were the symptoms of grey speck produced even when the plants were supplied with 10 p.p.m. of iron and no manganese.

In these first two experiments the main aim was to produce the characteristic symptoms of grey speck disease of oats. As this was not accomplished, it was decided at the time not to analyze the plants. However, at the close of the research as one plant from each group of experiment 2 had been retained, iron and manganese analyses were carried out.

The analyses were carried out on the shoots of the plants and the resulting data are given in Table III.

From the analyses data given in Table III for the shoots of the plants used in this experiment certain facts emerged. The absorption of iron by the plants was greater when manganese was absent from the culture solution than when it was present even though the iron supply was the same in both. (See groups I and II). The presence of iron in the solution appears

TABLE III

ANALYSES OF TAMA OAT PLANTS GROWN IN THE CULTURE EXPERIMENTS SHOWN IN TABLE II

Group No.	P.P.M. Fe in nut. soln.	P.P.M. Mn in nut. soln.	Mg. Fe per gram dry plant material	Mg. Mn per gram dry plant material
I	5.0	2.5	0.635	0.278
II	5.0	0.0	0.877	0.000
III	0.0	2.5	0.000	1.055

to have an antagonistic action on the absorption of manganese by the plants. There is also some indication that the presence of manganese probably antagonizes absorption of iron.

Due to the fact that only 1 plant from each group was analyzed, no definite conclusions can be reached. However, a mutual iron-manganese antagonism was suggested, iron having a greater antagonistic effect on the absorption of manganese by the plants than manganese had on the absorption of iron.

Experiment 3. - The effects of growing Tama oat plants in culture solutions in which iron and manganese were supplied intermittently.

Because of the difficulties encountered in retaining the iron in solution when supplied as ferrous sulfate, iron and manganese were intermittently supplied as separate solutions. In the group receiving both iron and manganese, the two elements were in the same solution.

Three micro solutions were prepared, the first containing iron and manganese, the second iron only and the third

manganese only. These solutions were buffered with 7 ml. of 1/10 M sodium tartrate and 1 ml. of 1/10 M tartaric acid. The addition of such a buffer not only simplified the preparation of the micro solutions but also kept the pH of the solutions fairly constant at pH 4.5.

The method of germination and the basic nutrient solution used were the same as for experiment 2.

When the plants were 6" high they were transferred to the basic nutrient solution.

For the duration of the experiment the oat plants were grown in the Potting room.

Initially the plants were removed from the basic nutrient solution and placed in their respective micro solution, as shown in Table IV, on Mondays and Fridays for 4 hour periods. However, on analyses of the solutions for iron, it was found that the plants were absorbing only minute quantities of this element in 4 hours. From the fourth week therefore until the close of the experiment, the plants were left in the micro solutions for periods of 24 hours. Aeration was continued throughout the periods when the plants were in the micro solutions.

The results of these experiments are shown in Table IV.

From the data given in Table IV, it will be observed that while these plants in this experiment were not as healthy as those in the previous experiment, the characteristic

appearance of each group was essentially the same in both experiments.

Though the plants in group I of this experiment were rather poor in appearance, this was believed to be due to a fungal infection which developed on the roots and lower parts of the shoots.

The plants in group II showed the best development being large and green. However, they were not sturdy. The roots were well developed and the fungal infection only slight.

The plants in group III were stunted, possessed narrow curled leaf blades and were almost dead by the end of the experiment.

At the beginning of this experiment, it was intended to analyze the solutions for iron and manganese, but as the analytical method for manganese was not at that time perfected, the idea was abandoned. This was because while the permanganate color could be developed in the solution, it disappeared on colling and diluting.

The method of intermittently supplying the plants with iron and manganese did not prove satisfactory as plant growth and development was poor. The addition of sodium tartrate and tartaric acid while being an excellent buffer, encouraged the growth of fungi which in turn infected the plants. The unsatisfactory plant growth and development was very likely partly due to the fungi present.

TABLE IV - Experiment 3

THE EFFECT OF GROWING TAMA OATS IN CULTURE SOLUTIONS IN WHICH IRON AND MANGANESE WERE SUPPLIED INTERMITTENTLY

Duration of Experiment	Group No.	No. of plants in group	P.P.M. Fe per L. solution	P.P.M. Mn per L. solution	Root development	Shoot development	Specific deficiency symptoms
Oct. 25th to Dec. 14th 1950	I	4	10.0	5.0	poor (infected with fungi)	fair to poor	browning and shrivelling of leaf tips. normal green of leaves
50 days	II	4	0.0	5.0	good (slightly infected with fungi)	fair	browning and shrivelling of leaf tips. normal green at leaves
	III	4	10.0	0.0	poor (infected with fungi)	-very poor -stunted -almost dead at end of experiment	browning and shrivelling more rapid than in Group I and II -leaves pale green

In this experiment, deficiency symptoms appeared which were not caused by lack of either iron or manganese. These symptoms which were rapid browning of the leaves from the tip downwards, were thought to be due to either potassium or nitrogen deficiency or to a too high calcium content in the culture solutions.

Analyses of the shoots of the plants grown in these culture experiments were carried out and the results are shown in Table V.

TABLE V

ANALYSES OF TAMA OAT PLANTS GROWN IN THE CULTURE EXPERIMENTS SHOWN IN TABLE IV

Group No.	P.P.M. Fe in nut. soln.	P.P.M. Mn in nut. soln.	Average dry wt. of shoot per plant g.	Mg. Fe per gram dry material	Mg. Mn per gram dry material
I	10.0	5.0	0.01965	0.223	0.239
II	0.0	5.0	0.0309	0.0	1.485
III	10.0	0.0	0.01635	0.88	0.0

From the analyses data given in Table V for the shoots of the plants grown in this experiment, it was observed that the results were similar to those obtained in the previous experiment. The absorption of iron by the plants was depressed in the presence of manganese, it being almost 4 times greater in the absence than in the presence of manganese. Again, iron exerted a greater effect on the absorption of manganese by the plants, than did manganese on iron absorption. The absorption

of manganese was 6 times greater in the absence than in the presence of iron.

As was found in the previous experiment, there is a definite suggestion of the existence of a mutual iron-manganese antagonism, iron exerting a stronger antagonistic effect on the absorption of manganese than did manganese on the absorption of iron.

Another fact observed, was that the average dry weight of shoot per plant was greater in the group receiving manganese only than in the group receiving both elements.

Experiment 4. - The effect of growing Tama oat plants in culture solutions containing different iron-manganese ratios, the iron being supplied either as ferrous sulfate or as iron humate.

In this instance two parallel experiments were carried out; in one the iron was supplied in the form of the organic iron humate, and, in the other it was supplied in the form of inorganic ferrous sulfate as shown in Table VI and Table VII.

Grey speck disease of oats tends to be aggravated on alkaline, organic soils. The use of iron in the form of the organic humate allowed the pH of the nutrient solutions to be initially adjusted to pH 6. When iron was supplied as ferrous sulfate, it was precipitated by the phosphate ion regardless of pH; as a result the pH of the culture solution receiving iron in the form of ferrous sulfate was also initially adjusted to pH 6. As the plants absorbed the nutrient elements from

the culture solution, the solution tended to become more alkaline.

Because of the symptoms observed in all the previous experiments, namely browning and shrivelling, of the leaves from the tip downwards, Solution C (see page 37) was used to provide the macro elements. This solution provided higher concentrations of both potassium and nitrogen and a lower calcium concentration. Iron and manganese were added directly to the nutrient solution. It was thought that by diluting the basic nutrient solution once, there would be less tendency for the complete precipitation of iron. This was found to be not so. However, shoot analyses, as shown in Table VIII, indicated that the precipitated iron must have been slightly soluble as the plants were found to have absorbed some iron.

The seedlings were germinated on moist filter paper using method (iii) previously described on page 43. When the seedlings were 5" to 6" high, they were then transferred to the aerated culture solutions.

The solutions were changed every two weeks.

These culture experiments were carried out in the laboratory on the third floor of the Science Building. The plants were grown along south facing windows, hence, no artificial light was necessary.

At the close of the experiment, photographs were taken of the plants. These are shown in Figures 9, 10, 11, and 12.

The results of these culture experiments are given in

TABLE VI
Experiment 4 - Set I

THE EFFECT OF GROWNING OAT PLANTS IN CULTURE SOLUTIONS CONTAINING MANGANESE AND IRON SUPPLIED AS INORGANIC FERROUS SULFATE

Duration of Experiment	Group No.	No. of plants in group	P.P.M. Fe in nut. soln.	P.P.M. Mn in nut. soln.	Root develop-ment	Shoot develop-ment	Remarks
May 11th 1951 to June 20th 1951	I	12	5.0	2.5	good	-good -3 to 4 spiklets per plant -long inter-nodes -plant tillered	browning and shrivelling of leaves pre-vented. plants healthy and green
41 days	II	12	5.0	1.0	good	-good -3 to 4 spiklets per plant -long inter-nodes -6 plant tillered	browning and shrivelling of leaves prevented.

TABLE VI - (continued)
Experiment 4 - Set I

THE EFFECT OF GROWING OAT PLANTS IN CULTURE SOLUTIONS CONTAINING MANGANESE AND IRON SUPPLIED AS INORGANIC FERROUS SULFATE

Duration of Experiment	Group No.	No. of plants in group	P.P.M. Fe in nut. soln.	P.P.M. Mn in nut. soln.	Root develop- ment	Shoot develop- ment	Remarks
May 11th 1951 to June 20th 1951	IIIA	6	5.0	0.00	good	-good -3-3 spiklets per plant -5 plants tillered	definite grey speck symptoms especially in the tillers
	IIIB	6	5.0	0.0	good	-good -2-3 spiklets per plant -4 plants tillered	Plants healthy
41 days	IV	6	0.0	2.5	fairly good	-small -sturdy -dead by end of experi- ment -3 to 4 spiklets/ plant	plants chloratic and completely straw colored by end of experiment

TABLE VII
Experiment 4 - Set II

THE EFFECT OF GROWING OAT PLANTS IN CULTURE SOLUTIONS CONTAINING MANGANESE, AND IRON SUPPLIED AS ORGANIC IRON HUMATE

Duration of Experiment	Group No.	No. of plants per group	P.P.M. Fe in nut. soln.	P.P.M. Mn in nut. soln.	Root development	Shoot development	Remarks
May 11th 1951 to June 20th 1951	I	12	5.0	2.5	good (fungal infection)	good -3 to 4 spiklets per plant -long internodes -9 plants tillered	-browning and shrivelling of the leaves prevented -healthy green plants
41 days	II	12	5.0	1.0	good (fungal infection)	good -3 to 4 spiklets per plant -long inter-nodes -10 plants tillered	-browning and shrivelling of leaves prevented -healthy green plants

TABLE VII - (continued)
Experiment 4 - Set II

THE EFFECT OF GROWING OAT PLANTS IN CULTURE SOLUTIONS CONTAINING MANGANESE, AND IRON SUPPLIED AS ORGANIC IRON HUMATE

Duration of Experiment	Group No.	No. of plants per group	P.P.M. Fe in nut. soln.	P.P.M. Mn in nut. soln.	Root development	Shoot development	Remarks
May 11th 1951 to June 20th 1951	IIIA	6	5.0	0.0	good (fungal infection)	-fairly good -2 to 3 spiklets per plant -3 plants tillered	grey speck symptoms especially in the tillers -3 plants stunted -3 good size
41 days	IIIB	6	5.0	0.0	good (fungal infection)	fairly good -2 to 3 spiklets per plant -5 plants tillered	-3 plants with long internodes -1 stunted

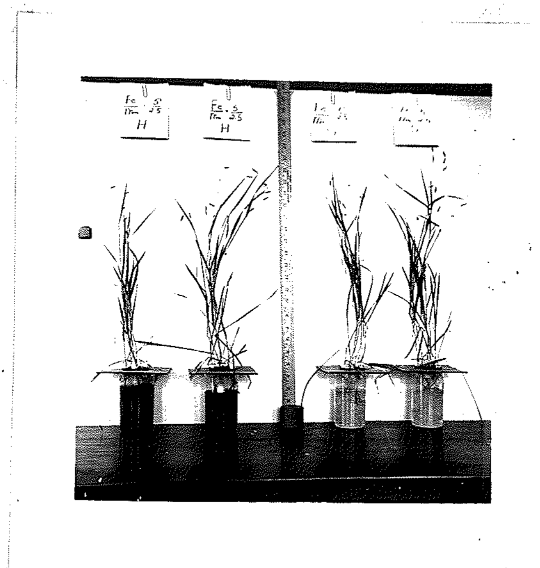


Figure 9.

THE EFFECT OF GROWING TAMA OAT PLANTS IN CULTURE SOLUTIONS CONTAINING IRON (5 P.P.M.) AND MANGANESE (2.5 P.P.M.) WITH IRON SUPPLIED IN THE HUMATE (H) OR SULFATE (S) FORM

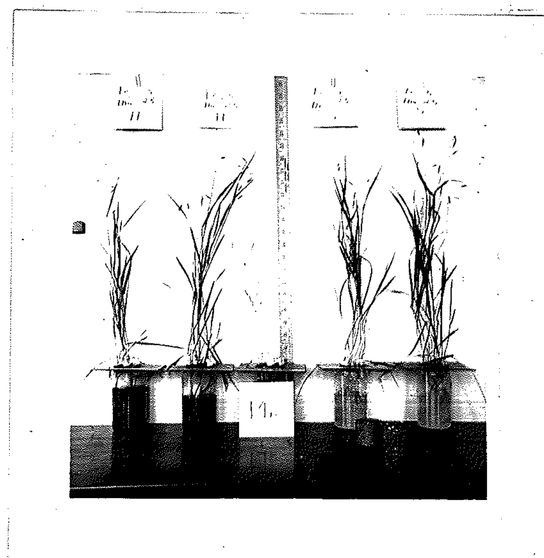


Figure 10.

CENTRE BEAKER SHOWS THE EFFECT OF GROWING OAT PLANTS IN CULTURE SOLUTIONS SUPPLIED WITH MANGANESE ONLY

Figure 11.

THE EFFECT OF GROWING TAMA OAT PLANTS IN CULTURE SOLUTIONS CONTAINING FE (5 P.P.M.) AND MN (1 P.P.M.) WITH IRON SUPPLIED IN THE HUMATE (H) OR SULFATE (S) FORM

Figure 12.

THE EFFECT OF GROWING TAMA OAT PLANTS IN CULTURE SOLUTIONS CONTAINING IRON (5 P.P.M.) ONLY, SUPPLIED IN THE HUMATE (H) OR SULFATE (S) FORM

Table VI and Table VII.

It will be seen that the general health and vigor of the plants in this experiment were very much better than in the three previous experiments.

In group A of set I and set II there were very definite grey speck symptoms on the leaves and especially on the tillers.

Root development was best in those plants receiving iron in the sulfate form. This was probably due to the fact that those receiving iron in the humate form had greater fungal infection.

Solution C, definitely gave healthier plant growth and development. However, it cannot be said that these healthier plants resulted from a better nutrient balance. In these experiments, the temperature, light and humidity were much more favourable to growth than in the previous experiments.

A series of analyses were carried out on the shoots of the plants used in this experiment and the resulting data are given in Tables VIII and IX.

From the data given in Table VIII it will be seen that the average dry weight per shoot was the same in group I and II but the average dry weight of the roots was greater in group I. The dry weights of the shoots and roots in group IIIA, where the grey speck symptoms occurred, were lower than the dry weights of those in group IIIB where no symptoms were observed.

There was not the definite mutual iron-manganese antagonism observed in this set as there was in experiments 1, and 2. In group I there was less iron absorbed than in group II where there was a lower manganese content in the culture solution.

From the analyses data given in Table IX it will be seen that both the dry weights of the shoots and the roots were greater in the groups receiving iron and manganese than in those receiving iron only. The dry weights of the shoots and roots of the plants in group IIIA infected with grey speck was less than that of the plants in group IIIB.

In groups IIIA and IIIB of both sets, the plants were found to contain detectable quantities of manganese. These quantities were unaccounted for unless they were provided by the endosperm as every precaution was taken to prevent such contamination. However, it will be observed that the manganese content was greater in the cases where no grey speck was produced than in the cases where the plants developed grey speck symptoms.

<u>Experiment 5</u>. - <u>The effect of growing Tama oat plants in culture solutions inoculated with root tips obtained from grey speck diseased oat plants.</u>

In order to test the validity of the theory of Gerretsen (15, 16), two parallel experiments were carried out. In one set the culture solutions were inoculated with root tips obtained

TABLE VIII
Experiment 4 - Set I

ANALYSIS OF OAT PLANTS GROWN IN CULTURE EXPERIMENTS SHOWN IN TABLE VI

Group No.	P.P.M. Fe in nut. soln.	P.P.M. Mn in nut. soln.	Average dry wt. per plant Shoot	Root	Ash wt. of Shoot per gram dry plant material	Mg. Fe per gram dry plant material	Mg. Mn per gram dry plant material
I	5.0	2.5	.138	.0239	.1293	.105	.188
II	5.0	1.0	.138	.0203	.1354	.174	.22
IIIA	5.0	0.0	.117	.0147	.1236	.124	.01
IIIB	5.0	0.0	.138	0.185	.123	.175	.04

TABLE IX
Experiment 4 - Set II

ANALYSIS OF OAT PLANTS GROWN IN CULTURE SOLUTIONS SHOWN IN TABLE VII

Group No.	P.P.M. Fe in nut. soln.	P.P.M. Mn in nut. soln.	Average dry wt. per plant		Ash wt. of Shoot per gram dry plant material	Mg. Fe per gram dry plant material	Mg. Mn per gram dry plant material
			Shoot	Root			
I	5.0	2.5	.1535	.0209	.1468	.188	.13
II	5.0	1.0	.169	.0174	.1330	.186	.18
IIIA	5.0	0.0	.118	.0136	.1190	.138	.01
IIIB	5.0	0.0	.127	.0163	.1380	.114	0.03

from grey speck diseased oat plants grown in the field while in the other set the culture solutions were not inoculated. According to Gerretsen (15, 16) healthy plants can be grown with a very low manganese content (5 to 10 p.p.m.) if the nutrient medium is kept sterile. However, if the medium is infected with a few diseased root tips, the plants will produce grey speck symptoms.

The method of germination was the same as in experiment 4. When the seedlings were 5" to 6" high, their endosperms were removed, and they were transferred to aerated culture solutions.

Solution C (see page 37) provided the macro elements. Iron was supplied in the form of the organic humate and manganese as manganese sulfate. As the humate iron was used the macro solution was not diluted as in the previous experiment. The pH of the culture solution was initially adjusted to pH 6.

The culture solution was not changed during the course of the experiment.

The oat plants were grown in the laboratory along south facing windows.

At the close of the experiment photographs of the plants were taken as shown in Figures 13, 14, and 15.

The results of these culture experiments are shown in Table X and Table XI.

TABLE X

SET I - THE EFFECT OF GROWING TAMA OAT PLANTS IN INOCULATED CULTURE SOLUTIONS

Duration of Experiment	Group No.	No. of plants per group	P.P.M. Fe in nut. soln.	P.P.M. Mn in nut. soln.	Root development	Shoot development	Remarks
August 10th 1950 to Sept. 24th 1950	I	12	5.0	2.5	good	-good -healthy green plants -2 to 3 spiklets on most plants -9 plants tillered	-plants healthy but not as healthy as those in the same group in Set II
44 days	II	12	5.0	1.0	good	-good -healthy green plants -1 plant fruiting -10 plants tillered	plants healthy but not as healthy as those in Group I
	III	12	5.0	0.0	fair	-fair to poor -no spiklets -4 plants tillered	-all 12 plants showed symptoms of grey speck -tillers severely infected

TABLE XI

SET II - THE EFFECT OF GROWING TAMA OAT PLANTS IN NON-INOCULATED CULTURE SOLUTIONS

Duration of Experiment	Group No.	No. of plants per group	P.P.M. Fe in nut. soln.	P.P.M. Mn in nut. soln.	Root development	Shoot development	Remarks
Aug. 10th 1950 to Sept. 24th 1950	I	12	5.0	2.5	very good	-very good -2-3 spiklets on most plants -10 plants tillered	-Largest and healthiest plants of either Set I or Set II
44 days	II	12	5.0	1.0	good	-good -no spiklets -healthy green plants -10 plants tillered	-plant development similar to those of the same group in Set I
	III	12	5.0	0.0	fair	-fair to poor -no spiklets -8 plants tillered	-3 plants dead by end of experiment -all plants showed definite grey speck symptoms -tillers severely infected

Figure 13.

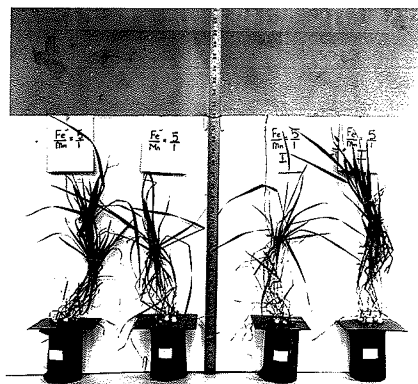


Figure 14.

Figure 15.

PHOTOGRAPHS SHOWING OAT PLANTS GROWN IN CULTURES OF DIFFERENT FE/MN RATIOS. CULTURES INOCULATED WITH GREY SPECK INFECTED MATERIAL MARKED I.

TABLE XI
Experiment 5 - Set I

ANALYSIS OF OAT PLANTS GROWN IN CULTURE SOLUTIONS SHOWN IN TABLE X

Group No.	P.P.M. Fe in nut. soln.	P.P.M. Mn in nut. soln.	Average dry wt. per plant Shoot g	Root g	Ash wt. of Shoot per gram dry material g	Mg. Fe/G dry material	Mg. Mn/G dry material
I	5	2.5	.197	.022	0.1356	.0545	.177
II	5	1.0	.167	.018	0.1601	.0625	.212
III	5	0.0	.0698	.007	0.121	.086	0.00

TABLE XII
Experiment 5 - Set II

ANALYSIS OF TAMA OAT PLANTS GROWN IN CULTURE SOLUTIONS SHOWN IN TABLE XI

Group No.	P.P.M. Fe in nut. soln.	P.P.M. Mn in nut. soln.	Average dry wt. per plant Shoot g	Average dry wt. per plant Root g	Ash wt. of Shoot per gram dry material g	Mg. Fe per gram dry material	Mg. Mn per gram dry material
I	5	2.5	.208	.023	.1238	.062	.0725
II	5	1.0	.160	.021	.1570	.074	.145
III	5	0.0	.056	.005	.117	.137	0.00

From the observational results given in Tables X and XI it will be seen the plants in Set II group I were by far the best plants and next were those in Set I group I. The plants in group II of both sets showed no differences in general appearance.

In group III of both set I and set II severe grey speck symptoms were produced in all the plants regardless of whether the solutions were inoculated or not.

A series of analyses were carried out on the shoots of the plants used in this experiment and the resulting data are given in Tables XI and XII.

From the analysis data, it will be seen that in groups I, II and III of both sets, the dry weight of the shoots and the roots increased as the manganese content in the culture solution also increased.

In all circumstances, as the manganese concentration in the culture solution decreased, the iron content of the plants increased. This was most noticeable in the non-inoculated set.

Experiment 6. - Analysis of field grown Tama oat plants exhibiting symptoms of grey speck disease.

Dr. Hagborg, of the Dominion Laboratory of Plant Pathology, Winnipeg, conducted plot experiments with Tama oats grown in a field near Oak Bank, Manitoba, in 1949. This is a grey speck disease area with neutral, black soil.

The field was divided into plots, each plot receiving treatment with iron, manganese or water. The plots receiving manganese were sprayed with a 0.65 per cent commercial manganese sulfate spray. Those receiving iron, were sprayed with an equivalent amount of a ferrous sulfate spray. The remainder of the plots received water only. The plants were sprayed June 15th and cut July 19th. All the plants exhibited symptoms of grey speck.

In some cases the plots were sprayed with the manganese sulfate spray only; in others the manganese was applied with a UL600 latex (Goodrich) solution spreader. The iron was always applied with this spreader.

Analysis of the leaves for iron and manganese was carried out for all the samples as shown in Table XIII. The seed and stems of four sam ples were analyzed, also, for iron and manganese, as shown in Table XIV and Table XV.

In the three tables, plots sprayed with manganese sulfate only will be designated as Mn; those sprayed with manganese sulfate and the spreader will be designated as MnGR; those sprayed with ferrous sulfate and the spreader will be designated as FeGR; and those receiving water only will be designated as H_2O. From the analyses data given in Table XIII it will be seen that generally speaking the leaves of the plants sprayed with manganese had a slightly higher manganese content than those sprayed with iron or water and

TABLE XIII

ANALYSIS OF THE LEAVES OF FIELD GROWN TAMA OAT PLANTS EXHIBITING SYMPTOMS OF GREY SPECK

Treatment	Wt. of ash per gram dry material	Mg. of Fe per gram dry material	Mg. of Mn per gram dry material
Plot 7 FeGR	0.1500	0.3750	.0071
Plot 3 FeGR	0.1470	0.2300	.0069
Plot 15 FeGR	0.1350	0.1450	.0064
Plot 19 FeGR	0.1460	0.1375	.0086
Plot 11 H_2O	0.1340	0.2000	.0079
Plot 8 H_2O	0.1370	0.1650	.0100
Plot 16 H_2O	0.1350	0.3620	.0071
Plot 2 H_2O	0.1480	0.1425	.0084
Plot 12 Mn	0.1310	0.1275	.0121
Plot 18 Mn	0.1280	0.1070	.0129
Plot 1 Mn	0.1360	0.1850	.0121
Plot 6 Mn	0.1340	0.1400	.0121
Plot 10 MnGR	0.1160	0.1350	.0139
Plot 13 MnGR	0.1350	0.0375	.0139
Plot 5 MnGR	0.1270	0.1250	.0100

TABLE XIV

ANALYSIS OF THE STEMS OF FIELD GROWN TAMA OAT PLANTS EXHIBITING SYMPTOMS OF GREY SPECK

Treatment	Wt. of ash per gram dry material	Mg. of Fe per gram dry material	Mg. of Mn per gram dry material
Plot 19 FeGR	0.0854	0.025	0.002
Plot 16 H_2O	0.0778	0.025	0.0028
Plot 13 MnGR	0.0816	0.019	0.005
Plot 12 Mn	0.0672	0.015	0.004

TABLE XV

ANALYSIS OF THE SEED OF FIELD GROWN TAMA OAT PLANTS EXHIBITING SYMPTOMS OF GREY SPECK

Treatment	Wt. of ash per gram dry material	Mg. of Fe per gram dry material	Mg. of Mn per gram dry material
Plot 19 FeGR	0.0470	0.037	0.017
Plot 16 H_2O	0.0465	0.042	0.0064
Plot 13 MnGR	0.0440	0.041	0.0136
Plot 12 Mn	0.0435	0.0465	0.0190

the leaves of the plants sprayed with iron or water had a slightly higher iron content than those sprayed with manganese Stem analyses, shown in Table XIV, show similar results.

The most important point that is brought out by the leaf, stem and seed analyses is that in no instance is the manganese content greater than 0.019 mg. of manganese per gram of dry material.

GENERAL DISCUSSION AND CONCLUSIONS

Although the primary purpose of the present investigation was to determine the relationship between iron and manganese supply and grey speck disease of oats, the experiments described have revealed a certain amount of additional important information.

No definite conclusions could be drawn with regard to the relationship between the iron-manganese ratio in the substrate and grey speck disease of oats. There was evidence, however, of a definite antagonism between the absorption of iron and manganese by the experimental plants. In the majority of the experiments, analysis data indicated that as the manganese concentration in the culture solution decreased the iron content of the plant increased, even though the iron concentration in the culture solution remained unaltered.

A fact of considerable interest was revealed in the course of the work. In the early experiments as has been described, the experimental plants were grown in the Potting room during the winter months. That is, they were illuminated by low intensity daylight from a north window, supplemented by fluorescent lamps; the temperature unavoidably fluctuated between wide limits and the atmospheric humidity was frequently very low. As a result these plants showed a very poor general state of nutrition. In none of these poorly developed plants

was it possible to produce grey speck symptoms. On the other hand the plants which were grown in the later experiments during summer months in a laboratory with a southern exposure and favorable temperature conditions were larger and showed a very much better general nutritional condition. In these a deficiency in manganese supply invariably caused the appearance of grey speck symptoms. Thus, it would appear that generally favourable culture conditions regarding temperature and light are necessary before manganese deficiency will produce symptoms of the disease.

The experiments in which the culture solutions were inoculated with grey speck infected root material seem conclusively to prove that bacterial infection in addition to manganese deficiency is not the cause of grey speck as suggested by Gerretsen (15, 16). Rather, the disease is definitely associated with manganese deficiency alone. This is further born out by the plant analysis data obtained both from the plants grown in solution culture and from those provided by Dr. Hagborg's field experiments. In all cases, grey speck is associated with a low manganese content in the plants showing symptoms of the disease.

SUMMARY

Investigations were carried out with *Avena sativa* var. Tama, using the water culture technique, to determine the relationship between iron and manganese and grey speck disease of oats, and to determine whether grey speck disease of oats was directly, or indirectly, a result of manganese deficiency.

Various analytical methods were attempted in order to find a suitable procedure for the analysis of plant material for iron and manganese. A modification of the spectrophotometric methods of Sandell (50) were devised for the quantitative determinations of iron and manganese in plant material.

There was a definite suggestion, as a result of these experiments, that manganese antagonized the absorption of iron by the plants.

The conclusion was arrived at that optimum external conditions for growth such as light, temperature and humidity were also the optimum external conditions for the development of grey speck symptoms.

The experimental data indicate that grey speck disease of oats is purely a nutritional disease caused directly by manganese deficiency.

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