Soil Manganese in Relation to Plant Growth

E. G. MULDER AND F. C. GERRETSEN

Agricultural Experiment Station and Institute for Soil Research, T.N.O., Groningen, The Netherlands

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I. Introduction

Among the earlier workers who studied the so-called stimulating effect of manganese on plant growth the names of Loew (1903), Bertrand (1905), and particularly Mazé (1914) must be mentioned. The last named carried out nutrient solution experiments with corn (*Zea mays* L.).

Of much importance was the discovery by Sjollema and Hudig (1909) (see also Hudig, 1911), that oat plants suffering from an unknown "soil disease" could be cured by the addition of 50 kg. of manganese sulfate per hectare. This "disease" was found to occur in the northern part of the Netherlands, particularly on reclaimed peaty soils which had become neutral or slightly alkaline through liming or application of Chilean nitrate as a nitrogen fertilizer. Hudig was unable to decide whether the beneficial effect of manganese had to be attributed to neutralization of some unknown injurious soil constituent or to its "stimulating influence" on plant growth.

A number of papers, mostly by Western European authors, appeared subsequently to Hudig's paper. It was shown that the "Dörrfleckenkrankheit" ("Gray Speck disease," as the oat disease was called first by Clausen, 1910), was common on many neutral sandy and peaty soils. One of the most remarkable of these papers was published by Hiltner (1924). He showed that the "Dörrfleckenkrankheit" is not limited to oats growing on neutral or alkaline soils, but may also be found in plants growing in acid nutrient solutions. In both cases it was cured by manganese. These experiments were very important for they demonstrated that the disease was not primarily caused by organic substances or by an alkaline reaction. Although these results may be considered as evidence that the gray speck disease is brought about by manganese deficiency, this conclusion was not drawn by Hiltner. Since carbon dioxide treatment of oat plants suffering from gray speck disease was found to

have a similar effect as supplying manganese to the nutrient medium, Hiltner (1924) attributed the beneficial effect of manganese to improved carbon dioxide assimilation by the plants, so that the disturbed equilibrium CO_2-assimilation-mineral substance uptake, which was supposed to be the cause of the disease, thereby would be corrected.

The essentiality of manganese as a micronutrient element for green plants became apparent shortly after 1920 (McHargue, 1922). The first authors who recognized that the symptoms of gray speck disease are essentially the symptoms of manganese deficiency were Samuel and Piper (1928). They carried out culture solution experiments with oats in comparison with pot and field experiments with "sick" soils. Very low values for manganese were found in diseased plants in comparison with healthy plants from sterilized "sick" soil or from "healthy" soil. Although clear differences in soil manganese were found, the methods used were inadequate for determining the plant-available manganese in soil, so that no explanation was given of the fact that plants growing on soils which contain large amounts of total manganese are unable to absorb the small quantities of this element that are required for normal development. Apparently Samuel and Piper were unaware of the important investigations by Beijerinck and Söhngen, who as early as 1913 and 1914, respectively, carried out a number of microbiological experiments concerning the oxidation of manganous salts to manganese dioxide. From his investigations Söhngen (1914) concluded that the unavailability of manganese in neutral or alkaline soils was due to the conversion of Mn^{++} to insoluble manganic oxides. In a later paper Piper (1931) demonstrated the importance of both soil reaction and oxidation-reduction equilibrium in rendering manganese unavailable to plants. The papers of Beijerinck and Söhngen were overlooked by soil scientists until their experiments were repeated and extended by Gerretsen (1936, 1937). Since that time a considerable number of papers have appeared dealing with the various aspects of soil manganese. Some of the investigators, for example, Gerretsen (1937), Leeper (1947), Quastel *et al.* (1948) are of the opinion that the conversion of available Mn^{++} to insoluble Mn^{+++} or Mn^{++++} is brought about by soil microorganisms; others believe that this is not the case because of the great rapidity with which this process takes place (Fujimoto and Sherman, 1948).

The fact that manganese, when supplied to plants in excessive amounts may cause toxic symptoms, has appeared to be of great importance in agriculture and horticulture. It has been shown, particularly during the last decade, that many acid soils may contain amounts of plant-available manganese large enough to bring about manganese

toxicity of the plants growing on it. In fact, manganese injury has been shown to be one of the main causes of soil acidity damage in many plant species on many acid soils. Since many papers deal with this subject, a relatively large portion of this review is devoted to the topic of manganese toxicity.

Many authors have considered the function of manganese in living organisms, plants, bacteria, and fungi, as well as animals. It appears that manganese plays the role of an activator in many enzymatic reactions. Practically all these investigations have been carried out *in vitro* so that it is as yet impossible to decide which enzymatic reactions are involved when manganese deficiency occurs *in vivo*. There is increasing evidence that manganese plays an important role in the photosynthetic apparatus of green plants and together with iron controls the oxidation-reduction potentials in the cells during illumination and in the dark.

II. Manganese Determination

Manganese in soils and plants can be determined by colorimetric, spectroscopic, polarographic, and biological methods. Plant material is generally ashed or wet-digested, though in some cases spot tests are used, which aim to estimate the manganese content of leaves, stems, fruits, etc., directly. The determination in soils is complicated by the fact that the several manganese compounds differ widely in availability, which to a great extent depends on the pH and the redox potential of the soils. Moreover, microorganisms take a very active part in transforming soluble manganese compounds into insoluble oxides and vice versa; consequently the manganous-manganic equilibrium in the soil depends largely on the activity of the soil flora, a fact which often has been overlooked by soil chemists.

Microbiological transformation may affect the amount of available manganese in a very short time, so storage of wet samples has to be avoided. Also purely physical treatments, such as drying or sterilizing, can effect drastic changes in the available manganese content of a soil; these facts should be born in mind when taking soil samples for manganese determination. Sherman and Harmer (1942) stress the point that samples should be analyzed immediately and in their natural field condition, especially because some soils release larger amounts of exchangeable manganese if they are air-dried before extraction. Boken (1952) showed that not only the amount of exchangeable manganese increases considerably when wet soil samples are dried at higher temperatures, but also that this is the case during storage of air-dried soil samples even at room temperature. The increase in the manganese

values is a function of both the length of the storage period and of the storage temperature.

1. *Colorimetric Methods*

In many cases the original method of Willard and Greathouse (1917) as modified by Richards (1930) is still used; it involves the destruction of the organic material by ashing, the removal of the chlorides by digesting with sulfuric acid, the elimination of the disturbing effect of iron by addition of phosphate and finally the oxidation of the manganese to permanganate by means of periodate. The red color of the permanganate is measured photometrically. Strickland and Spicer (1949) have made an extensive study of the kinetics of the formation of permanganate using periodic acid as an oxidant and give a detailed account of the probable mechanism of the oxidation, at the same time recommending suitable conditions for an effective absorptiometric method.

According to de Wael (1941) Richards' method does not give correct results; digestion with sulfuric acid causes errors (up to 18 per cent) due to evaporation, whereas the ash retains some manganese because of the formation of insoluble manganic oxides. Coppenet (1949) showed that the silicic acid in the ash of cereal straw combines with manganese causing 27.4 to 40.5 per cent of the manganese present to escape determination. To avoid this the author dissolves the silicate in 2–3 ml. hydrofluoric acid. Strickland and Spicer (1949) found permanganic acid in the distillate from boiling sulfuric acid containing manganese solutions. This error can be avoided by the substitution of 85 per cent H_3PO_4 for the 12 N H_2SO_4 commonly used.

Smith (1950) emphasizes the point that the oxidation to permanganate by means of periodate is less rapid than is generally believed. He recommends holding the temperature of the solution near the boiling point for at least thirty minutes after the addition of KIO_4. Nydahl (1949) claims that by using ammonium peroxidisulfate instead of periodate the oxidation is completed in a few seconds, whereas with periodate no more than 86 per cent of 1 μmol manganese is converted into permanganate after boiling for one hour.

As the accuracy of the colorimetric determination of permanganate diminishes markedly at very low concentrations, several authors attempted to revise existing methods or to perfect new ones. Sideris (1937) uses the formaldoxime reagent (H_2C : NOH) of Deniges, (1932) which instantaneously develops a wine red color directly proportional to the amount of manganese in the solution. After some improvements with regard to the interfering ions of iron and phosphate (Sideris, 1940), amounts of manganese varying from 0.005 to 0.01 mg. in a 10-ml. sample

can be determined with an accuracy of approximately 2 per cent. The same reagent is used by Waldbauer and Ward (1942) and Kniphorst (1946), whereas Wiese and Johnson (1939) prefer benzidine ($NH_2 \cdot C_6H_4 \cdot C_6H_4 \cdot NH_2$) which gives a blue color with permanganate, quickly changing into a stable yellow-green color.

The most sensitive reaction for manganese is the oxidation of 4,4′-tetramethyldiaminodiphenylmethane by $KMnO_4$. According to Gates and Ellis (1947) the organic material is ashed at 600°C., dissolved in a HNO_3-H_3PO_4 mixture, oxidized with KIO_4, and methane base added. Chlorides, vanadium, and cerium interfere; recoveries of manganese added to various materials ranged from 90–112 per cent. The sensitivity range according to these authors lies between 0.05 and 0.5 μg. Mn. According to Cornfield and Pollard (1950) this method is 300 times as sensitive as the permanganate method; they were able to determine the manganese content in the ash of 0.05–0.1 g. oven-dried plant material.

In the widely used Morgan soil testing system (Lunt *et al.*, 1950) manganese is determined in the soil extract either with benzidine and NaOH or with KIO_4 and methane base (tetra-base); the resultant blue color is compared with a standard color chart, rating from 5 to 40 p.p.m. in soil.

Chemical tissue tests are very useful to reflect the nutrient status of the plant. Leaf analysis for practical diagnostic purposes was introduced by Lagatu and Maume (1932). Roach (1944, 1946) at the East Malling Research Station in England applied the method for the detection of manganese deficiency: 25 mg. of dried leaf were burned directly in an acetylene flame, and the line intensity was determined with a flame spectrometer. Roach and Roberts (1945) used an ingenious method for diagnosing manganese deficiency in leaves by injecting or spraying a growing leaf with a solution of $MnSO_4$ (0.025 per cent). Deficient leaves respond by improving the chlorotic color of the tissues in a few days.

Nicholas (1948) has developed a quick and more-or-less precise quantitative determination of the manganese content of crop plants which may be used for the diagnosis of deficiencies in the field, as well as for the detection of cases where excess may be expected. With Morgan's acetate-acetic acid buffer at pH 4.8 0.5 g. of leaf is extracted. The reagents used for the test are trioxymethylene sulfate and NaOH, when manganese is in excess, or KIO_4 with methane base for deficiency levels. In the latter case the sensitivity ranges from 1 to 100 parts per 1000 million.

2. *Spectrochemical Methods*

The desirability of greater rapidity, permitting analyses of large numbers of soil samples, has resulted in the development of spectrochemical procedures for manganese. Flame spectrography has been perfected by Lundegårdh (1951) to such an extent that a number of important elements can be quantitatively determined by means of an automatic robot machine of very ingenious construction. Of biological interest is the high sensitivity in the flame of heavy metals, e.g., manganese. The manganese line (4030 Å) can be separated from the near-by potassium line (4044, 2 Å) by means of a spectrographic slit with a width of 0.005 mm. The accuracy of the determination in leaves is approximately 3 per cent, and the error is in general definitely less than 5 per cent. Heidel (1946) also uses a spectrochemical procedure for the determination of manganese.

3. *Polarographic Methods*

A promising method seems to be the polarographic determination which has been used by Zàk (1942) in an investigation of the fluctuation of the manganese content of lucerne during the season. Manganese shows a pronounced step in the polarographic curve at -1.53 v. and may be determined in this way with an accuracy of 0.01 mg. per cent. Zàk shows that the silicic acid of the ashed plant material binds up to 20 per cent of the manganese present, and repeated evaporation to dryness with HCl is necessary to avoid these losses. The polarographic method has the advantage that in a single polarogram different ions can be determined one after another.

Riches (1946) proposes to isolate different trace elements from a mineral acid digest by the use of synthetic resins. With a small column packed with granules of Amberlite 1 R 100 it was possible to retain quantitatively copper, cadmium, zinc, nickel and manganese. Recovery after elution with *N* HCl followed by polarographic determination amounted to 87–96 per cent.

4. *Biological Procedures*

The objection that the crude ways in which soils are generally extracted for chemical determinations differ very much from the progressive mild extraction exercised by the plant roots does not hold true for biological tests, which come much nearer to natural conditions.

As early as 1912 Bertrand and Javillier demonstrated the favorable influence of manganese ions on the growth of *Aspergillus niger*. Niklas and Toursel (1941) showed that 0.001 per cent of $MnSO_4$ increased the

weight of the mycelium as much as 83 per cent. Steinberg (1936) obtained an increase in weight of the mycelium of *Aspergillus niger* of approximately 30 per cent by the addition of manganese. He showed, however, that the optimal heavy metal concentration varies with the acidity of the solution, being approximately twentyfold in the solution at pH 8.01 as compared to that at 7.35. Löhnis (1944-45), who made a very thorough investigation of the influence of manganese on yield and sporulation of *Aspergillus niger,* came to the conclusion that, though *Aspergillus* is quite sensitive to traces of manganese under highly acid conditions, an *Aspergillus* standard will not be suitable for soil tests. Nicholas (1950), however, uses the *Aspergillus* method for the determination of manganese in soils, but adjusts the basal solution to pH 7.5 with dilute ammonia before addition of the soil sample. The effective range per 50-ml. culture solution lies between 0.01 and 10 μg.

An interesting microbiological method has been developed by Bentley, Snell, and Phillips (1947). *Lactobacillus arabinosus* responds to manganese by producing more lactic acid, which is measured by titration. The basal medium is freed from traces of manganese by preabsorption with the test organism. Within 73 hours 0.3 μg. manganese causes an increase in acid production of 6 ml. 0.1 *N* acid. Recovery of added manganese was quantitative within the experimental error of approximately 10 per cent.

III. Manganese in the Soil

Manganese occurs in the soil in different forms with widely divergent solubilities. Therefore one has to distinguish between total manganese and available manganese. The former, which may be determined by treating the soil with strong acids (Alten and Weiland, 1933, even use *aqua regia*), is of secondary importance for plant growth. Of much more significance is the available or "active" manganese as it is called by Leeper (1935, 1947). It includes water-soluble and exchangeable Mn^{++} and those forms of manganic oxide which are easily reducible by hydroquinone at pH 7. Those oxides which oxidize hyposulfite at pH 7 and hydroquinone at pH 2, but not at pH 7 are considered by Leeper moderately active, whereas the rest of the manganic oxides, which require more drastic treatment for solution may be considered inert and of no value for plant growth.

1. Effect of pH on Soil Manganese

The occurrence of manganese in various forms depends on a number of factors of which pH and organic matter content are the most im-

portant. Soils with pH values lower than 5.5 may contain a large amount of their manganese in water-soluble or exchangeable form. With increasing pH Mn^{++} will be converted into manganic oxides (Mn^{+++} and Mn^{++++}), as a result of which it becomes far less available to plants so that under certain circumstances manganese deficiency may occur. This conversion presumably depends largely either directly or indirectly on the activity of microorganisms. The oxidation of Mn^{++} which in the test tube takes place at pH values above 8, proceeds in the soil at much lower values probably owing to the presence of hydroxy acids and perhaps pyrophosphate (Söhngen, 1914; Heintze and Mann, 1947). Mattson *et al.* (1948) were unable to observe a stimulating effect of hydroxy acids on Mn^{++} oxidation. Since the experiments of Söhngen have been confirmed by the present authors a more detailed investigation of this problem seems urgent. Microorganisms appear to be able to oxidize Mn^{++} at pH values of the soil above 5.5.

Fixation of added manganese in an unexchangeable form in many neutral or alkaline soils proceeds rapidly. Wain *et al.* (1943) observed fixation of the bulk of the added manganese within a few days after its application. Similar results have been obtained by Sherman and Harmer (1942). The rapidity with which the exchangeable manganese content of a soil can fall after liming is clearly shown in Table I derived from Heintze (1946). Increasing amounts of lime were added to a clay loam and after one week's incubation pH values and exchangeable manganese were determined.

TABLE I

Exchangeable Manganese of a Clay Loam after One Week's Incubation with Lime (After Heintze, 1946)

Treatment	pH	Manganese (mg. %)
Control	4.5	10.2
1 ton lime per acre	5.5	10.0
3 tons lime per acre	6.3	8.4
8 tons lime per acre	7.5	5.0
12 tons lime per acre	7.9	2.0

In the light of these facts it becomes intelligible that the application of manganese sulfate to manganese-deficient soils generally has only a transitory effect, unless at the same time measures are taken to reduce the pH of the soil.

In this connection it is of interest to note that the application of lime, not as $CaCO_3$ but as CaO, to manganese-deficient clay soils rich in organic matter may result in a temporary improvement and even in

a complete recovery (Maschhaupt, 1934; Popp *et al.*, 1934). The former author even used from 15,000 to 20,000 kg. CaO per hectare which fully suppressed the deficiency symptoms on a so-called Roodoorn soil, a sea clay soil, devoid of $CaCO_3$, with a pH of 5.7 and a humus content of 12.5 per cent. In a couple of years, however, on this soil wheat showed severe manganese-deficiency symptoms. A similar beneficial effect of treating manganese-deficient soils with high amounts of CaO on growth and health of oats was noted by Gisiger and Hasler (1948). At high pH values soil organic matter apparently may reduce higher manganese oxides to Mn^{++} (Mattson *et al.*, 1948).

The reduction of manganic oxides to Mn^{++}, may proceed either by direct reaction with organic matter or by biological processes. Reduction by organic matter is more likely at low pH values since the oxidizing power of the higher oxides increases rapidly with acidity. Hydroxy acids again play an important part in this reduction (see below). Biological reduction can take place in acid as well as in alkaline soils if the oxygen tension is low through waterlogging.

2. *Availability of Soil Manganese and Its Estimation by Chemical Analysis*

Initially it was thought by Leeper (1935, 1947) as well as by Sherman and Harmer (1942) that the active manganese brought into solution by 0.2 per cent hydroquinone in normal ammonium acetate of pH 7.0 would be a reliable measure of plant-available manganese and thus for distinguishing between healthy and deficient soils. In alkaline soils easily reducible manganese should be at least 100 p.p.m. in order to maintain an adequate level of exchangeable manganese for plant growth. In such soils the quantity of exchangeable manganese should be > 3 p.p.m.

Heintze (1946) in an extensive study of "marsh spot" in peas failed to distinguish between soil samples from healthy and diseased parts in the same fields when using Steenbjerg's method (1935) for the determination of exchangeable manganese or Leeper's method with hydroquinone and calcium nitrate.

Dion, Mann, and Heintze (1947) investigated the factors controlling the reducibility of higher manganese oxides. Estimation of the easily reducible manganese appeared to be dependent on the pH of the system, the nature of the salt solution, the nature of the reducing agent, the time of contact in addition to the amount and nature of the higher oxides of manganese present. Pyrolusite (MnO_2) and a synthetic preparation of $Mn(OH)_3$ were found to be easily reducible. Manganite ($MnO(OH)$) and hausmannite ($MnMn_2O_4$) are apparently difficultly reducible forms.

The substitution of hydroxylamine hydrochloride for hydroquinone in Leeper's procedure was found to avoid the troublesome destruction of hydroquinone prior to the colorimetric manganese determination.

Jones and Leeper (1950, 1951a), investigating the availability of manganese in various synthetic manganese oxides, could not correlate the response of oats and peas obtained in pot experiments with the quantities of manganese in the soil as determined by means of a solution of hydroquinone in normal ammonium acetate. With this reagent the amounts extracted from manganite, hausmannite, and pyrolusite were of the same order. Yet the manganite and pyrolusite when added to manganese-deficient soils gave healthy plants, whereas hausmannite failed. The combined action of a concentrated electrolyte and a reducing agent seemed to be too drastic. Much better results were obtained with 0.05 per cent watery hydroquinone solutions (one-hour contact). The method as adopted by these authors includes stirring of the soils with 50 per cent alcohol containing 0.05 per cent hydroquinone, the alcohol being used to flocculate the colloids. Hydroquinone is removed by washing with 50 per cent alcohol, and the manganese now present as the exchangeable ion is replaced with a semimolar calcium nitrate solution at pH 7. The results obtained by this method by Jones and Leeper (1951a) have given the best correlation with plant response.

Electron microscope photographs of a number of synthetic manganese oxides showed that all the beneficial manganese oxides consisted of very small particles. Obviously a large specific surface is needed to ensure activity. This was found to be true of several highly oxidized forms: manganite, pyrolusite, cryptomelane, and manganous manganite; hausmannite, however, was found to be inert and unavailable to oats even when the particles were as minute as those of the active forms. X-ray analysis showed that the latter was highly crystalline, whereas in the others the degree of crystallinity was low. Difference in crystallinity was suggested by Jones and Leeper (1951a) to be also the cause of the divergent results with respect to manganite, for which Dion, Mann, and Heintze (1947) reported a low reducibility in hydroquinone solutions.

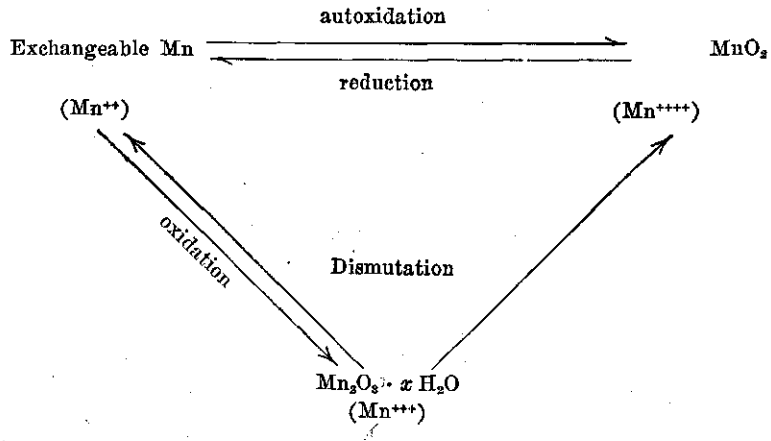
Curing manganese-deficient soils with active oxides did not increase the content of exchangeable manganese of the soils. This shows clearly that a reserve of the bivalent manganese is not needed for healthy plant growth.

In experiments with Australian soils active manganese oxides were found to preserve their activity for a considerable number of years (Jones and Leeper, 1951b). Presumably they have to be reduced to Mn^{++} at the root surface before the manganese can be absorbed. Re-

version of active oxides to a less available form is thought to be due mainly to aging, i.e., the surface becomes more ordered and less active.

3. *Occurrence of Trivalent Manganese in Soil*

An important contribution to our knowledge of the different states in which manganese may be present in the soil comes from Dion and Mann (1946). They showed that in alkaline soils a significant part of the soil manganese may be present in the trivalent state, which exists as the more or less highly hydrated manganese oxide $Mn_2O_3 \cdot xH_2O$. This is considered to be the first product of oxidation of divalent manganese in soils. According to these authors the following manganese cycle may be represented:



Autoxidation of divalent manganese to MnO_2 will probably be of significance only at pH values above 8. In less alkaline soils oxidation of divalent manganese is assumed to result in the production of trivalent manganese. In a percolation experiment with three soil samples 80.3 to 98.5 per cent of the added manganese sulfate which had been circulated for two weeks through the soil was recovered as trivalent manganese.

In acid solutions manganic hydroxide dismutes to give an ion of divalent manganese and a molecule of MnO_2, according to the following equation:

$$2Mn(OH)_3 + H_2SO_4 \rightarrow MnSO_4 + MnO_2 + 4H_2O$$

Under soil conditions Dion and Mann (1946) expect the same dismutation to take place. To show the effect of pH on this process they carried out an experiment to measure the amount of Mn^{++} produced in one week as a result of storage of $Mn(OH)_3$ at different pH values. At pH

7.50, 10.7 per cent dismutation was observed after one week, whereas at pH 6.18 a value of 82 per cent was found.

With sodium or potassium pyrophosphate the trivalent manganese may be extracted from the soil forming a stable complex to which the formula $Na_3(Mn(H_2P_2O_7)_3)$ may probably be ascribed.

The results of Mattson *et al.* (1948) confirm the concept of Dion and Mann as to the occurrence in alkaline soils of a large part of the soil manganese in the trivalent state.

4. Effect of Organic Matter on Soil Manganese

The role which organic matter plays in the conversion of the Mn^{++} to insoluble manganese compounds in the soil is not clear. That the presence of a certain amount of organic matter is required for the appearance of manganese deficiency of the plants has been shown by many workers. In the Netherlands manganese deficiency will be found on sandy soils containing a certain amount of organic matter and on reclaimed peaty soils when the pH becomes higher than 6 or 6.5. On neutral or alkaline clay soils, however, manganese deficiency is seldom found, unless they contain a certain amount of organic matter.

It may be possible that the effect of organic matter is due to the presence of hydroxy acids which according to Söhngen (1914) and Heintze and Mann (1946) play an important part in the conversion of soil manganese.

Heintze and Mann (1949) advance the hypothesis that manganese deficiency of plants on neutral and alkaline soils of high organic matter content and of adequate total manganese content is due to the formation of complexes of Mn^{++} with the organic matter which are dissociated only to such a slight extent that the available manganese in the soil is insufficient for the needs of the plants. The formation of such complexes was shown by these authors. Jones and Leeper (1951b) found no evidence for the existence of such complexes in their experiments.

Hudig (personal communication) made the interesting observation that in the northern part of Holland, on the borderline between acid sandy peat soil and clay soil, gray speck disease of oats was a common phenomenon, whereas on both sides of this line oats were perfectly healthy. The most plausible inference is that in the diseased area the pH of the peaty soil had been raised by the clay to a level at which manganese is made insoluble. The question, however, as to why on alkaline clay soils manganese is much more available than on sandy soils of the same pH needs further elucidation.

5. *Manganese in Calcareous Soils*

The French workers Boischot and Durroux (1949) have studied the fate of manganese in calcareous soils; manganese was found to be fixed by adsorption on the surface of the calcium carbonate particles. In contact with a 0.1 per cent neutral solution of ammonium humate the adsorbed manganese was set free in a form accessible to plants. This would explain why crops growing on calcareous soils generally do not suffer from manganese deficiency; if it does occur on such soils it is not caused by adsorption of the manganese on the calcium carbonate, but, according to these authors, has to be ascribed to biochemical oxidative processes.

IV. The Role of Microorganisms in Transforming Manganese Compounds

1. *Oxidation of Manganous Compounds*

The oxidation of manganous compounds to brown manganic oxides by bacteria and fungi was first shown by Beijerinck (1913). A small micrococcus and motile rods were isolated, forming small brown colonies on agar with 0.05 per cent manganous lactate as sole carbon source (*Bacillus manganica*). Amongst the fungi were representatives of Botrytis, Mycogone, Tricholadium; one of the most active fungi was *Papulospora manganica,* which feeds on traces of organic substances present in plain agar and which is able to oxidize manganous carbonate at relatively large distances from the mycelium.

Söhngen (1914) made a very valuable contribution to the problem and showed that a great variety of microorganisms, including *Pseudomonas fluorescens, Azotobacter chroococcum, Escherichia coli, Oidium lactis* and different species of Saccharomyces were able to transform soluble manganous compounds into insoluble, brown manganic oxides, when grown on agar media containing neutral salts of hydroxy acids (gluconic, malic, citric, lactic, and tartaric acids). However, the same oxidation could be performed by applying a drop of a sterile Na_2CO_3 or $NaHCO_3$ solution to the surface of these agar media.

On the other hand, it was shown by this author that in agar plates, containing sugars or cellulose and MnO_2, the latter compound is transformed into soluble manganous salts through the intermediary of the hydroxy acids produced by different cellulose or sugar-decomposing microorganisms. Whether manganous salts will be converted into manganic oxides or the reverse reaction will take place largely depends on the pH of the medium.

The agricultural aspect of the problem rested for a quarter of a century until Gerretsen (1936, 1937), by using soil agar plates in the center of which agar with 1 per cent $MnSO_4$ was placed, showed that brown rings of MnO_2 were formed, which consisted of numerous small colonies of bacteria and of fungi in which MnO_2 had been precipitated. The intensity of the precipitation rapidly diminished when the pH of the agar was raised or lowered, the limits lying between pH 6.3 and 7.8.

Leeper and Swaby (1940) using Gerretsen's technique, slightly modified, confirmed his results. Though brown spots generally developed most rapidly in plaques of pH between 6.0 and 7.5 they also observed brown spots due to microbial oxidation on plaques of which the final pH ranged from 4.8 to 8.9, the initial pH being 5.5. MacLachlan (1941) by means of the same soil agar plaque method was able to isolate a greater number of manganese oxide-precipitating bacteria from a manganese-deficient soil than from a normal soil and attributed manganese deficiency in the soil in question to the activity of microorganisms. According to Bromfield and Skerman (1950) neither the agar used by Beijerinck nor that by Gerretsen to isolate the active bacteria was satisfactory for the purpose, because pure cultures of bacteria isolated from Gerretsen's medium failed to oxidize Mn^{++} on plain soil agar media. Bromfield and Skerman, however, used meat infusion agar as an intermediate culture medium when isolating these bacteria and overlooked the fact that according to different authors most manganese oxidizing bacteria either do not thrive on such media or lose their ability to oxidize manganous compounds (Beijerinck, 1913; von Wolzogen Kühr, 1927). Bromfield and Skerman (1950) isolated two bacteria which were unable to oxidize manganous compounds separately, but did so in association on soil agar plaques. In contrast to these statements Timonin (1950a) isolated several microorganisms on Gerretsen's calcium citrate-manganous sulfate agar which were capable of oxidizing manganous salts on artificial media as well as on soil agar manganese sulfate plaques without further addition.

The present situation with regard to the microbiological oxidation of manganous compounds to insoluble manganic oxides is not yet sufficiently cleared up. There is positive evidence that microorganisms of widely divergent origin are able to produce hydroxy acids from cellulose and other substrates of vegetable origin and that the manganous salts of these acids are readily oxidized chemically by the oxygen from the air when the pH is above 7. In accordance with this fact the application of organic material may result in inducing manganese-deficiency symptoms or in aggravating them, with lower yields of grain than on untreated soil. Hudig and Meyer (1919) in their fundamental experiments

on gray speck disease incorporated different organic materials in their sand cultures (cotton, glucose, filter paper, etc.) which resulted in the appearance of severe manganese deficiency symptoms which could be prevented in some cases by applying $MnSO_4$, indicating that by the addition of organic material available Mn^{++} had been immobilized. Timonin (1946) using straw mulch obtained similar results and de la Lande Cremer (Agricultural Experiment Station, Groningen, private communication) using aqueous extracts of wheat straw observed distinct symptoms of manganese deficiency in his pot cultures.

However, the nature of the organic material incorporated in the soil seems to be of great importance. When Hudig and Meyer (1919) added oat leaves instead of cellulose to their pot cultures, the plants remained healthy; extracting the leaves with sodium hydroxide or with acids annulled the effect, and the disease symptoms were as heavy as with cellulose. Fujimoto and Sherman (1948) observed a considerable increase in available manganese of Hawaiian soils after treatment with sucrose, ground pineapple leaves, and sugar cane leaves with a high carbon nitrogen ratio. Hurwitz (1948) in studying the effect of applying oat straw and alfalfa meal at a carbon-nitrogen ratio of 30:1 to a soil observed a considerable increase in exchangeable manganese which after three days' incubation at 37° and 47°C. reached a maximum value. Thereupon it decreased to about the initial value after about two weeks' incubation. The increase in exchangeable manganese as described by these authors apparently has to be attributed to the very rapid breakdown of the organic matter as a result of which the oxygen supply became limited and the redox potential was lowered. In the case of sucrose a decrease in pH may also have occurred.

Mann and Quastel (1946) percolating neutral or slightly alkaline soils with 0.02 *M* $MnSO_4$ showed that the manganese usually disappears at an increasingly rapid rate and is found in the soil in an oxidized form. Biological poisons such as sodium azide (0.001 *M*) inhibit the oxidation. They concluded that oxidation under the given experimental conditions is wholly or almost wholly accomplished by proliferating microorganisms.

Though the oxidation of manganese compounds is exothermic, there is as yet no evidence of the existence of true autotrophic manganese oxidizing bacteria. The precipitation of the manganic oxides has been observed inside the bacterial and fungal cells as well as outside (Beijerinck, 1913; von Wolzogen Kühr, 1927; Gerretsen, 1936). Whether in all cases oxidation takes place through the intermediary of hydroxy acids and an accompanying rise of the pH or that a more direct oxidation by means of special enzymes occurs as well is still to be investigated.

As manganese-oxidizing microorganisms of the former class require only small quantities of organic materials and representatives are to be found among the most common groups of soil microbes, it is evident that in most soils the conditions for microbial immobilization of manganese will be fulfilled as soon as the pH rises above neutrality. Moreover organic compounds excreted by the roots may favor the development of manganese-precipitating microorganisms in the rhizosphere. Timonin (1946) showed that a susceptible variety of oats harbored in its rhizosphere five to thirteen times as many manganese-oxidizing bacteria as a resistant variety. The application of cyanogas as a soil disinfectant reduced the numbers of bacteria in the rhizosphere to about 7 per cent of their original value and tripled the yield of the plants.

It is interesting to note that according to von Wolzogen Kühr (1927) the removal of manganese from dune water in the filter beds of the waterworks is essentially a bacteriological process, analogous to that described above for the soil. This author attributes to the precipitated manganic oxide the property of absorbing manganous compounds on its surface which are readily oxidized by microorganisms; in this way manganic concretions of increasing size are built up. It is alluring to suppose that a similar process takes place in the soil by which manganese deposits grow in size and gradually become less available to the plants. The formation of iron-manganese concretions in the A_2 horizons of certain poorly drained prairie soils which are waterlogged for a considerable part of the year, perhaps may be attributed to a similar process. These concretions which may have diameters from 0.1 to 15 mm. contain from fifteen to seventy-five times as much manganese and from two to more than four times as much iron as the surrounding soil in which they are found; the manganese is present as the higher oxides (Drosdoff and Nikiforoff, 1940).

2. *Solubilization of Manganic Oxides*

The solubilization of unavailable manganic oxides can be accomplished by microorganisms in different ways: by changing the redox potential or the oxygen tension of the soil, or eventually by producing reducing organic substances which cause the manganic-manganous equilibrium to shift in the direction of the latter compounds. The favorable effect of waterlogging on manganese-deficient soils is to be ascribed to these activities of microorganisms. In addition to a high water content which greatly limits the entrance of oxygen from the air, a certain amount of assimilable organic matter is necessary for the growth of the microorganisms to enable them to accomplish their diverse metabolic activities. Söhngen (1914) has convincingly shown that precipitated

brown MnO_2 may be brought into solution by hydroxy acids, produced by microorganisms, e.g., from cellulose, but not by fatty acids, such as acetic, propionic, or butyric acids. Similar results have been obtained by Heintze and Mann (1947). They found, however, a sharp difference between $Mn(OH)_3$ and MnO_2, the former being dissolved to a large extent by various hydroxy acids whereas the latter was practically unattacked.

The pH may influence manganese availability in different ways. The reduction of higher manganic oxides by organic matter is more important at low pH values, since the oxidizing power of these oxides increases rapidly with acidity. The production of strong inorganic acids especially sulfuric acid by the sulfur-oxidizing bacteria and nitric acid and sulfuric acid by nitrifying bacteria favorably influences the amount of manganese available to the plants.

3. Microorganisms in Relation to Leaf Spots of Manganese-Deficient Oats

Microorganisms may play another important role in connection with the development of the typical symptoms of manganese deficiency. Gerretsen (1937) carried out extensive studies with sterile cultures of oats, in soil, sand, and nutrient solutions containing inadequate quantities of manganese for normal growth and showed that, when these cultures were kept sterile, the plants did not show typical symptoms of gray speck disease, though generally they remained smaller than normal plants.

Infection of these cultures, either with a small quantity of the original diseased soil (5 per cent) or even with a diseased root tip of a diseased plant resulted in the appearance of the typical symptoms. Alkaline products produced in the decaying roots by the infecting saprophytic microorganisms are carried in the transpiration stream to the leaves, where they produce the gray spots.

According to Gerretsen it is necessary to distinguish between the direct physiological effect of manganese deficiency (etiolation, reduced photosynthesis, necrosis) and the typical leaf spots related to the infection of the roots.

In the light of these facts one aspect of the distinction between susceptible and resistant varieties of oats might be traced back to an increased unspecific resistance of the roots toward invading microorganisms, hereditary "axeny," as Gäumann (1946) calls it, which is a common quality of resistant varieties in general. On the other hand, diminished photosynthesis is the reason why manganese-deficient plants contain less carbohydrates than normal plants (see Sec. X,4), and it

is to be expected that in consequence the roots not only remain smaller but also have a lower degree of resistance to invading microorganisms.

In accordance with this view Timonin (1946) succeeded in growing healthy susceptible oats on a diseased plot which had been treated with cyanogas, a soil fumigant. He ascribes the improvement, however, to the eradication of the manganese-oxidizing bacteria in the soil. Hasler (1951) showed that the dry weight of the roots of a number of grasses was much more depressed than that of the leaves on manganese-deficient soils, the ratio of the dry weights of the roots of the diseased plants as compared to those of the healthy ones being 1:5, whereas that of the leaves was 1:2.5.

4. Resistant Oat Varieties

The fact that the resistant varieties do not show the typical gray speck disease symptoms may point to an increased resistance of the roots toward invading saprophytic microorganisms.

There is no doubt that the resistant varieties need manganese too for normal growth, though they might be adapted to a lower level of this element than the ordinary varieties. Accordingly the yield of the resistant varieties is also unfavorably affected by manganese deficiency. Rademacher (1935) growing a resistant variety on a manganese-deficient soil obtained a lower yield (45 per cent) than on a healthy soil. Though in Timonin's experiments (1946) the resistant variety ACTON produced 40 per cent more grain than the susceptible variety on a diseased plot, the yield of the former was doubled by fumigating the soil with cyanogas. It is supposed that in this case the oat plants have had more manganese at their disposal, as, according to Timonin, the greater part of the manganese-oxidizing bacteria had been killed by the soil disinfectant. It is the impression of the authors that there is as yet insufficient evidence to draw a definite conclusion with regard to the real causes of the resistance of certain oat varieties to manganese deficiency.

V. SYMPTOMS OF MANGANESE DEFICIENCY IN PLANTS

Cereals, oats (Wallace, 1944; Samuel and Piper, 1929). Among cereals, oats is the most susceptible crop. Marginal gray-brown colored necrotic spots and streaks appear first on the third highest leaves, particularly on the basal half. The streaks tend to elongate and coalesce. At the distal ends of the affected basal part the necrotic spots may soon extend across the blades so that the upper half or two-thirds of the leaf falls over with a sharp kink at the collapsed portion. The distal ends of the leaves remain green for a considerable time. On older leaves

the collapse may be confined to the lower quarter, and oval spots of necrotic tissue may appear irregularly on the leaf blade, though less frequently toward the tip end. Streaks of tissue collapsing at the margins of the leaves are also very characteristic. The root system is poorly developed and is more affected by microorganisms than is the case with an adequate manganese supply.

In *wheat* and *barley* the symptoms are not so characteristic as in oats. The leaves are somewhat pale green and may show only faint chlorotic streaking and yellowing. In severely affected barley irregular brown necrotic spots occur on the leaves running especially between the veins. In some cases these necrotic spots may be situated on the middle parts of the leaves with falling over of the distal halves similar to oats. In other cases the spots are located on the basal half and sometimes on the distal half. In wheat interveinal white lesions and streaks may develop. In rye and maize the necrotic tissues are also white colored. In severely attacked rye large parts of the leaves may collapse. The general appearance of manganese-deficient cereals is very limp. In the field rye is considerably less susceptible than the other cereals. Of ten varieties of winter and spring wheat tested by Gallagher and Walsh (1943) three appeared to be nearly immune to manganese deficiency, six others were particularly sensitive, and one was intermediate. Similar large differences were noted in oats.

Grasses. Grasses growing on soils where oats showed severe symptoms of manganese deficiency did not exhibit the typical leaf symptoms (Hasler, 1951). Some leaves had brownish tips while grayish spots were situated on the leaf blades. Of the fifteen species tested by Hasler, *Alopecurus pratensis, Arrhenatherum elatius,* and *Bromus erectus* gave the highest increases in yield upon manganese treatment. *Anthoxanthum odoratum, Cynosurus cristatus, Festuca pratensis* and *Trisetum flavescens* gave intermediate responses. The following species responded slightly or not at all to added manganese. *Agrostis alba, Dactylis glomerata, Festuca rubra, Lolium italicum, Lolium perenne,* and *Poa pratensis.* The root systems of manganese-deficient grasses were poorly developed.

Beans (*Phaseolus vulgaris*) (Townsend and Wedgworth, 1936). At first the trifoliate leaves show a faint mottled pattern, the tissue near the veins remaining green longer than that between the veins. The cotyledonary leaves remain green until late in the development of the disease. A few days after the appearance of the first mottling the entire leaf blade may turn golden yellow. Small necrotic brown spots can be seen parallel to each side of the midrib and principal veins which may extend to the tips and margins of the leaves. Subsequently, the under-

surface of affected leaves appears to be cupped between the veins, while the upper surface of the same areas appears water-soaked and soon breaks down. New growth from the apical bud becomes slower as the disease progresses, and the buds eventually die. All leaves become brown and withered by the time the bud dies. Frequently there is secondary growth from lateral buds.

Peas (Wallace, 1944). The plants may appear quite healthy or, with a severe deficiency, may show a somewhat chlorotic condition in the foliage. In the seeds a very characteristic condition of the seed occurs, known as marsh spot. When the seed coat is removed and the two cotyledons are separated, small brown specks or larger circular brown areas are seen on the flat surfaces. The areas may become hollowed out in very severe conditions.

More severely affected plants show a brownish discoloration of the young tendrils and the youngest internodes at the top of the stem (Samuel and Piper, 1929). The youngest leaves fail to expand, becoming yellowish with small discolored areas between the veins. Slightly older but not fully expanded leaves acquire a characteristic mottled appearance due to the mesophyll between the small veins becoming yellow, whereas the veins themselves remain green. The lower leaves retain their normal green color. The growing tips and the youngest leaves will be completely dead in a short time.

Potato (Wallace, 1944). The tip leaves lose their luster and turn pale. They tend to be small and rolled toward the upper surfaces. Small blackish brown spots may be developed on the leaves along the veins, and although these are more numerous on the pale leaves near the tips of the shoots, they may also be present on older leaves which are still green. In severe conditions the plants show much browning and yellowing, especially in the young foliage.

Tobacco (McMurtrey, 1944). The first visible symptom is a loss of color in the young leaves. Between the veins the tissue is light green to almost white while the veins themselves remain darker. The leaf has a checkered appearance, because of the contrast between the green veins and the tissues that have lost their color. In the latter tissues necrotic spots may develop which may drop out giving the leaf a ragged appearance. Usually this spotting is not confined to the tip and margins, as in the case of potassium deficiency, but involves parts scattered over the entire leaf. The plant as a whole may be considerably dwarfed.

Tomato (Skinner, 1944). The earliest symptom is a lightening of the green color, which gradually turns to yellow in the leaf areas farthest from the major veins. As the condition progresses the yellow becomes more marked and extensive. The veins remain green, which gives a char-

acteristic mottled appearance to the leaf. Eventually the foliage may become completely yellow, and in many cases necrosis sets in, appearing at first as small brown pinpoints centering in the yellow areas farthest from the veins and expanding until larger dead areas indicate complete breakdown of the tissue. Growth is spindling, little or no blossoming takes place, and no fruits form.

Sugar beet and mangold (Wallace, 1944). The leaves tend to be more upright than usual and somewhat triangulate in outline, due to curling of the margins toward the upper surfaces. The interveinal tissue becomes chlorotic. Brownish spots may appear in the interveinal areas and the brown tissue may die and fall out leaving small holes.

In *Brassica* crops (Wallace, 1944), the symptoms first appear as an interveinal chlorotic marbling. With severe deficiencies the whole of the leaves may be practically bleached, only the veins remaining green (kale) or some necrosis may develop in the mottled tissue, when it takes on a dull brownish gray appearance (savoy cabbage).

Other vegetable crops. The general foliage symptoms are a chlorosis between the veins, in many cases extending from the margin inward. The tissue along the veins and midrib remains green much longer.

Ornamental plants. Dickey and Reuther (1938) describe manganese deficiency symptoms in a great number of ornamental plants. In most cases the affected leaves show a yellow green chlorosis extending inward from the margins between the primary veins, the tissue immediately surrounding the midrib and primary veins remaining green.

Fruit plants (apples) (Wallace, 1944). The leaves develop an interveinal chlorosis which begins near the margins and extends toward the midrib, and finally only the veins remain green. Strongly growing young shoots may be only little affected, which is a point of difference from iron deficiency, in which the chlorotic condition is always most severe in the young tip foliage. In pears, plums, peaches, and cherries the symptoms are similar to or only slightly different from those of apple (see Wallace, 1944). In citrus the leaf pattern is also that of a network of green veins on a lighter green background (Camp *et al.*, 1944).

VI. Manganese Content of Plants

1. *Normal Plants Growing under Natural Conditions*

The manganese content of plants grown under natural conditions varies enormously. This is mainly due to the fact that the availability of soil manganese varies in much the same way. Since the latter has been shown to be dependent to a large extent on soil pH and oxidation-

reduction potential (see Sec. III), it may be expected that plants growing on acid soils and on waterlogged soils are rich in manganese.

Olsen (1934) determined manganese in leaf blades of *Holcus lanatus, Oxalis acetosella, Asperula odorata,* and beech (*Fagus silvatica*) growing on Danish soils at a wide range of pH values. A close correlation was found between soil pH and manganese content of these plants. Above pH 7 values lower than 100 p.p.m. were found, whereas on the most acid soils the leaves contained more than 1600 p.p.m. in the dry matter. Plants growing on swampy soils contained high manganese values even at pH values higher than 7. The uptake of manganese by different plant species growing under the same conditions may differ widely. This appears from the extensive investigations of Erkama (1947), who analyzed a great number of naturally occurring plant species.

The analytical data of Mayer and Gorham (1951) show that the manganese content of naturally occurring plants varies with the individual species, the pH of the soil, and the water content of the soil. The correlation with pH, as observed by these authors was less pronounced than was the case in the investigation of Olsen (1934).

2. *Manganese Content of Healthy and Manganese-Deficient Plants*

There is little agreement in the literature as to the critical amount of manganese in plants below which deficiency symptoms may be found. Samuel and Piper (1929), Piper (1931), and Leeper (1935) found 14 to 15 p.p.m. in the whole plant at the flowering stage to be the lowest value in healthy cereals. Rye, ryegrass, and a tolerant barley variety were found to be healthy when containing only 10 to 11 p.p.m. manganese on the dry basis. Gerretsen (1937) succeeded in growing healthy oat plants in sterile culture media very low in available manganese. Values as low as from 5 to 10 p.p.m. Mn in such plants were found.

In manganese-deficient winter wheat (tops) analyzed in early spring Coïc and Coppenet (1949) found values of 20.3 and 22.5 p.p.m. and in healthy plants 34.5 and 48.5 p.p.m. Goodall (1949) determined manganese in various parts of wheat plants at different growing stages. He found no response to manganese treatment when the manganese content of the older leaf blades at the beginning of shooting was higher than 34 p.p.m.

Nicholas (1949) determined manganese in the leaves of a number of crop plants grown on a soil poor in available manganese. Oats with 7 p.p.m. Mn had the lowest value and showed pronounced deficiency symptoms; wheat and barley containing 14 and 12 p.p.m. of Mn respectively

were slightly deficient, whereas maize on the same soil containing 15 p.p.m. Mn in the dry leaves was healthy. From the other plants investigated, peas with 8 p.p.m. and sugar beet and mangold both with 10 p.p.m. showed moderate deficiency symptoms. In flax, radish, carrot, and lucerne with 15 p.p.m. or over, visual signs of the deficiency were absent. These values are much closer to those of the Australian workers than to those of Goodall and Coïc and Coppenet.

Hasler (1951) found the following concentrations of manganese in manganese-deficient grasses: *Arrhenatherum elatius,* 37 p.p.m.; *Festuca pratensis,* 44 p.p.m. A large variation in the manganese content of various grass species grown on the same soil has been recorded by Beeson *et al.* (1947). Under the conditions of their experiments *Agrostis alba* contained 815 p.p.m. Mn whereas *Poa pratensis* had values of 108 and 164 p.p.m. Seekles (1950) determined manganese contents of grass grown on different soils in the Netherlands. Grass from clay soils had the lowest manganese content viz. 114 p.p.m. in the dry matter that from peaty soils had 152 p.p.m. and that from sandy soils 191 p.p.m. These values are averages of a great number of analyses in samples originating from different parts of the country.

VII. Correcting Manganese Deficiency

Manganese deficiency can be corrected in a number of ways.

1. *Application of Manganese Sulfate to the Soil*

Amounts from 50 to 100 kg. per hectare are being recommended by various authors, although on alkaline peat soils larger amounts may be required for obtaining healthy plant growth. Since on many manganese-deficient soils the added manganese sulfate will be readily converted into practically unavailable oxides, the duration of the improvement is often very short. This was already noted by Hudig (1911), who had to apply manganese sulfate simultaneously with the sowing of oats in order to be sure that the plants did not suffer from gray speck disease.

2. *Spraying the Foliage with a Dilute Solution of Manganese*

This method is more economical since much smaller amounts can be employed. From 0.2 to 0.5 per cent manganese sulfate solutions are being used (from 500 to 1000 l. per hectare). Gallagher and Walsh (1943) employed 1 per cent manganese sulfate sprays in correcting manganese deficiency in oats. Harmer and Sherman (1943) added manganese sulfate to Bordeaux mixture at the rate of 1 kg. $MnSO_4$ per 500 l. Bordeaux mixture. Manganese deficiency in fruit trees was cor-

rected by Thompson (1944) by injecting a solid manganese salt into the trunk.

3. *Treating the Soil with Acidifying Substances*

The beneficial effects of treating the soil with acidifying substances such as sulfur or sulfate of ammonia are often due to the formation of pockets of relatively high acidity in which the manganese becomes much more soluble than in the bulk of the soil. That this statement is true may be concluded from the experiments of Hudig (1911) in which mixing of a peaty soil with sulfuric acid or acetic acid in amounts equivalent to those present in ammonium sulfate had no effect on the deficiency symptoms of oats, whereas a treatment with sulfate of ammonium gave practically healthy plants. Similar results have been obtained by Gerretsen in an unpublished experiment with sulfur of a different degree of fineness. The best results were obtained with coarse particles.

Treating the soil with acid peat may also give good results.

A method which has given perfect results is mixing the applied manganese sulfate with one of the above mentioned substances.

4. *Flooding*

The flooding of soils sometimes may give a much improved manganese supply to the plants due to the reduction of part of the manganic oxides to available manganese. Under certain circumstances, however, flooding may have the reverse effect. This has been demonstrated in the Dutch and Belgian sea polders which were inundated during World War II. After the flooding manganese deficiency was more frequently observed than before.

VIII. Manganese Nutrition and Fertilizer Interactions

The effect of compounds which bring about considerable changes in pH (lime, sulfur) will not be considered here, since their effect has been discussed in detail in Sec. III,1.

1. *Nitrogen Applications*

The beneficial effect of ammonium sulfate as contrasted to the harmful influence of nitrate, particularly sodium nitrate, on the availability of soil manganese is well known. This effect is entirely indirect and results from the changes in pH which accompany the uptake of NH_4^+ and NO_3^- by the plants. Since the shift in pH is greater with sodium nitrate than with calcium nitrate, the former affects the availability of soil manganese more strongly.

It may be expected, however, that the nitrogen compounds will exert a direct effect on the uptake of manganese. Furthermore the change in pH of the nutrient medium may also affect the uptake of manganese ions.

Several authors have observed that nitrate nitrogen may favor the manganese uptake by plants. Olsen (1934) growing barley in nutrient solutions of pH 6–7 found 90 p.p.m. in the dry matter of the leaf when the plants had been supplied with nitrate and 23 p.p.m. when ammonium nitrogen had been employed. Coïc *et al.* (1950) studying manganese deficiency in cereals in Brittany obtained much more healthy plants when the crop was treated with calcium nitrate than in the absence of added nitrogen. The manganese content of the grain from manganese-deficient plants was 14 p.p.m. without nitrogen fertilization and 19.4 p.p.m. when treated with a moderate amount of calcium nitrate. When treated with manganese the values were 15.5 and 21.3 p.p.m. of manganese respectively.

Timonin (1950b) reports a beneficial effect of calcium nitrate in correcting manganese deficiency under field conditions.

The favorable effect of nitrates on manganese uptake by plants is also apparent from papers reporting on studies of manganese excess (Millikan, 1950; Löhnis, 1951).

Manganese uptake in relation to pH of the nutrient medium was studied by Olsen (1934) in culture solution experiments with barley, mustard (*Sinapis alba*), *Zea mays*, and *Lemna polyrrhiza*. An optimal uptake was found to take place at pH 6 and 7. Similar results were obtained by Burström and Boratynski (1936) in nutrient solution experiments with wheat. From pH 3.5 to 6.5 a clear increase in manganese uptake was observed.

The increased uptake of Mn^{++} (or other cations) at decreasing hydrogen-ion concentration of the nutrient medium may be explained by an enhanced affinity of Mn^{++} for the root surface as a result of the increased Ca/H ratio on the root surface exchange complex (complementary ion effect). If under similar conditions of decreased hydrogen-ion concentration a base-adsorbing solid substrate, e.g., clay, is present, two systems are operating with opposite effects on the availability of Mn^{++}. The release of Mn^{++} from the surface of the solid exchange material will be diminished, whereas the affinity of Mn^{++} for the root surface is enhanced. Epstein and Stout (1951) studying the uptake of manganese from bentonite-containing suspensions observed an increased absorption of manganese with increasing Ca/H ratio on the bentonite. Apparently the increased affinity of Mn^{++} toward the root surface at increased Ca/H ratio outweighed the diminished exchangeability of manganese on

the bentonite. Under soil conditions the complementary ion effects will be obscured by changes in the concentration of available manganese.

2. *Effect of Phosphate Fertilization*

A beneficial effect of superphosphate on uptake of applied manganese was observed by Steckel *et al.* (1948) when both fertilizers were mixed together. The beneficial effect is stated to be due to the precipitation of the manganese as the manganous phosphate, which would retard the oxidation of the manganese and would provide a constant though small supply of divalent manganese. The somewhat increased acidity caused by the superphosphate surrounding the manganese would also be of some importance in preventing rapid oxidation of the added manganese.

On the sandy soils of central Florida which are almost exclusively composed of siliceous sand, application of large amounts of phosphate at pH values from 5.5 to 6.0 may give rise to the accumulation of calcium phosphate, presumably "hydroxy apatite" which retains applied manganese and magnesium so that they are not so readily leached by high rainfall as is the case from these soils without the presence of accumulated phosphate. Both manganese and magnesium thus adsorbed are available to citrus (Wander, 1950).

Albrecht *et al.* (1941) in studying the effect of calcium carbonate and phosphate applications to a well-weathered prairie soil of pH 5.5 found that calcium carbonate when mixed throughout all the soil lowered the manganese content of sweet clover, lespedeza, *Poa pratensis,* and notably *Agrostis alba,* as might be expected. Mixing the carbonate into the surface one-fourth of the soil and leaving three-fourths untreated gave a considerably higher manganese content of the crop as compared with the untreated plants. Phosphate dressings were found to exert a beneficial effect on manganese uptake of the plants.

3. *Effect of Copper on Manganese Nutrition*

Hudig *et al.* (1926) have stated that copper treatment of neutral soils on which the crop suffered from copper deficiency would correct the copper deficiency but would promote the occurrence of manganese deficiency.

Mulder (1938) studying the phenomena related to copper nutrition of plants and copper treatment of soils has carried out a few experiments, the results of which tend to confirm the above statement of Hudig *et al.* (1926). The microbiological oxidation of manganous salts to MnO_2 by fungi was found to be clearly stimulated by the addition of a trace of copper to the nutrient medium of the microorganisms. The copper-manganese relationship in green plants was studied in nutrient

solution experiments with cereals. In one experiment with rye an increased demand for manganese was observed with increased copper supply. In a similar experiment with barley such a copper-manganese interaction did not occur. In the presence of an excessive amount of manganese, however, the barley roots of copper-supplied plants showed an intensive browning presumably due to MnO_2, particularly in the upper parts, which was absent when the plants suffered from copper deficiency. Although the plants were not growing under sterile conditions, the browning occurred so uniformly that it was improbable that microorganisms were involved. The results of these experiments show that copper may activate the biological oxidation of manganous compounds by fungi and probably by root cells from barley plants.

Kenten and Mann (1949) were able to extract from horseradish roots an enzyme system which may oxidize manganese in the presence of hydrogen peroxide to Mn^{+++} or Mn^{++++}. The system consists of peroxidase and peroxide substrate (Kenten and Mann, 1950). Although it is unknown whether the system may accumulate MnO_2 *in vivo,* it is probable that at a high concentration of Mn^{++} this may be the case. It is unknown whether this system may be less active in copper-deficient plant roots.

The effect of copper sulfate on the oxidation of manganese in acid soils treated with calcium carbonate was studied by Sherman *et al.* (1942). In contrast to the biological oxidation reported above, a retarding effect of copper on manganese oxidation was demonstrated, as a result of which oat plants were suffering much less from manganese deficiency than in the absence of applied copper sulfate. Both soils used by Sherman *et al.* (1942) were clay soils which presumably were not deficient in copper. The results of Ødelien (1948), working on copper-deficient soils are in agreement with those of Hudig *et al.* (1926).

4. *Iron-Manganese Relationships*

According to Shive (1941), Somers and Shive (1942), Somers, Gilbert, and Shive (1942), and Stiles (1946), the ratio of iron to manganese in the nutrient medium of soybean plants and presumably also of other plant species should lie between 1.5 and 2.5 to assure optimal plant growth. If the ratio is above 2.5, symptoms of iron toxicity (= manganese deficiency) would occur; if it is below 1.5, the plant would suffer from manganese toxicity (= iron deficiency). Since the values for active (soluble) manganese and iron within the plant tissues associated with good growth covered about the same range of values as did those in the external medium, the observed phenomena were thought

to be due to the existence of a close interrelationship between iron and manganese within the living cell.

In a large number of papers either published earlier than those of Shive and collaborators, or later, the existence in plants of a more or less pronounced iron-manganese interdependency has been reported (see the review by Twyman, 1946). According to most authors, however, the range of iron-manganese ratios of the nutrient medium at which normal plant growth is possible is much wider than that mentioned by Shive *et al.* In contrast to the statement of these authors that more importance should be attached to the ratio of manganese to iron than to their absolute concentrations in the nutrient medium, Hewitt (1948c) found that the growth of oats and sugar beet was affected by absolute levels of these elements rather than by their ratios. Similar results were obtained by Morris and Pierre (1947) in nutrient solution experiments with lespedeza. Increase of the concentrations of both iron and manganese caused pronounced symptoms of manganese toxicity notwithstanding that the ratio of iron to manganese in the nutrient solution was unchanged. Ouellette (1951) found both the concentration of iron and manganese in the culture solution and their ratio of importance in attaining optimal growth of soybeans. Although iron chlorosis due to manganese excess is reported by many authors, there is sufficient evidence available in the literature to state that manganese excess is not identical with iron deficiency and that iron excess is not identical with manganese deficiency (see Sec. IX).

Some authors have presented clear evidence that an increased iron supply to the plants may depress the uptake of manganese (Somers and Shive, 1942, for soybeans; Morris and Pierre, 1947, for lespedeza; Burström, 1939, for wheat). An increased manganese supply may also depress the uptake of iron, but the effect is less clear than the reverse (Somers and Shive, 1942). Friederichsen (1944) studied the effect of increasing manganese concentration of the nutrient solution on the iron content of roots and leaves of spinach and barley plants. With nitrate as the nitrogen source high concentrations of manganese did not affect the iron content of the leaves. The roots, however, were much lower in iron. When the plants were supplied with ammonium nitrogen, manganese depressed not only the iron content of the roots but also that of the leaves.

IX. Manganese Toxicity in Plants

It has been shown, mainly during the last decade, that excess of manganese in the nutrient medium may cause toxicity symptoms. Since the concentrations of manganese at which the toxicity symptoms become

visible are relatively low, many acid soils may be found on which the manganese supply to the plants is high enough to bring about some crop injury. From the results of several investigations which will be discussed below, it has appeared that manganese toxicity is one of the main causes of soil acidity injury to plants. Aluminum toxicity and lack of available calcium, magnesium, or molybdenum may also affect plant growth on certain acid soils.

Before excess of available manganese was recognized as the cause of injury to plants growing on certain acid soils (Jacobson and Swanback 1932; Bortner, 1935; Wallace *et al.*, 1945; Hewitt, 1945; Hale and Heintze 1946; Löhnis, 1946, 1951), manganese toxicity of plants had been studied by soil scientists working on manganiferous soils, i.e., soils rich in manganese. Such soils have been described as early as 1909 by Kelley (see Sherman *et al.*, 1949; Jacobson and Swanback, 1932) in the drier regions of the Hawaiian Islands. Manganese contents ranging from 1 to 4 per cent may be found in such soils. Kelley (1909) indicated the relationship between the poor growth of pineapple in these regions and the high manganese content of the soils. Extensive pot experiments with similar soils have been carried out in Puerto Rico by Hopkins *et al.* (1944).

That manganese toxicity may be the cause of injurious effects observed on acid soils was shown by Jacobson and Swanback (1932) working with tobacco on Connecticut soils, and Bortner (1935) studying abnormally growing tobacco in Kentucky. Extensive investigations have been carried out by workers of the Long Ashton Research Station in England (Wallace *et al.*, 1945; Hewitt, 1945, 1946, 1947, 1948a). They compared the visual symptoms of acidity injury of different plant species growing in the field with those of sand cultures at different levels of certain added micronutrient elements. A striking resemblance was found to exist between field symptoms of acidity and excess of manganese in the sand cultures, particularly at low calcium levels. This was found to be true of several agricultural and horticultural crops (including runner bean (*Phaseolus vulgaris*), cauliflower, savoy cabbage, swede, and kale). In potato, calcium deficiency was also frequently seen as an acidity symptom. Oats, barley, celery, carrot, beet, and particularly sugar beet and mangold appeared to be much more sensitive to excess of aluminum than to excess of manganese so that acidity injury to these crops may resemble aluminum toxicity. Hale and Heintze (1946), studying cases of field acidity from various parts of England came to conclusions similar to those of Wallace *et al.* (1945).

The poor growth of legumes on many acid prairie soils in the United States appeared to be due to high amounts of soluble manganese in these

soils (Morris and Pierre, 1947, 1949; Morris, 1948). On certain acid soils of northern Wisconsin potatoes may suffer from "stem streak necrosis," a disease which Berger and Gerloff (1947a,b) showed to be due to excess of available manganese. Schmehl *et al.* (1950) growing alfalfa in an acid soil from New York observed symptoms of manganese toxicity. According to these authors the poor growth of their plants was only partly due to manganese excess. Aluminum toxicity apparently was of more importance. Similarly Heslep (1951) found phosphorus deficiency, manganese toxicity and presumably a third factor as the causes of poor growth of lettuce in two acid Californian soils.

Although magnesium deficiency is often found to be the cause of acidity damage of cereals and potato on sandy and peaty soils in the Netherlands (Smit and Mulder, 1942), manganese toxicity may also occur (Löhnis, 1946, 1950, 1951). In 1940 the disease was observed for the first time in *Phaseolus vulgaris* growing on a sandy soil which had been fertilized with ammonium sulfate for a number of years as a result of which the pH had dropped to a very low value. Its cause remained unknown until 1943 when visual symptoms identical with those noted in the field were induced by the addition of excess manganese to plants growing in nutrient solutions or in neutralized soil. To obtain more evidence as to the cause of the disease, young full grown leaves of *Phaseolus vulgaris* from both injured and normal plants, grown on the same soil treated with lime, were analyzed for manganese. The values found in a large number of samples from injured plants, collected during three successive years, ranged from 1104 to 4216 p.p.m. Mn in young foliage and from 40 to 904 p.p.m. in healthy plants of the same age. Although in different years apparently owing to different climatic conditions widely varying concentrations of manganese were found, the threshold values above which the foliage was always visibly affected was almost constant in the successive years, that is approximately 1200 p.p.m. on a dry weight basis. This value depends on a number of various factors, and it may be expected that changing the circumstances under which the plants are growing also will change the amount of manganese at which toxicity symptoms become visible. Temperature was found by Löhnis to have an important effect. Bean plants containing manganese in such amounts that severe symptoms of injury might be expected at moderately high temperature remained healthy at a high temperature. Millikan (1951) in nutrient solution cultures of peas found a remarkable difference in tolerance between older and younger leaves of the same plant. Manganese concentrations much higher than those which

were found in the affected younger leaves did not cause injury to the lower leaves.

Of further plant species tested by Löhnis (1951), vetch and lucerne appeared to be very susceptible to excess of manganese. The minimum amount of manganese in affected plants (field experiments) was for vetch 500 p.p.m. in 1948 and 1117 p.p.m. in 1949 and for lucerne 477 p.p.m. in 1948 and 1083 p.p.m. in 1949. White and red clover and flax were found to be slightly susceptible. Mangold was depressed in growth in acid soils but appeared to be insensitive in nutrient solutions. This agrees with the results of Hewitt (1948a) that the injury by acidity in sugar beet and mangold is often due to aluminum toxicity. Potatoes, tobacco, mustard, oats, and strawberry appeared insensitive in field tests. In contrast to the results of Löhnis potatoes have been found to suffer from manganese toxicity on certain northern Wisconsin soils [stem streak necrosis, Berger and Gerloff (1947a,b)], while tobacco growing on certain acid soils in Kentucky was found to show typical manganese toxicity symptoms (Bortner, 1935). Whether these different results in potatoes and tobacco have to be attributed to the different environmental conditions under which the plants were growing or to varietal differences is unknown.

Morris and Pierre (1949) working with nutrient solutions found lespedeza and sweet clover to be much more sensitive to manganese toxicity than peanuts. Cowpeas and soybeans held intermediate positions. Since the peanuts contained much lower amounts of manganese than the other plant species supplied with the same amount of manganese, the tolerance of this plant apparently is due to a limited absorption of manganese. Lespedeza absorbed large amounts of manganese, whereas the sensitiveness of sweet clover apparently was due to its inability to endure relatively low amounts of manganese.

In accordance with these results Löhnis concluded from the manganese contents of the various plants tested for manganese toxicity that a tolerance for a high level of available manganese in the soil may be due (1) to a weak absorption of manganese (oats, mustard, mangold) and (2) to a strong tolerance within the plants (tobacco, flax, strawberry, potato, and presumably broad bean). Striking examples were found to be oats, which contained only 325 p.p.m. of Mn when growing in an acid soil, and tobacco, which contained nearly 3000 p.p.m. Both plant species showed no visual symptoms of manganese toxicity. Jacobson and Swanback (1932) recorded values of 5250, 6470, and 11670 p.p.m. of manganese in affected tobacco grown on acid soils.

1. *Manganese Toxicity in Relation to the Supply of Some Nutrient Elements*

a. Effect of Nitrogen Compounds. The effect of nitrogen nutrition on manganese toxicity was studied in detail by Millikan (1950) in nutrient solution experiments with flax. With nitrate and urea as the nitrogen sources severe symptoms of manganese injury were noted which were absent in the case of ammonium nitrate and ammonium sulfate and in plants subjected to nitrogen deficiency. Toxicity symptoms were affected by these treatments in the following range of increasing severity nitrate>urea>NH_4NO_3>$(NH_4)_2SO_4$>nitrogen deficiency. Although the manganese content of the plants was much higher in the case of nitrate nutrition than when supplied with ammonium compounds, it was presumably not the only cause of the large difference in toxicity symptoms. The beneficial effect of the presence of ammonium ions in the nutrient solutions on the appearance of manganese toxicity symptoms was also observed by Löhnis (1951). The foliage of beans treated with nitrate alone contained five times as much manganese as in the presence of ammonium nitrate.

b. Effect of Phosphorus. A beneficial effect of phosphate fertilization of acid soils on tobacco plants suffering from manganese toxicity was found by Bortner (1935). In nutrient solutions with high manganese, phosphate gave a similar effect. These results were not confirmed by Morris and Pierre (1947), who found a greater depression of growth of lespedeza owing to excessive manganese in culture solutions with a higher phosphate concentration. Walsh *et al.* (1950) observed a favorable effect of applications of superphosphate and basic slag in preventing manganese toxicity of swedes.

c. Effect of Calcium. There is no agreement in the literature as to the effect of calcium ions on manganese injury. Hewitt (1945) considers calcium as an element that clearly antagonizes the intake of manganese under sand culture conditions. With increasing calcium supply (as the sulfate) the symptoms of manganese toxicity became less severe.

Morris and Pierre (1947) in culture solution experiments with lespedeza were unable to show an alleviation of manganese toxicity by the addition of calcium. If acid soils were treated with calcium sulfate, the symptoms of manganese deficiency in lespedeza and sweet clover were found to be aggravated (Morris, 1948). This was shown to be due to a lowering of the pH as a result of which the content of water-soluble manganese in the soil increased. Water-soluble rather than exchangeable manganese of the soil was found to be a reliable indicator of manga-

nese toxicity. A similar unfavorable effect of calcium sulfate application on soil pH and manganese toxicity was found by Berger and Gerloff (1947a) with potato and by Schmehl *et al.* (1950) in pot experiments with alfalfa.

The well-known beneficial effect of liming (increase of pH) on manganese toxicity is due to the conversion of soluble manganese to unsoluble manganese oxides (see Sec. III). The uptake of Mn^{++} by the plants is favored, however, as was shown by Olsen (1934) in nutrient solution experiments with barley, *Zea mays,* mustard, and *Lemna polyrrhiza.* Optimal absorption was found to occur at pH 6 to 7.

d. Manganese Excess in Relation to Iron Deficiency. If the statement of Somers and Shive (1942) that normal plant growth depends on a certain iron-manganese ratio within the plant would appear to be true, it may be expected that manganese toxicity is identical with iron deficiency. Several authors have discussed this problem; some of them agree to a certain degree with Somers and Shive, others do not give support to their views.

Evidence that excess of manganese may produce symptoms of iron deficiency comes from those authors who have studied manganese excess in pineapple on manganiferous soils in Hawaii and Puerto Rico. On these soils growth of pineapple is very poor apparently owing to a large absorption of manganese. The plants become very chlorotic, and they respond clearly to sprays of ferrous salt solutions. (Johnson, 1917; Hopkins *et al.,* 1944; Sideris and Young, 1949). When Phaseolus beans were planted in such soils, they showed severe chlorosis within ten days, and no further growth of the plants took place (Hopkins *et al.,* 1944). Applications of iron as humate or liming the soil to pH 6.2, which precipitated a great deal of the soluble manganese, gave normal plant growth. In addition to these experiments with soil, Hopkins and collaborators carried out extensive investigations with beans, tomato, and pineapple, growing in culture solutions in order to elucidate the iron-manganese relationship. They presented substantial evidence that under the conditions of their experiments the injurious effect of excessive manganese might be counteracted by iron. This was found to be true not only of the chlorosis but also of necrotic spotting which occurred on the leaves.

Similar results were obtained by Millikan (1947, 1949), who studied the effect of a number of heavy metals, including manganese, on the growth of flax in nutrient solutions. Although his main interest lay with the antagonizing effect of molybdenum on manganese toxicity, Millikan carried out treatments with iron salts to show that the chlorosis and top

necrosis of his flax plants were due to iron deficiency. Other symptoms of excessive manganese, such as the dwarfed growth and the lower leaf necrosis were not prevented by iron. Warington (1951) confirmed Millikan's results with flax in foliage painting tests with dilute ferrous sulfate solutions.

The results of Hopkins *et al.* (1944), Millikan (1947, 1949), and Warington (1951) indicate that at least part of the symptoms of manganese toxicity may be considered as manganese-induced iron deficiency.

Morris and Pierre (1947) were unable to confirm these results with lespedeza. Symptoms of iron deficiency were found to differ widely from those of manganese toxicity. Nevertheless they observed a considerable alleviation of manganese toxicity by addition of iron. This was found to be due to an approximate 50 per cent reduction in the manganese content rather than to an increase of total iron in the plant. In a further set of experiments (Morris and Pierre, 1949) it was shown that the symptoms of manganese toxicity in soybeans, cowpeas, and peanuts were entirely different from those of iron deficiency.

Manganese toxicity of potato (stem streak necrosis) was found by Berger and Gerloff (1947a), to be entirely different from iron deficiency, and it was unaffected by treating the plants with dilute ferrous sulfate solutions.

Although Hewitt (1948b) in a single case reports the occurrence of iron deficiency (in sugar beet) upon treatment with several heavy elements, including manganese, he was unable to obtain any response to ferric citrate when painted on the leaves of Phaseolus showing manganese toxicity symptoms (1945). Similarly, Löhnis (1951) in her extensive studies on manganese toxicity did not observe a beneficial effect of ferrous sulfate treatments of manganese-injured plants. Symptoms of iron deficiency in beans when grown in an iron-deficient nutrient solution appeared to be entirely different from those of manganese toxicity. In a single experiment, however, in which the manganese injured bean plants grew at a high temperature, a clear response to iron treatment was found. The appearance of the plants was whiter than usual. Since Hopkins *et al.* (1944), who obtained a very clear response of manganese injured Phaseolus to ferrous sulfate, were working under tropical conditions, it may be possible that the temperature factor can explain the controversy between various workers as to the relation between iron deficiency and manganese toxicity.

e. Manganese Toxicity in Relation to Molybdenum Supply. In a large number of nutrient solution experiments Millikan (1947, 1949, 1950, 1951) has investigated the effect of molybdenum on toxicity symp-

toms caused by excess of some heavy metals, including manganese. In flax plants a marked reduction in the severity of the injury was observed. Ammonium molybdate appeared to be more effective than sodium molybdate (Millikan, 1947, 1949).

In a further set of experiments Millikan (1951) studied the effect of sodium and ammonium molybdates on manganese uptake from high and low manganese solutions by flax, peas, cabbage, and tomato and its distribution in the plant tissues, using a tracer technique. A marked accumulation of manganese in the tops of the leaves of flax at both manganese levels was observed. This concentration corresponds with the lower leaf necrosis which commences at or near the tip of the leaf. The presence of excess of molybdenum appeared to have a marked effect not only on the total manganese content, which was much reduced, but also upon its distribution in the leaves. The manganese content of the leaf ends was relatively much more depressed than that of the base of the leaves. In peas, cauliflower, and tomato similar relationships were found to exist between manganese content, manganese distribution in the leaves, occurrence of toxicity symptoms, and addition of molybdates.

From the results of his experiments and from the similarity of visual symptoms of manganese excess and molybdenum deficiency, Millikan concludes that a narrow relation exists between both phenomena. It should be stressed, however, that comparatively large amounts of molybdenum are required to affect manganese toxicity in Millikan's experiments, whereas in molybdenum studies extremely small amounts of this micronutrient element have been found to give normal plant growth. In contrast to Millikan's results with flax, Hewitt (1948c) found that molybdenum may accentuate chlorosis caused by excess of manganese in sand cultures of sugar beet. Löhnis (1951) was unable to show any effect of molybdenum on manganese toxicity of flax growing in nutrient solutions. Warington (1951) showed that molybdenum in amounts similar to those employed by Millikan may intensify the chlorosis induced by manganese excess in flax and soybean. These results are in agreement with those of Hewitt with sugar beet. In contrast to molybdenum, vanadium was found to be able to alleviate the symptoms of manganese toxicity.

To test Millikan's hypothesis as to the relation between manganese toxicity and molybdenum deficiency the senior author carried out the following experiment (unpublished). Manganese determinations were carried out in white clover grown in acid soils in a number of which the plants responded clearly to small amounts of added molybdenum. If a relation between manganese toxicity and molybdenum deficiency exists, it would be expected that plants which responded to added molybdenum

would have higher manganese contents than those which did not so respond. This appeared not to be the case.

*2. Symptoms of Manganese Toxicity in Some Crop Plants.**

Beans (*Phaseolus vulgaris*) (Löhnis, 1951; Hewitt, 1945). The first symptoms in younger leaves appear as marginal and later smooth interveinal chlorosis between major veins. When older, the leaves become somewhat crinkled and are spotted with small yellowish and later whitish areas. Finally minute brown necrotic spots appear. Petioles of the seed leaves and of the first trifoliate leaves are speckled with small superficial purple brownish spots. Severely injured plants remain stunted and produce hardly any flower or seed. Less injured plants may recover later in the season, when only traces of the initial injury still occur in the older leaves.

Peas (Millikan, 1951). First symptoms, necrosis along the edges of the third or fourth leaflets in the form of small grayish spots in the interveinal tissues. These marginal spots soon coalesce, and the necrotic edge may inroll. The tendrils of the affected leaves show a necrosis at the tips. The upper leaves of the plant may show symptoms like iron deficiency chlorosis followed by necrosis.

Vetch (Löhnis, 1951). The young leaves are chlorotic and a very marked dark purplish discoloration occurs along the margins of the full grown leaves. Sometimes small orange-red sunken spots may be found in the leaf margins, and the upper surface of the leaves may be speckled with minute dark spots. The plants remain small and spindly.

Soybeans and cowpeas (Morris and Pierre, 1941). In soybeans pale green irregular areas occur between the main veins of the leaves. Most of the affected areas become brown. In cowpeas small reddish purple spots are distributed uniformly over the leaf area. In both plant species growth is markedly decreased.

Alfalfa (Hewitt, 1948a). Growth is reduced and there is marginal paling of midstem leaves, followed by pale brown or buff spotting near margins; younger leaves are distorted at tips, with wavy margins or twisting of lamina. Later a yellow-green or gray-green paling at leaf tips occurs; margins become brown or bronzed and irregularly speckled.

Red clover (Hewitt, 1946). Marginal chlorosis of leaves. When older, leaflets show marginal crinkling and forward curling, slightly cupped; paling becomes yellow-green, spreading interveinally; light brown necrotic spotting appears between veins around inner border of pale marginal regions.

* This list covers only the most important agricultural and horticultural crops of which a rather clear-cut description has been found in the literature.

Sweet clover (Morris and Pierre, 1949). The distal leaf margin shows marked chlorosis, usually accompanied by a definite crimping of the leaf; no spotting.

Lespedeza (Morris and Pierre, 1949). Dark reddish brown leaf spots, very distinct on the underside of the leaves, cover in more severe cases as much as 50 per cent of the leaf area. Leaf margins are chlorotic. Considerable shedding may occur.

Potato (Berger and Gerloff, 1947b). First symptoms are the appearance of dark brown streaks on the lower stem at the base of the petioles. A pale yellow chlorosis develops in areas between the veins on the lower leaves although the veins themselves remain green. Quite often small brown necrotic areas, irregular in shape, appear between the veins near the midrib on the leaflets. As the necrosis becomes more severe, many long, narrow brown streaks are found on the lower portions of the stem and even on the petioles. The necrotic streaks also affect the inner tissues of the stem. The affected parts become very brittle, the petioles break off with a slight touch, and the chlorotic leaves finally dry and fall from the plant. The streaking of the stem and subsequent leaf dropping progress upward on the plant until the terminal bud becomes necrotic and the plant dies prematurely.

Cauliflower (Hewitt, 1946). There is marked forward cupping of margins of middle and older leaves. A marginal paling occurs, spreading interveinally between major veins, followed by dark brown spotting in pale areas. There is also severe marginal and interveinal crinkling and distortion.

Savoy cabbage (Hewitt, 1946). Growth is stunted. Expanding leaves show a marked paling in a narrow marginal zone ("rim effect"), becoming cream or dull white. Occasionally a slight mottling spreads inward interveinally for a short distance. Slight forward cupping of first affected older leaves may occur, but is absent in later leaves. Older leaves later develop indigo or black tinting along margins and in veins in marginal zone, and there is interveinal dark brown necrotic spotting.

Flax. Although this plant may tolerate relatively large amounts of manganese without any harmful effect, severe damage is affected by excessive amounts. The growth of the plants is stunted, and a severe apical chlorosis occurs. A brown necrosis may develop at the middle of one or both edges of the second lowest pair of leaves. This necrosis soon involves the whole of the distal half of the leaf. Other lower leaves will also become damaged, the necrosis mostly commencing at the tips of the leaves and extending downward, so that soon the top half of the leaf will be involved. The lower halves of the leaves will remain normal green in color. Sometimes numerous dark brown necrotic spots may be

found on the lower leaves. On the stems sometimes numerous small brown spots may be found, which may coalesce to form larger areas.

Carrot (Hewitt, 1948a). Growth is slightly reduced; chlorosis of leaf margins is followed by bronze necrotic speckling along leaf margins and scorching.

Lettuce (Hewitt, 1948a). Growth is reduced. Foliage is pale and dull yellow around leaf margins.

Peach (Thornberry, 1950). Excessive manganese causes "internal bark necrosis," i.e., occurrence of necrotic lesions localized internally without any observable change on the surface. These lesions may converge into necrotic areas which tend to advance until the trunk or bark is girdled. At later stages of the disease there is surface darkening and subsequent splitting of the outer bark along with the production of gum.

X. Function of Manganese in Plants

Manganese plays an important role as a cofactor in various enzymatic reactions. Many of these reactions have been studied *in vitro* with more or less purified enzymes obtained from normal organisms, plants as well as animals. Control experiments employing tissues or enzymatic preparations derived from organisms deficient in manganese are missing in most of these investigations.

In several enzyme studies concentrations of manganese far higher than those required in nutrition experiments with plants or microorganisms have been employed. Another fact which deserves more attention is the specificity of the metals. In many enzymatic reactions *in vitro* manganese may be replaced my magnesium, cobalt, nickel, or still other metallic ions. Although it may be possible that in the living organisms this replacement also occurs, little is known of it so far. The fact that manganese deficiency of green plants occurs mainly on neutral or alkaline soils in which the magnesium supply is ample and magnesium deficiency on acid soils in which available manganese is present in large amounts is not in favor of the replaceability of both metals in their major functions in higher plants. Whether a tendency in that direction occurs in any "natural" enzymatic reaction may only be decided by experiments in which different combinations of manganese, magnesium, cobalt, etc., have been supplied. The tissues of such plants will have to be used for enzymatic studies.

The only experiments of this type of which the authors are aware are those of Nilsson *et al.* (1942), who studied the replacement of magnesium by manganese in a number of enzymatic reactions and in some growth studies with three species of bacteria, namely, *Azotobacter chroo-*

coccum, Bacterium radiobacter, and *Bacterium prodigiosum.* Although the results obtained demonstrate that under the conditions of these experiments manganese may be substituted for magnesium in Azotobacter and presumably also in *B. radiobacter* and *B. prodigiosum,* the time during which the rate of growth of these bacteria was ascertained was so abnormal, that is, two and five months respectively, that the conclusions have only limited value. A further objection to these experiments is that no cultures with both manganese and magnesium added were included, so that it cannot be concluded to what extent the growth in the solution with either magnesium or manganese may be considered as normal.

Although it is not the purpose of the authors to give a detailed review of all the enzymatic reactions described in the literature in which manganese may function as a cofactor, some of the reactions which are of fundamental importance in higher and lower plants will be reported here. In addition an attempt will be made to connect the results of these enzymatic studies with those of plant physiological and bacteriological investigations.

It has been shown that manganese catalyzes not only various reactions of carbohydrate breakdown and organic acid metabolism but also a number of important conversions involved in nitrogen metabolism and phosphorus metabolism.

1. Manganese in Relation to Carbohydrate Breakdown

a. Glycolysis. The breakdown of carbohydrates to tricarbonic acids (glycolysis) by the cells of higher plants, microorganisms, and animal cells occurs in a number of graded steps, each of which is catalyzed by different enzymes. Some of these reactions are activated by manganese, magnesium, or cobalt ions, namely, phosphoglucomutase, which catalyzes the interconversion of glucose-1-phosphate and glucose-6-phosphate, and enolase, which catalyzes the formation of phosphoenolpyruvate from D-2-phosphoglycerate. Mg^{++}, Mn^{++}, or Zn^{++} are active as a cofactor in this case (see the review of Lardy, 1949). In fermentation experiments with purified apozymase from *Saccharomyces cerevisiae,* Nilsson *et al.* (1942) observed practically no breakdown of glucose to ethanol and carbon dioxide in the absence of either manganese or magnesium. Both metals gave about the same activation. Optimal activity was obtained with 0.01 *M* $MnCl_2$.

b. Organic Acid Metabolism. It is a well-known fact that organic acids play an important role in cell respiration as well as in the reverse process, carbon dioxide assimilation. The former process, the breakdown of the end products of glycolysis to carbon dioxide and water, proceeds

through a number of enzymatic reactions involving different organic acids known as the Krebs cycle (Green, 1949; Bonner, 1950). Although these reactions have been studied in detail mainly in animal tissues and tissue extracts and in microorganisms, there is both direct and indirect evidence available that similar reactions occur in higher plants.

One of the important enzymes of the tricarboxylic acid cycle of Krebs, isocitric dehydrogenase, which catalyzes the transformation of isocitric acid to α-ketoglutaric acid, requires either Mn^{++} or Mg^{++} (von Euler *et al.*, 1939; Adler *et al.*, 1939; Ochoa, 1948). This conversion proceeds in two steps:

$$\text{D-Isocitric acid} + \text{TPN}_{ox.} \xrightleftharpoons[\text{oxalosuccinic carboxylase}]{\text{isocitric dehydrogenase}} \text{Oxalosuccinic acid} + \text{TPN}_{red.} \quad (1)$$

$$\text{Oxalosuccinic acid} \xrightleftharpoons[+Mn^{++}]{} \alpha\text{-Ketoglutaric acid} + CO_2 \quad (2)$$

Reaction (1) depends on the presence of triphosphopyridine nucleotide (TPN). According to Ochoa, Mn^{++} is required for reaction (2) and not for (1). Since both reactions (1) and (2) are reversible, this system perhaps can play an important part in the biological fixation of carbon dioxide as a first step in photosynthesis.

The experiments of Ochoa have been carried out with extracts from animal tissues. Von Euler *et al.* (1939) have found the isocitric dehydrogenase system in animals, yeasts, and higher plants. In the absence of Mg^{++} or Mn^{++} the reaction does not proceed. The activity of Mg^{++} was found to be less than that of Mn^{++}. The optimal concentration of Mn^{++} is 5.10^{-4} *M*, and that of Mg^{++} is $2.5.10^{-3}$ *M* (extract of animal tissues). Berger and Avery (1944) investigated the isocitric dehydrogenase of the Avena coleoptile. It was shown to be activated by Mn, Mg, and Co ions and to a lower extent by Zn and Ni. In accordance with von Euler *et al.* (1939) a Mn concentration of 5.10^{-4} *M* was found to be optimal.

A further example of a carboxylase depending for its activity on manganese is the oxalacetic carboxylase studied in detail by Vennesland *et al.* (1949). This enzyme, which splits oxalacetic acid in pyruvate and carbon dioxide has been found in parsley root. Its activation by various cations has been studied by Speck (1949). In the absence of metal ions oxalacetic carboxylase from parsley roots was found to be nearly inactive. The optimal effect was obtained with Mn^{++} at a concentration of 0.01 *M*. Ca^{++}, Co^{++}, Cd^{++}, and Zn^{++} showed also a notable effect while Pb^{++}, Ni^{++}, Fe^{++}, Mg^{++}, Cu^{++}, and Ba^{++} acted only slightly.

The preparation of oxalacetic carboxylase from a number of different

plants (wheat germ, carrot, beet, spinach, parsley, parsnip, peas) invariably showed the capacity of catalyzing the reversible reaction:

$$\text{L-Malate} + \text{TPN}_{ox.} \rightleftharpoons \text{Pyruvate} + \text{CO}_2 + \text{TPN}_{red.} \qquad (3)$$

an activity referred to as "malic enzyme." This reaction, which may be of great importance in the fixation of carbon dioxide, is catalyzed by Mn^{++} as well as Co^{++} (Conn, Vennesland, and Kraemer, 1949).

A manganese-activated "malic enzyme" system in animal tissues has been described by Ochoa *et al.* (1948; see also Salles *et al.*, 1950).

The malic enzyme system has recently been studied in connection with carbon dioxide assimilation of green plants (Vishniac and Ochoa, 1951; Tolmach, 1951; Arnon, 1951). It was shown that illuminated isolated chloroplasts may greatly enhance the carbon dioxide fixation in the malic enzyme reaction (3). This effect apparently is due to the photochemical reduction of TPN by the illuminated chloroplasts as a result of which reaction (3) is shifted to the left. It is questionable, however, whether this reaction represents a true model of photosynthesis since the photochemical reduction of TPN or DPN may also be coupled with other reactions which require the presence of the reduced pyridine nucleotides.

In addition to the enzymatic reactions described above many more dealing with organic acid metabolism which are activated by manganese may be found in the biochemical literature.

2. *Manganese in Relation to Nitrogen Metabolism*

The activity of manganese as a cofactor in enzymatic reactions is not limited to the carbohydrate and organic acid metabolism, but is of similar importance in the nitrogen metabolism of plants and animals. Many of the simple peptidases of plants, microorganisms, and animals do not operate in the absence of metallic ions. These may be manganese ions but also cobalt, ferrous, or magnesium ions. L-Leucine-aminoexopeptidase, a peptidase which splits L-leucylglycine and other L-leucine containing di- and tripeptides is activated by manganese and magnesium, (Smith and Bergmann, 1944; Smith, 1951). Apparently high concentrations of these cations are required to give full operation of the reaction. It was observed that the effect of manganese on the activity of peptidase was considerably greater when enzyme and cations were incubated together for some time before the substrate was added. This indicates that the metal reacts with the enzyme protein in forming the active enzyme.

The peptidase which hydrolyzes glycylglycine is specifically activated by cobalt and to a much smaller degree by manganese (Maschmann,

1941; Smith, 1951). In this case, however, no time lag in the activation of glycylglycine dipeptidase by cobalt has been observed. It was found that cobalt formed a complex with the dipeptide. From these and a number of similar observations it was suggested (Smith, 1951) that the role of the metal ion in the enzyme system could be explained by assuming an interaction of this ion with the substrate on one hand and with the protein on the other.

Very interesting is the observation by Bamann and Schimke (1941a, b) that the "L-peptidase" activity of animal and plant tissues may be increased by both Mn and Mg ions whereas the "D-peptidase" reaction is activated by Mn^{++} but not by Mg^{++}. In their experiments the authors tested seedlings of lentil, peas, oats, wheat, barley, and rye; leucylglycine was used as the substrate in the enzyme reactions. (For a review of the literature on metal activated peptidases see Smith, 1951 and also Hewitt, 1951).

Further manganese-activated enzymes which play a part in the nitrogen metabolism of plants are arginase and glutamyl transphorase. Arginase splits the amino acid arginine into ornithine and urea. It may be activated by Mn^{++}, Co^{++}, Ni^{++}, or Fe^{++} (Hellerman and Perkins, 1935; Stock, Perkins, and Hellerman, 1938; Anderson, 1945; Folley and Greenbaum, 1948; Greenberg, 1951).

Glutamyl transphorase plays a part in the amide metabolism of plants. It has been found to catalyze the reaction:

$$\text{Glutamine} + \text{Hydroxylamine} \longrightarrow \text{Glutamohydroxamic acid} + \text{Ammonia}$$

ATP (adenosinetriphosphate) or ADP (adenosinediphosphate), phosphate or arsenate, and manganese (not replaceable by magnesium, zinc, copper, ferrous iron, aluminum, or cobalt) are essential components of the complete system. The enzyme has been isolated from seedlings of *Cucurbita pepo* (Stumpf *et al.*, 1951). It has been found present in a wide variety of algae and plants, particularly in nodules of clover and lupine. The same enzyme system has been found to catalyze the exchange of isotopic ammonia with the amide group of glutamine (Delwiche *et al.*, 1951).

The question may be raised whether the observed effect of manganese on the above-mentioned enzymatic reactions may explain the results of those investigations in which the relation between manganese nutrition and nitrogen metabolism has been studied *in vivo*. In experiments with excised roots of wheat plants and with macerated roots, Burström (1939, 1940) has shown that the rate of nitrate assimilation may be increased considerably by addition of small amounts of manganese to the nutrient solution. Since manganese has an activating effect on the

so-called basal respiration of wheat roots (Lundegårdh, 1939), the beneficial effect of added manganese on both nitrate assimilation and basal respiration of roots may be a result of a manganese-activated enzymatic reaction of the glycolysis or tricarboxylic acid system.

Nance (1948), in experiments with wheat roots, failed to demonstrate a stimulating effect of manganese on nitrate reduction. This may have been due to a higher manganese content of the seed than was the case in the experiments of Burström (1939). Jones *et al.* (1949) in culture solution experiments with soybeans observed an accumulation of nitrite in the nutrient solution when no manganese was added. The plants developed yellow leaves, ascribed by the authors to nitrogen deficiency. Supplied with small amounts of manganese, no nitrite accumulated, and the plants were green and healthy. These results would indicate that manganese activates the reduction of nitrate to nitrite. The presence of nitrite in culture solutions of plants deficient in manganese has also been observed by Eversmann and Aberson (1927) and Gerretsen (1937). The latter author, however, ascribes its presence to the fact that the roots and especially the root tips of manganese-deficient plants are easily attacked by microorganisms which in the presence of decaying organic material easily reduce nitrate to nitrite.

From the fact that spinach plants, growing in nutrient solutions with either ammonium or nitrate nitrogen, showed no substantial difference in manganese deficiency, Friederichsen (1944) concluded that manganese plays no part in nitrate reduction of this plant. Alberts-Dietert (1941) studied the effect of manganese on growth of *Chlorella* in culture solutions with different nitrogen compounds. In the absence of added manganese, nitrate and ammonium nitrogen gave the same effect when *Chlorella* was grown under autotrophic conditions. In the presence of glucose, however, nitrate was found to be inferior to ammonium salts, apparently owing to a poor nitrate reduction. Supplied with manganese this difference was not observed. These results would indicate that under autotrophic conditions the effect of manganese deficiency on nitrate reduction was overshadowed by its effect on carbon dioxide assimilation.

High concentrations of nitrate in manganese-deficient plants have been recorded by Leeper (1941) and Hewitt *et al.* (1949). Although these results in accordance with those of Burström (1939) may indicate a possible function of manganese in nitrate reduction, it may also be explained by assuming a reduced carbohydrate content in manganese-deficient plants as a result of a poor carbon dioxide assimilation (see Gerretsen, 1949).

Hewitt *et al.* (1949) in the case of manganese-deficient cauliflower

found an accumulation of nitrate as well as of amino acids, particularly aspartic and glutamic acids, in young leaves. These results are hard to reconcile with the postulated role of manganese in nitrate reduction. They may indicate, however, that the function of Mn may be sought in catalyzing some reaction in the chain between amino acids and protein, e.g., the above-mentioned peptidase activity or the glutamyl transphorase. The accumulation of nitrate possibly might result from the accumulation of amino acids. The results of Hewitt *et al.* (1949) are in agreement with earlier investigations by Friederichsen (1944) who showed that manganese-deficient spinach leaves had a considerably higher ammonia and amino acid content than leaves from normal plants; nitrate was also higher in manganese-deficient leaves, but the difference was much less pronounced than in the roots. The protein content was slightly lower in manganese-deficient plants. Gerretsen (1936, 1937) found also a higher ammonia content in manganese-deficient leaves of oats than in normal plants.

The effect of manganese in comparison with that of iron and molybdenum on the assimilation of nitrate in excised wheat roots has been studied recently by Burström (1949) in inhibition experiments with di-n-amylacetic acid. This substance in concentrations as low as 10^{-6} *M* was found to be able to inhibit nitrate assimiliation almost completely. Addition of manganese or iron in amounts of 5 p.p.m. restored the nitrate assimilation, whereas molybdenum had no effect. From these experiments it may be concluded that manganese activates a reaction in wheat roots essential for the nitrate assimilation which may be blocked by di-n-amylacetic acid. These results need not to be in conflict with the view of the senior author that molybdenum has to be considered as an essential element in the reduction of nitrate by *Aspergillus niger*, denitrifying bacteria, and green plants (Mulder, 1948). Molybdenum which exerts its activity in concentrations a thousand times smaller than those employed for manganese has undoubtedly been present in adequate amounts in the roots employed by Burström. At least two views exist: (a) both manganese and molybdenum are essential for nitrate assimilation in roots of wheat; (b) the mechanism of nitrate assimilation of roots is different from that of leaves of higher plants and that of microorganisms. The latter concept apparently holds for *Aspergillus niger*, which according to unpublished experiments by the senior author does not require Mn for nitrate reduction.

The view that at least two different mechanisms of nitrate assimilation exist in green plants is in accordance with the results of recently published investigations by Mendel and Visser (1951). These authors demonstrated that the nitrate reduction which occurs in tomato leaves

in the dark is dependent upon respiration for a source of energy, while in the light the reduction may well be independent of respiratory processes with the necessary energy supplied by light. This was concluded from the fact that iodoacetate, a well-known inhibitor of cell respiration, almost completely inhibited nitrate reduction in the dark, whereas it was completely without effect in the light.

3. *Manganese Nutrition and Ascorbic Acid Content of Plants and Animals*

The question whether manganese may affect the formation of ascorbic acid in plants and animals cannot be answered definitely. In a number of investigations pronounced differences in ascorbic acid content between manganese-deficient and normal plants have been found. Several other workers, however, were not able to confirm these results.

The first observation of a beneficial effect of manganese on the ascorbic acid content of plants was made by Rudra (1939a). He investigated the effect of dilute solutions of manganese sulfate and manganese chloride on germinating seeds of *Cicer arietinum*, *Phaseolus mungo*, wheat, barley, and oats. After six days germination considerably higher values for ascorbic acid were found in the manganese-treated seedlings of Cicer and the cereals than in those germinated in water. The optimal concentration of manganese was found to be 0.001 per cent in the case of Cicer and 0.01 per cent with the cereals. In a further paper the same author (1939b) claims that the inability of guinea pigs to synthesize ascorbic acid may be attributed to insufficient manganese in the tissues of these animals. Simultaneous injection of mannose and manganese was found to increase the ascorbic acid content of some tissues, particularly liver and jejunum. Without manganese, mannose would not affect the ascorbic acid content (see also Rudra, 1944). These experiments have been repeated by Skinner and McHargue (1946) with scorbutic as well as normal guinea pigs. No effect of injections of mannose and manganese on the scorbutic condition of the animals nor on the amount of indophenol-reducing substances of their livers was observed by these authors.

In another paper Rudra (1943) described on slender evidence the occurrence of a manganese-activated hexose dehydrogenase in the small intestines of animals, which would be able to produce ascorbic acid from aldohexoses.

In experiments with tomatoes growing on manganese-deficient soils Hester (1941) observed an increased content of ascorbic acid in the fruits from plants treated with manganese. These results have not been confirmed by Lyon *et al.* (1943), who in carefully controlled nutrient

solution experiments found manganese-deficient tomatoes as rich in ascorbic acid as fruits from control plants. A similar negative effect was obtained by Gum *et al.* (1945). The ascorbic acid contents of neither the foliage nor the fruit of tomatoes, growing in nutrient solutions, showed any consistent differences with manganese treatment. In contrast to these results, von Bronsart (1950) in Germany obtained consistently higher values for ascorbic acid (sum of reduced and oxidized forms) in tomatoes from manganese-treated plants than in the fruits from untreated control plants.

A positive effect of manganese additions or sulfur treatments of manganese-deficient soil on growth and ascorbic acid content of spinach, Sudan grass, and oats has been reported by Harmer and Sherman (1943). In Sudan grass and oats the reduced form of ascorbic acid was affected mainly, whereas in spinach the oxidized form was increased to a very large extent by an improved manganese supply. Rangnekar (1945) found a notably higher concentration of ascorbic acid in the leaves of manganese-treated *Amaranthus gangeticus* than in the leaves of control plants.

Maton (1947), working in Hoagland's laboratory, found a remarkable effect of the manganese supply to some plants growing in culture solutions on the ascorbic acid content of their leaves. In sunflower 42 μg. ascorbic acid per gram fresh weight of leaves was found in the absence of manganese against 74 μg. in leaves of plants supplied with 0.125 p.p.m. of Mn. In tobacco the differences were even more pronounced: 20 μg. per gram fresh weight of Mn-deficient leaves against 145 μg. in leaves of manganese-supplied plants. Tomatoes on the other hand did not show any difference in ascorbic acid content of the leaves at different manganese levels notwithstanding the occurrence of great differences in total yield.

No correlation was found by Hivon *et al.* (1951a) between manganese nutrition of soybeans grown in the field and ascorbic acid content of the leaves. In an extensive nutrient solution experiment with green pod beans the same authors (1951b) determined ascorbic acid (reduced form) in the leaves of manganese-deficient and normal plants at 27, 34, 41, 48, 55, 62, and 69 days from planting. At 30 days the plants with restricted manganese showed definite chlorosis which became increasingly severe at later sampling date. No effect of manganese supply on ascorbic acid content occurred.

The discussion of the above-mentioned papers clearly demonstrates the great lack of uniformity in the results obtained by the various authors. Although several workers have reported definitely negative results, the number of papers in which a positive effect has been shown

is too large to deny the effect which, under certain circumstances, manganese may have on the ascorbic acid content of green plants. Since weather conditions, notably illumination, have been shown to be of great importance in controlling ascorbic acid content of plants, it may be possible that they play an important part in affecting the manganese-ascorbic acid relationship. This point is also stressed by Hamner (1945) in a discussion of minor element-vitamin relationships in plants. The fact that some authors are working under field conditions and others under greenhouse conditions, either with nutrient solution cultures or with soils, may also be considered as a point which deserves more attention.

The relationship between manganese and the content of some other vitamins or vitaminlike substances has been studied in a few cases only. Gum *et al.* (1945) found lower concentrations of carotene in manganese-deficient tomato leaves than in those from normal plants. The fruits of these plants showed no differences. For riboflavin slightly lower values were found in the leaves and fruits from manganese-deficient tomato. These results are in accordance with those of Burger and Hauge (1951), who analyzed manganese-deficient and normal soybean plants for carotene, choline, and tocopherol (vitamin E). Plants from manganese-deficient soil which exhibited symptoms of chlorosis and necrosis were found to be notably lower in carotene and tocopherol, but higher in choline. A similar beneficial effect of manganese treatment on carotene content of the leaves was found in wheat and oats.

4. *The Role of Manganese in Photosynthesis*

The evidence is increasing that manganese is an essential constituent of the photosynthetic apparatus in green plants. In this connection it is interesting to note that as early as 1924 Hiltner in his extensive investigations on gray speck disease of oats relates two remarkable experiments, which hitherto received little attention. (1) Increasing carbon dioxide assimilation of oat plants by enriching the surrounding atmosphere with carbon dioxide was found to prevent the outbreak of the gray speck disease on a manganese-deficient soil; reducing the carbon dioxide content of the air aggravated the symptoms (see Table II). (2) Reducing the carbon dioxide assimilation by shading the plants also intensified the Mn-deficiency symptoms, whereas the effect of manganese additions was much more pronounced on the shaded plants than on the plants grown in normal daylight. Both experiments point to an intimate connection between carbon dioxide assimilation and manganese activity in the plant.

A quarter of a century later Gerretsen (1949) showed that CO_2

TABLE II

(After Hiltner, 1924)

Treatment	Number of Grains	Total Dry Weight, g.	Symptoms
O Mn, no CO_2 added *	0	3.12	Pronounced Mn-deficiency symptoms
+ Mn, no CO_2 added *	23	5.73	No symptoms after the appearance of the fourth leaf
O Mn, CO_2 added	168	10.97	No symptoms at all
+ Mn, CO_2 added	138	9.53	No symptoms at all

* Reduced CO_2—content of the air.

assimilation of leaves from oat plants, grown on manganese-deficient soil or in culture solutions poor in manganese, is reduced to 25–40 per cent of the normal rate. As special care had been taken not to use leaves which were chlorotic or which showed any pronounced outward signs of manganese deficiency, it is unlikely that in this case chlorosis had been the cause of a reduction of the photosynthetic activity by 60–75 per cent.

To investigate the role manganese eventually plays in photosynthesis, Gerretsen (1950a, 1951) used cell-free watery suspensions of crushed oat leaves in which he determined the redox potential in the dark and during illumination.

It was shown that the change of the E_h upon illumination was much more pronounced with normal leaf material than was the case with manganese-deficient leaves. The addition of small amounts of manganese as $MnSO_4$ in the latter case restored the E_h changes to normal values. Addition of manganese to normal leaf extracts in the dark had no effect, but upon illumination the potential rose with 100 to 150 mv. to values which make the formation of peroxides and in particular hydrogen peroxide highly probable.

Addition of trivalent iron had the opposite effect—the equilibrium $Fe^{+++} \rightleftarrows Fe^{++}$ was shifted to the right by illumination and to the left in the dark. Gerretsen concluded that in the plant a photosensitive oxidation-reduction system exists in which manganese and iron act as complementary oxidation-reduction agents. They keep each other in equilibrium at a fixed potential during illumination and at another potential in the dark. As the potential of such a combination is determined in the first place by the ratio of the components and much less by the absolute quantities, it is evident that an excess of one element over the other is likely to disturb the oxidation-reduction equilibrium

in the plant. As early as 1930 Hopkins suggested that manganese tends to control the ratio $Fe^{+++} \rightleftarrows Fe^{++}$ in the cell, and in a more recent paper he and his co-workers are led to believe that the interaction of manganese, iron, and light controls to a large extent the oxidation-reduction potential of green plants (Hopkins *et al.*, 1944). Thatcher (1934) grouping the elements according to their function in the plants placed Mn and Fe amongst the oxidation-reduction regulators and called them "mutually coordinating catalysts for oxidation-reduction reactions."

In this connection the observation of Shive (1941), Somers, Gilbert, and Shive (1942), and Somers and Shive (1942) that the dry weight of their soybean plants was related to the iron-manganese ratio and not so much to the absolute concentrations of the elements seems to fit very well into this scheme.

Although several workers repeating the experiments of Shive and his collaborators were unable to confirm their results as to the narrow ratio of iron to manganese required for optimal growth, most of them observed an antagonizing effect of manganese on iron (see Sec. VIII).

The scope of this review does not permit us to go into details of the complicated photochemical reactions which constitute photosynthesis. It will suffice to say that by virtue of his experiments Gerretsen (1950a) assumes that in the dark manganese and iron exist respectively in the divalent and trivalent forms. Owing to illumination, under absorption of light energy, the situation becomes reversed and now Mn^{+++} and Fe^{++} cooperate in the photolysis of water, which is regarded as the first step in photosynthesis. The ultimate effect is that Mn^{+++} accepts one electron from OH^-, ($H_2O \rightleftarrows OH^- + H^+$) which becomes a $\overline{OH}$ radical. The manganese ion is reduced to Mn^{++}, whereas the $\overline{OH}$ radical finally gives rise to the evolution of oxygen ($2\overline{OH} \rightleftarrows \frac{1}{2}O_2 + H_2O$). At the same time, by light induced electron transfer, Fe^{++} cedes an electron to H^+, which becomes a highly reactive $\overline{H}$ atom, the iron being oxidized to Fe^{+++}. The $\overline{H}$ atom immediately combines with an intermediate acceptor to a more stable $\overline{HX}$ reduction product, which will be used in the formation of carbohydrates. Manganese and iron are now back in their original valency states, and the process can start anew.

It should be stressed that other workers demonstrated the importance of manganese in the fixation of carbon dioxide in certain light-induced reactions. Apparently the reduced carbon dioxide assimilation as observed by Gerretsen in manganese-deficient oat leaves may be due to reduced primary photochemical reactions or to reduced activity of enzymes of the tricarboxylic acid cycle or to both.

The fact that manganese deficiency, iron deficiency, and manganese

toxicity are all accompanied by chlorosis does not imply that the cause of this chlorosis is the same in all these cases. In the latter two cases the junior author is inclined to ascribe the chlorosis to photooxidation of chlorophyll, which is a secondary effect of the rapid destruction of the protective protein substances surrounding the chloroplasts, in consequence of the high redox potentials which are characteristic for the illuminated Mn-Fe redox systems in the plant, when Mn is in excess (Gerretsen, 1950b).

This view is supported by the fact that injury due to manganese excess is greater in more intense light, which has been observed by different workers, McCool (1913, 1935), Hopkins *et al.* (1944), Morris and Pierre (1949), whereas Gericke (1925) reports that wheat plants growing in solutions deficient in iron showed excellent growth and normal green color in the shade and little growth and chlorosis in bright sunlight.

Accepting reduced carbon dioxide assimilation as one of the main consequences of manganese deficiency enables us to interpret a number of divergent characteristic symptoms from a central point of view.

1. The reduced content of sugar and starch in the leaves of oat plants (McHargue, 1926) and of tomatoes (Eltinge, 1941) deficient in manganese is a direct result of reduced photosynthesis.

2. The same holds true for the retardation of growth, the smallness and slackness of the leaves, the weakness of the leaf ribs which causes the leaves to drop over with a kink.

3. Reduced frost resistance (Gerretsen, 1949; Lloyd Frisbie, 1947) is closely related to the turgescence of the cells and the latter to the content of sugars in the cell sap.

4. The presence of large quantities of nitrates in the leaves of manganese-deficient oat plants and canary grass as observed by Leeper (1941) fits well into the scheme, as it is obvious that accumulation of inorganic nitrogen must take place in leaves, in which insufficient carbohydrates are synthesized to combine with the nitrogen to build up proteins.

5. The entrance of toxic substances into the cells of manganese-deficient oat leaves (Gerretsen, 1937) with subsequent loss of turgescence and poisoning of the protoplasts is closely linked with increased permeability of the cell walls and the reduced size of the protoplasts due to lack of assimilates.

6. The reduced volume of the root system (Gerretsen, 1937; Hasler, 1951) and the susceptibility to invading saprophytic microorganisms can also be traced back to shortage of carbohydrates, the multiplication of the cells in the meristematic zone being much slower, as well as the

rejuvenation of the root cap tissue, which determines its protective function.

7. By increasing the carbon dioxide content of the surrounding air gray speck disease symptoms may be reduced or even disappear (Hiltner, 1924); in this case the increase of carbon dioxide assimilation neutralizes the decrease due to manganese deficiency.

8. Manganese-deficiency symptoms are aggravated by shading the plants; in this case reduced photosynthesis is further reduced by diminishing light intensity, which inevitably leads to a change for the worse.

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