[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, KENTUCKY AGRICULTURAL EXPERIMENT STATION]

THE ROLE OF MANGANESE IN PLANTS[1]

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Received May 1, 1922

The purpose of this investigation is to determine in as definite a way as possible whether or not manganese has any important function in plant economy. The aim of this paper is to describe in a brief way some of the observations and results obtained thus far in this investigation.

The method of attack has been the preparation of plant nutrient compounds and quartz sand free from manganese and the growing of plants in different portions of water cultures or sand cultures to which manganese was added or from which it was excluded. The plants were grown until they approached maturity. The green weights were determined at the time the plants were harvested and the dry weights at a later time. Chemical analyses were made of the plants grown under the two different conditions with respect to manganese.

The work has been done at the Kentucky Agricultural Experiment Station with the exception of last year during which time it was conducted in the laboratories of the Department of Soil Technology at Cornell University. Further work on this subject is now in progress at the Kentucky Agricultural Experiment Station.

During the last 20 years considerable attention has been given by investigators interested in plant nutrition and soil fertility to the occurrence, distribution and probable function of manganese in its relation to agriculture. The writer is aware of as many as 150 investigations pertaining in one way or another to this subject. In a review of this literature he was inclined to divide it into two classes. One class would include investigations the object of which was to determine whether or not an application of a manganese compound to the soil will increase the yield of a crop grown thereafter. The other class of investigations would include those in which it has been sought to determine whether or not manganese is an essential element in plant economy. While a few of the investigations included in the fertilizer class have afforded some indication of an economic relation existing between manganese and agriculture they do not afford sufficient evidence to answer the question: Is manganese a necessary element in plant growth?

In 1774, Scheele[2] first detected the presence of manganese in the soil and in the ash from plants that had grown in the soil. Apparently he made no effort to determine whether or not manganese had a necessary role in the growth of the plants. In the

[1] A paper read before the Division of Agricultural and Food Chemistry at the meeting of the American Chemical Society held at Birmingham, Alabama, April 3 to 7, 1922.

[2] Scheele, "Opuscula Chemica et Physica," Leipzig, 1788, vol. 1, p. 258.

century and a half that has elapsed since Scheele's discovery of manganese in the plant only a few investigators have sought to determine whether or not this element is necessary in plant economy. As early as 1864 Sachs[3] attempted to answer this very important question once and for all time. He succeeded in demonstrating that manganese could not replace iron in the growth of plants but was unable to satisfy his own mind that manganese was necessary for the growth of plants in the presence of iron. It appears, however, from his own statements that he did obtain some evidence which led him to believe that manganese did have a function to perform in plant economy, because in other experiments with plants which were conducted for a purpose other than to show the effect of manganese he always added a portion of some manganese compound to insure the presence of this element.

More recently Bertrand[4] has obtained evidence which led him to conclude that manganese is necessary for the growth of both autotrophic and heterotrophic plants.

After conducting experiments on the effect of different concentrations of manganese sulfate on the growth of barley seedlings, Miss Brenchley[5] concludes "that it seems probable that manganese may prove to be an element essential to the economy of plant life even though the quantity usually found in plants is very small."

Experimental

Some time ago the writer[6] became interested in determining the presence of barium in different parts of some plants which had grown in the soil under natural conditions and most of them in the vicinity of the Kentucky Agricultural Experiment Station. Upon the addition of dil. hydrochloric acid to the ash resulting from the incineration of brown hazelnut shells, a faint but distinct odor of chlorine was detected. This observation suggested that the ash must have contained an oxide of manganese which decomposed the hydrochloric acid and liberated chlorine. A test for manganese in an aliquot of the solution from the ash confirmed the presence of this element in moderate amount. This observation suggested further experiments in regard to the amount of manganese contained in the different parts of the hazelnut. Accordingly, a quantity of the nuts was obtained and separated into 3 parts, shells, seed-coats and cotyledons. A definite weight of each of these parts was ashed and manganese determined in each part separately. The determinations showed that the brown seed-coats contained the greatest amount of manganese when equal weights were taken. This fact was of sufficient interest to warrant similar determinations on like parts of other species of seeds. The results of this investigation showed that this relation obtained in all the seeds that were examined; namely, that the seed-coat contained a greater concentration of manganese than any other part of the seed.

Of the different seeds examined, wheat afforded more interest than any other. It was found that the chaff which surrounds the berry contained

[3] Sachs, "Handbuch der Physiologischen Botanik," Leipzig, **1865**.

[4] Bertrand, *Compt. rend.*, **124**, 1032–35 (1897).

[5] Brenchley, "Inorganic Plant Poisons and Stimulants," Cambridge Press, **1914**.

[6] McHargue, This Journal, **35**, 6, 826 (1913).

a very small amount of manganese and that the flour within the berry contained less than the chaff, but the bran or seed-coat contained approximately 0.02% of its dry weight of manganese. This fact was of sufficient interest to cause some wonder as to whether or not manganese has a useful role in the economy of this and other plants. This question suggested itself: What would be the ultimate effect on the growth of the plant if seeds were germinated and the resulting plants grown to maturity in a medium which contained all the known plant nutrient compounds but no compound of manganese? This suggestion was of sufficient interest to warrant the undertaking of some experiments with this end in view. In the first move in this direction a rather persistent obstacle was met with which in the mind of the writer has been the chief cause of the delay in unmasking the important function manganese appears to play in plant metabolism until this time. The contamination of chemical compounds that are used for plant nutrients with manganese was not suspected until tests revealed this fact, a point which appears to have been overlooked in previous investigations. This point is of fundamental importance because the compounds of calcium, magnesium and iron which are used as plant nutrients may contain enough manganese to supply a plant's requirements during growth. After having obtained the necessary number of manganese-free plant nutrient compounds, some of which could not be purchased free from manganese and had to be purified in the laboratory, a Pfeffer's nutrient solution was made and wheat seedlings grown in different portions of the nutrient solution with manganese added or withheld. During the first 6 or 8 weeks of the time the plants were making their growth no observable differences were noted between the sets of plants receiving different treatments with respect to manganese. A short time thereafter the plants from which manganese was being withheld began to give evidence of having reached a condition of unequal growth when compared with the plants to which it was available. The first observable difference was a lack in the development of chlorophyll in that the leaves became a yellowish-green instead of a deep green as maintained in the plants receiving manganese treatments. The differences between the two sets of plants became more pronounced as they approached maturity. The plants from which manganese was withheld made a stunted growth and produced no seed whereas those to which manganese was available grew normally and produced seed. There was an increase of 135% in the dry matter produced in the plants receiving manganese when compared with the dry weight of an equal number of other plants from which it was withheld.

In another experiment Alaska pea seedlings were grown under conditions similar to those in which wheat seedlings were grown until they approached maturity.

During the first 6 or 8 weeks of their growth no differences of any significance were to be observed in the growth of any of the plants. A short time thereafter the plants which did not have manganese added to the nutrient solution in which they were growing, developed a chlorotic condition in the young leaves and buds. This condition increased until the young buds and branches died back and no further growth of any consequence occurred in these plants. The plants to which manganese was added made a normal growth and produced flowers and young pods which, I have no doubt, would have produced mature seed had the experiment been continued to that stage. However, because the plants in the controls had ceased to show any further development, all the plants were harvested at this time and their dry weights determined. The increase in the dry weight of the plants receiving manganese was 66.90% over those plants from which it was withheld. A determination of the manganese contained in the two lots of plants was made. The plants to which manganese was available during their growth contained 0.179% of this element in the moisture-free material, whereas the plants to which no manganese was added contained only a trace which was probably no more than could have been derived from the manganese contained in the seed.

After having obtained the above results with nutrient solutions, it was thought that perhaps a better plan of procedure would be to grow the control plants in a manganese-free sand medium, as this plan would be less laborious and would permit the test being applied to a larger number of species of plants, and would have a still further advantage in that the plants would be grown under conditions more nearly like those that are maintained in the soil.

Accordingly, a quantity of quartz sand of medium sized grains was washed, first with water to remove a small amount of clay the sand contained, and then with a hot mixture of nitric and hydrochloric acids until the coating of iron and manganese which adhered to the grains was dissolved, and the residue of sand again washed with water until free from chlorides. The sand, which was snow-white, was then dried and definite portions were weighed out and mixed with appropriate amounts of available manganese-free compounds of the mineral elements which are commonly considered as being all that are necessary for the normal growth of plants. To one-half of the number of pots of sand 2 g. of manganese in the form of the carbonate was added in addition to the other necessary plant nutrient compounds.

The purified sand containing the plant nutrient compounds was placed in clean earthenware jars and soy bean seed planted. Enough distilled water was added to bring the moisture content to $^2/_3$ saturation, which was maintained near this point by frequent weighings and the addition of distilled water to replace that lost through transpiration during the time the plants were making their growth.

All the plants made a normal growth during the first 4 or 6 weeks. After this time those to which no manganese was added ceased to grow as rapidly as did those that were receiving it and a little later a chlorotic condition developed in the young leaves and buds. The increase in the dry weight in the plants to which manganese was available was 61.80%. The percentage of manganese in these plants was 0.032, and was only a trace in those from which it was withheld.

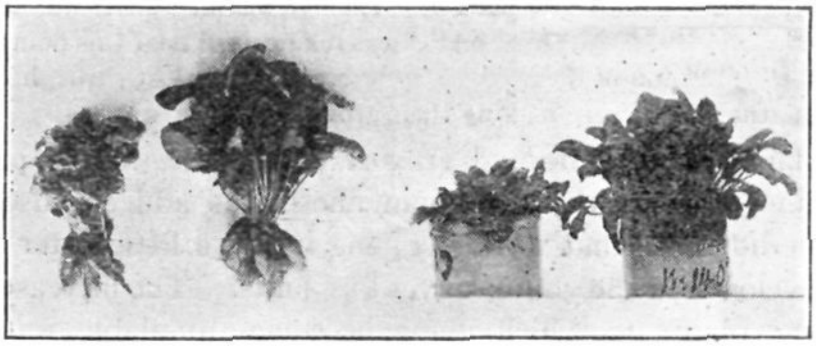
Similar results have been obtained with cowpeas which were grown in the same pots of sand after the soy beans were harvested, except that the difference in the dry weight of the plants receiving manganese in this case was 143%. The amount of manganese found in the plants to which it was available was 0.036% and in those from which it was withheld was 0.0031%, less than $^1/_{10}$ the amount in the plants to which it was added. From the chlorotic condition and the diminution in the production of dry matter it is evident that the manganese content in the control plants was not sufficient to carry on a normal metabolic process.

In another experiment yellow bantam sweet corn was planted in pots of sand under conditions similar to those described with soy beans and cowpeas. The plants were allowed to grow until tassels were produced, at which time the plants were harvested and the green and the dry weights determined. The increase in the dry organic matter produced in the plants to which manganese was available was 25.55%.

After having obtained this rather convincing evidence in regard to the necessity of manganese in the plant's growth, a more comprehensive experiment was planned. At the present time there are 20 different series of experiments in progress on this subject. Each series is made up of four 2-gallon pots of quartz sand from which manganese was eliminated previous to the addition of the mineral nutrient compounds. Two g. of manganese in the form of the carbonate was added to and mixed in with the other mineral nutrient compounds in half of the number of pots of sand. The remaining half of the pots of sand received an equal amount of manganese-free mineral plant nutrient compounds only, and served as the controls.

The following species of seeds have been planted since January 1 of this year (1922) in the different series of experiments: radish, Alaska garden pea, Canada field pea, cowpeas, lettuce, tomatoes, spinach, carrots, onions, garden beans, cabbage, wheat, oats, clover and velvet beans. These plants have been kept in the greenhouse at a temperature near 21°. The moisture content of the sand has been kept at approximately $^2/_3$ saturation by frequent weighings and the addition of distilled water to replace that lost through evaporation and transpiration.

The photographs show how seriously some of these plants have been affected by a lack of manganese when compared with the other plants to which this element was available.



Radish plants. Spinach plants.

Fig. 1.

Photograph No. 1 shows an equal number of radish plants and the growth attained with and without manganese. The plants on the right grew in sand containing manganese while it was excluded from the sand in which the plants on the left were grown.

It is to be observed that the plants to which manganese was available during their growth made a larger leaf and root growth than did the plants from which it was withheld. The leaves of the plants from which manganese was withheld became more or less chlorotic several weeks before the plants were harvested and this chlorotic condition continued to increase until the plants were harvested, while the leaves of those plants to which manganese was available maintained a normal deep green color during the time they were making their growth. Photograph No. 1 also shows 2 pots containing spinach plants which have not been harvested. Manganese was available to the plants on the right but none was added to the plants on the left. The plants on the right are larger and are apparently normal in every respect, while those on the left developed a chlorotic condition and have ceased to make a further growth of any consequence.

Photograph No. 2 shows Kentucky Wonder pole bean plants with which a very interesting result has been obtained with respect to the necessity of manganese in the processes of fructification of this plant. To the plants on the right manganese was available during their growth while to those on the left it was withheld. The plants on the right have developed in a normal way and have flowered and are bearing pods which were 6 to 10 inches in length at the time the photograph was made. The plants on the left produced vines that grew to be as tall as the plants on the right but the leaves on the plants from which manganese was withheld became chlorotic after they had grown for 6 or 8 weeks and no blossoms were produced on these plants; consequently they have borne no pods of beans, thus showing in a very striking way that manganese apparently has a very important function in the processes of the early stages of flowering and of fructification.

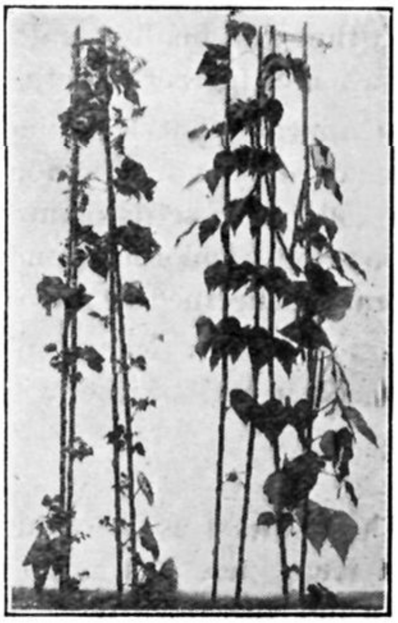


Fig. 2.—Kentucky Wonder pole bean plants.

Discussion

It will be recalled that the different species of plants mentioned in this paper which were grown in a medium that contained no manganese made a normal growth for 6 or 8 weeks but thereafter developed a chlorotic condition and failed to make further growth of any consequence. The normal condition of the plants during the first few weeks of their growing period is accounted for by assuming that the manganese which the seed contained was sufficient to maintain a normal metabolic process during this part of the plant's growth and that the chlorotic condition was a result of the lack of a further supply of available manganese, thus showing that with only a limited amount of manganese available normal growth will take place until that amount becomes used up in building new tissues, and thereafter the plant will cease to make further growth. This fact affords very strong evidence that manganese is essential for the normal growth and maturation of autotrophic plants. With plants that have a short and rapid growing period such as peas, radishes and beans it will require from 6 to 8 weeks of growth before the reserve supply of manganese contained in the seed is apparently used up in the plant's growth and before a chlorotic condition develops.

The first effect to be noted in the growth of plants from which manganese has been withheld is a lack in the development of chlorophyll in the newly formed tissues or the growing parts of the plant. This condition increases with time and finally results in the tips of the branches dying back and a cessation of further growth of any consequence in the plant.

It appears that leguminous plants are more sensitive to the lack of manganese than are the non-legumes because of the plants produced from pea and radish seeds planted at the same time, the pea plants were first to develop a chlorotic condition, thus suggesting that manganese is apparently concerned in nitrogen assimilation and the synthesis of proteins.

Apparently manganese plays the role of a necessary catalyst in plant metabolism and together with iron functions in the synthesis of chlorophyll.

Summary

The results thus far obtained in this investigation afford direct evidence that manganese has a function to perform in the formation of chlorophyll and consequently in carbon assimilation and possibly in the synthesis of protein. The results evidence a new and useful function of manganese in nature which has not heretofore been definitely established.

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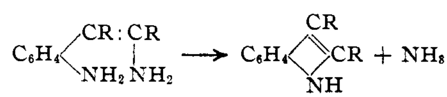
NOTE

The Mechanism of the Fischer Indole Synthesis.—For the mechanism of the Fischer indole synthesis (with which must be included Brunner's preparation of oxindoles from acid hydrazides[1] and the Drechsel-Baeyer method for tetrahydrocarbazoles[2]) four suggestions have been made.

Robinson and Robinson[3] supposed that an unsaturated azine was first formed, which underwent an *ortho*benzidine rearrangement followed by elimination of ammonia.

$PhNH.N\!:\!CR.CH_2R \longrightarrow PhNH.NH.CR\!:\!CHR \longrightarrow$



Cohn[4] suggested an *ortho*semidine change with subsequent loss of ammonia

[1] Brunner, *Monatsh.*, **17,** 479 (1896); **18,** 95, 527(1897); Ger. pats. 218, 477, 218, 727.

[2] Drechsel and Baeyer, *Ann.*, **278,** 105 (1894).

[3] Robinson and Robinson, *J. Chem. Soc.*, **113,** 639 (1918).

[4] Cohn, "Die Carbazolgruppe," **1919,** p. 12.