ACCUMULATED IRON IN THE NODES OF CORN PLANTS[1]

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(WITH ONE PLATE)

HOFFER and his colleagues (**1, 2, 3**) reported on the accumulation of iron in corn plants and suggested that this had a relation to the problem of root rots. HOFFER (**4, 5**) also suggested a relation between iron accumulation and potash deficiency, and that simple colorimetric tests for accumulated iron could be used to indicate potash needs. WELTON, MORRIS and GERDEL (**8**) did not find the relation between accumulated iron and potash deficiency close enough to permit using such tests to indicate need for potash fertilizer. SALTER and AMES (**7**) found a considerable variation in the percentage of total iron in the nodal tissues among individual corn plants. They found little or no relation between such quantitative determinations of total iron and the results of the colorimetric tests used by HOFFER. HOFFER and CARR (**1**) state that the accumulated iron occurs in the phloem of the vascular bundles.

This report is an attempt to find out more fully the form or kind of iron and its place of occurrence in the corn plant. No attempt is made to correlate iron accumulation with any mineral nutrient deficiencies.

Material and methods

The corn plants used in these investigations were of such varieties as happened to be growing under conditions where iron accumulation was high. Some plants from the vicinity of Wooster were used but usually had too little iron accumulation to be satisfactory. Corn from southwestern Ohio and from the muck lands at Lodi, Ohio, constituted the best material obtainable. Plants which were "fired" usually had the largest iron accumulations, but occasional plants without this symptom would contain a considerable amount. Discoloration of the nodal tissues served as a good preliminary indicator of iron accumulation, but was not infallible, as not all such discoloration is due to iron.

In addition to gross tests on the split stalks, the iron accumulations were studied microscopically, both in cut sections of the fresh stalks and in dried, pulverized nodal tissues. The sections were cut free hand with an iron-free knife. The dried tissue examined was first pulverized and passed over a

[1] Research cooperative between the Office of Cereal Crops and Diseases of the Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C., and the Departments of Botany and of Agronomy of the Ohio Agricultural Experiment Station, Wooster, Ohio.

300 mesh sieve. The fine material passing this sieve contained the iron accumulations freed from much of the surrounding tissues.

The potassium ferrocyanide, $K_4Fe(CN)_6$, and potassium ferricyanide, $K_3Fe(CN)_6$, tests given by KLEIN (**6**) were used. These tests are specific for iron, the one ($K_4Fe(CN)_6$) testing for ferric, and the other ($K_3Fe(CN)_6$) for ferrous iron. The blue products formed do not diffuse readily through the tissue and thus show very well the localization of the iron. The tests are more or less permanent if the mounts are properly prepared. HOFFER and CARR (**2**) used the potassium thiocyanate (KCNS) test for iron in their work. This test is very sensitive and is specific for ferric iron. The ferric thiocyanate (FeCNS) formed in the test is soluble however, and soon diffuses throughout the tissue so that localization can not be determined. Furthermore it shows the presence of ferric iron only. For these reasons it was used only occasionally to check the results of the other methods.

None of these tests show the presence of masked or organic iron, but are for the free or inorganic forms, the only kind here considered.

Observations on accumulated iron

Iron occurs in different amounts in different plants. Very few plants were found which did not show a trace of iron at some of the nodes. There was a variation in the amount of iron at the different nodes of a single plant, the amount usually being largest at the ear node. The accumulated iron was found only at the nodal plate, where the veins anastomose and extend into the leaf sheath.

Under the microscope it was found that the iron occurs in the cells of the bundle sheath and in the first layers of pith cells around the bundle. In no case was accumulated iron found in the conducting elements of the bundles. This fact is best shown by watching the section under the microscope as the test is applied. In plants which had very little accumulated iron at the nodes, microscopical examination of a thin section of the tissue before treatment with potassium ferrocyanide revealed minute granules in those cells where iron accumulates. These granules were shown to be iron or to contain iron, by their reaction as they came into contact with the test solutions. Where the iron occurs in larger amounts, the cells are found to contain either large irregular masses, which are red to reddish brown in color, or crystals which are red in color, or both. These masses and crystals gave the reaction for iron.

After standing for some time in the reagents, the masses and crystals disappear, but the blue color indicative of iron remains, diffusing throughout the containing cells. The blue substance which is formed in the reaction, however, does not diffuse through the cell wall, but eventually may be

adsorbed by the cell wall, the cell contents becoming colorless. Plate III, figures 1 and 2 show photo-micrographs of cross sections of a node of a corn plant having a large accumulation of iron. The sections are of the fresh corn stem mounted in water only, not stained for iron. The dark spots surrounding the bundles are the crystals and masses.

The crystals and masses test equally well for ferric or for ferrous iron. The masses are often as large as 30 μ and the crystals may be as large as 14–16 μ. The cells containing them are 60–80 μ in diameter.

Optical properties of the crystals and masses

The crystals and masses are isotropic; they are dark under polarized light in all positions, and remain so as the stage of the microscope is rotated. They therefore belong to the cubic crystal system. The refractive index was determined from material separated from dried, pulverized nodal tissue as already described. This material contained relatively few of the masses and crystals but enough to permit determining their refractive index by means of immersion oils. The refractive index of the crystals was 1.59 and that of the masses somewhat lower, 1.57–1.58. The crystals may be square, rectangular, or hexagonal in outline and cubic or octahedral in form (plate III, figures 3, 4, 5, 6).

Behavior of the crystals and masses with solvents

No solvent has been found which will entirely remove the iron from the tissue. The crystals disappeared in dilute acids and alkalies, in hot alcohol, in hot water, even in alcohol and glycerine in the cold after several days. The tissue which had been treated with these solvents still gave a very good test for iron, but only in those cells in which iron ordinarily occurs. Only a trace of iron could be removed by extraction with hot alcohol in a Soxhlet extractor for 20 hours. The explanation of this behavior seems to be that the substances are largely dispersed rather than dissolved. Concentrated acids and alkalies destroyed the crystals and also the whole tissue. The crystals and masses remained unaltered in tissue dried at ordinary temperatures.

Conclusion

A careful search of the literature failed to reveal a record of any compound of iron having the properties of these crystals and masses as detailed above. The problem was taken to Professor W. G. McCaughey, mineralogist of the Ohio State University, for advice. It was concluded that it is not a simple compound of iron, but probably a mixture or solid solution containing a compound of iron. The crystal itself is probably some other substance, the host substance, which contains some form of iron mixed with it in solid solution. Iron often occurs in this way, especially in organic

matter, and a trace of iron may color the host substance deeply, giving the appearance of a heavy deposit, or a pure compound of iron. Thus, no real measure of the quantity of iron present in such ways can be obtained from its color. The oxide and hydroxides of iron are the forms which usually occur in this way.

The properties of the crystals are similar to those of some of the protein crystaloids. Much more work would be needed however to permit any conclusions as to the nature of the host substance.

Summary

1. The iron studied in this work occurred only at the nodal plates of corn stems, and only in the bundle sheath and outer layers of pith cells around the bundle.

2. It accumulates as masses and crystals containing iron, which have definite optical and other properties.

3. The crystals and masses are isotropic. The crystals belong to the cubic crystal system, and have a refractive index of $n = 1.59$.

4. The iron is present probably not as a simple compound, but as an oxide or hydroxide of iron in solid solution in some host substance. The properties of the crystals suggest that the host substance may be protein in nature.

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EXPLANATION OF PLATE III

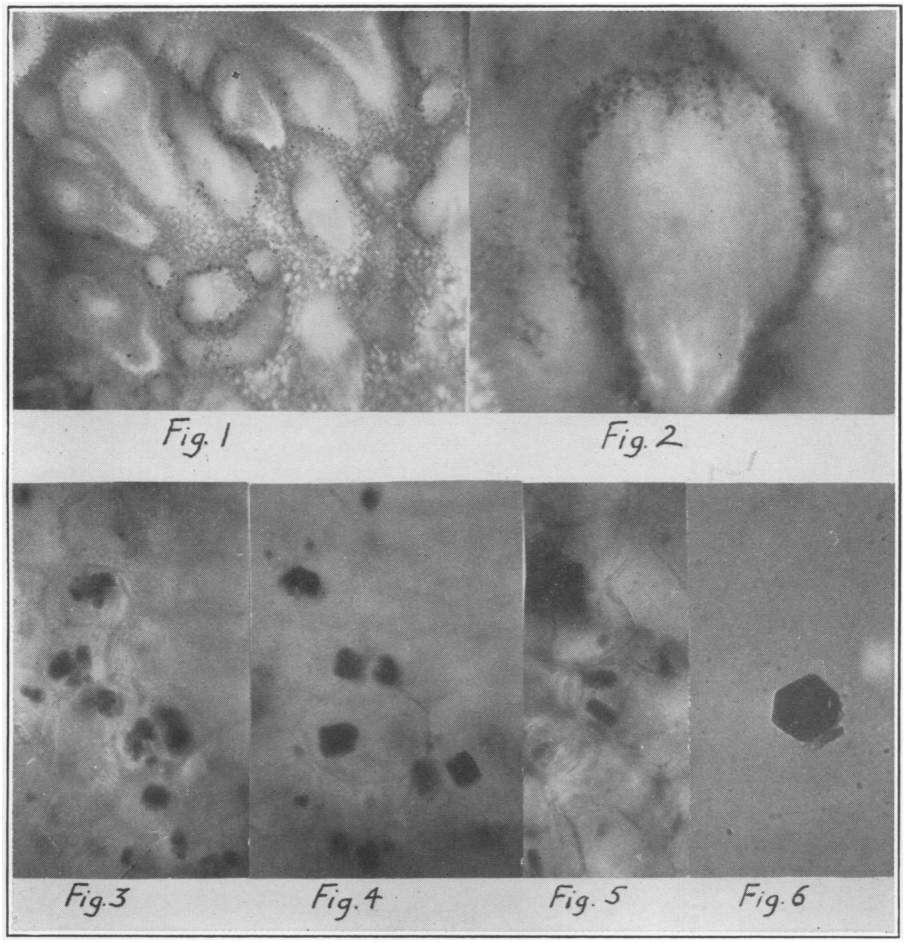
FIG. 1. Cross section of node of corn showing iron accumulated around the vascular bundles (×60).

FIG. 2. Cross section of a vascular bundle at the node of corn showing iron accumulated around the bundle (×110).

FIG. 3, 4, 5. Crystals and masses containing iron at the node of the corn stem (×260).

FIG. 6. Crystal containing iron at the node of the corn stem (×600).

All sections unstained, and mounted in water.



SAYRE—IRON ACCUMULATION