X-RAY PHOTOGRAPHY OF MINERAL ACCUMULATIONS IN PLANTS

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(WITH SIX FIGURES)

The accumulation of mineral salts in plants has heretofore been demonstrated mainly by microchemical means, a method which is not always satisfactory for quantitative ends, since some of the minerals may be masked through organic combinations, and since the amount of color produced, in reactions dependent on colors, is not necessarily proportional to the amount of mineral present in the tissues.

In recent years HOFFER1 and his coworkers have used the microchemical method to demonstrate the accumulation of iron and other elements in the nodal tissues of Zea mays in connection with corn root rot problems. The writer has employed an entirely different method, and has been able to demonstrate such mineral accumulations in the nodal tissues of sugar cane, Saccharum officinarum, with results that offer beautiful confirmation of HOFFER's discoveries with the microchemical methods in corn.

The purpose of this paper is to present a few facts concerning the method of detecting mineral accumulations by means of X-rays. It is hoped thereby to stimulate more work along this line, and to encourage the use of this method in studies involving mineral deposition, and mineral translocation in plants. As time goes on X-rays have found an ever widening field of usefulness in science, industry, and medicine. It seems quite probable that many other uses for these rays in biological work may be found.

GEORGE L. CLARK, in his "Applied X-Rays," mentions several of the less well-known applications, and says: "Although X-rays because of their short wave-length are much more able to penetrate matter than ordinary light, still they are differently absorbed by different substances; that is to say, all materials are not equally transparent to X-rays." This fact is the basis of the science of radiography. Broadly defined, the technique consists in passing a beam of X-rays through the object to be examined, and by means of a fluoroscope, or preferably a photographic plate, record the varying intensities of the emergent rays, thereby obtaining a shadow picture of the interior of the object. The first practical uses of the X-rays were probably of a radiographic nature, and today radiography is a most useful tool for the medical and industrial diagnostian.

The writer has simply employed the essential quality of the X-rays of being differentially absorbed by different substances, in detecting the localization of mineral depositions in plants. In developing the technique, some substances other than those found abundantly in plants were used, mainly to afford substances differing in opacity to the X-rays. Some of the chemicals used are quite opaque to X-rays, others relatively easily penetrated.

In performing the experiments, solutions of 1–2 per cent. strength were used, of the following salts: Ferrous sulphate, aluminum chloride, potassium sulphate, sodium chloride, lead nitrate, and barium chloride. The heavier metals, lead and barium, are quite opaque; following these are iron and aluminum, relatively considerably less opaque; finally, sodium and potassium are very easily penetrated by X-rays.

Normal average sugar-cane sticks were cut at the surface of the soil, the cut end placed at once into a beaker containing one of the mentioned solutions; control sticks were similarly placed in tap water. All sticks were allowed to remain in the solutions for about 48 hours before they were photographed. During this time, continuing transpiration and existing saturation deficits caused the absorption of some of the solution, which could easily be detected from the decrease of volume of solution remaining in the beakers.

The sticks were now laid across the film-holder containing an X-ray film, and were exposed to X-rays emanating from a Coolidge X-ray tube. In order to secure good photographs, the rays must be adjusted to a suitable penetrating power, and the time of exposure must also be adjusted to the results desired. The photographs reproduced in this paper, were made with a voltage of only about 27500, with 10 milliamperes of current. The exact voltage used depended somewhat upon the thickness of the sticks of cane. The time of exposure was usually about 3.5 minutes. The cane was placed as close to the film-holder as possible in order to obtain clear sharp images. In any given case the investigator will find it necessary to carry on preliminary experiments with different milliamperages and voltages with each object of research, to obtain the best results. In general, the voltage will be much lower than is easily obtained in most makes of apparatus, the average medicinal unit being designed for higher voltages.

The distance from the X-ray tube to the film is, of course, very important since the length of exposure varies with the distance. The photographs shown here are the results of a preliminary study. In fig. 1 is shown a stick which was allowed to take up barium chloride through the cut end. The barium was deposited most heavily at the node, or under the buds. Not only are the nodal deposits shown, but also the vascular system is clearly outlined. It shows better in the photograph than in the reproductions from it. One can see some of the root bands also, rather easily.
Fig. 1. Sugar cane stick, photographed by X-rays after having taken up barium chloride. Dark bands show nodal accumulation of BaCl₂.

Fig. 2 was made from the photograph of a stalk allowed to absorb lead nitrate solution. The control stick is at the left. The root bands show very
Fig. 2. X-ray photograph of sugar cane sticks; at the right, treated with lead nitrate; at the left, the control. The lead nitrate is very prominent in the root bands.
Fig. 3. Nodal accumulation of iron, from FeSO₄. Improperly exposed.
clearly here, and the vascular system in the internodal region is brought out quite well. In the control sticks two buds are seen at the nodes. Both figures 1 and 2 are made from stalks containing the most opaque salts used. Lead and barium are not very prominent constituents among the ash elements of plants, though probably never completely absent. Barium is rather universally distributed in soils and plants, but these elements do not occur in such abundance as to show up in the nodes of the control sticks. Iron and aluminum, however, are nearly always fairly abundant in soils. In fig. 3 is shown a photograph from an improperly conducted exposure of a stalk which had been treated with FeSO₄. The nodal accumulation, in spite of the improper exposure, is clearly shown, and even the vascular bundles can be seen. Better examples of iron accumulation are shown in fig. 6.

The lighter elements, potassium and sodium, are more readily penetrated by X-rays, and so appear to clear up the nodes. In fig. 4 we see a stick which was allowed to take up NaCl. The control stick, to the left, contrasts strongly with the treated one. The latter seems to have deposited the sodium salt sharply at the node. One might question whether it is the sodium, or chlorine, or both that are involved in the deposition. In the treated stick (right) one can see some spots of lighter color. These were found, on cutting the sticks, to be the cavities produced by a stalk borer.

In fig. 5 the control stick is to the right, and the treated stick (left) was allowed to absorb K₂SO₄. This also seems to clear out the nodal tissue, and to make it less dense than normal. The proper interpretation of this clearing of nodes by Na and K salts probably is that the sodium or potassium displaces some heavier metals, as Fe and Al, that normally are deposited in that region, and so prevents their union with the organic constituents of the nodes. These X-ray results are again in harmony with Hoffer's results in which he claims that potassium prevents the over-accumulation of iron or aluminum in the nodes of corn.

The final figure 6 is most interesting. These stalks were not allowed to absorb solutions, but were taken from soils in which the soluble mineral content was high. The photographs show what can be accomplished in checking up on soil experiments. The small stick to the right is a young cane grown on a soil containing high available aluminum, while the two to the left of it are from a soil with high available iron. The stalks from the iron containing soil showed slight indications of iron injury, but yet growth was practically normal. Tests made for iron and aluminum by other methods did not show them present, but the X-ray photographs clearly show the nodal accumulations. This method may therefore exceed in sensitivity the microchemical methods of detecting these accumulations. A very interesting feature of the stalks grown on the iron-rich soil is the clear indication in
Fig. 4. Sugar cane sticks: Right, after exposure to NaCl, nodal accumulation being indicated by clearer nodal tissue; left, control. Spots in the experimental stick are due to a borer.
Fig. 5. Sugar cane sticks: Left, treated with FeSO₄; right, control. Nodal accumulation is indicated by clearer nodal tissue.
Fig. 6. Sugar cane sticks grown on soils with high available minerals. Right, grown on high available aluminum soil. Center and left, on high available iron soils. The nodal accumulations are easily seen, as well as the vascular tissue of internodes. See text for additional details.

the middle stalk of fig. 6 of three bands of iron deposition at the nodes, one across the central part of the node, and one on either side. Professor Hoffer, who has examined these photographs, has found similar banded nodal deposits by microchemical means.

Additional work is in progress with the X-ray method in the hope that some of the problems connected with the translocation of salts in plants may be solved. It ought to be possible to determine whether xylem or phloem is mainly concerned in translocation of salts by a study of localization of minerals in plants which have absorbed the salts through unbroken root systems. Space does not permit the inclusion of photographs of some other plants, such as corn, tomato, and some quite good ones of Selaginella which seem to show the structure of the vascular system very well.
In conclusion, the writer desires to express his thanks and appreciation to Mr. W. S. Kendrick, of the Victor X-Ray Corporation, Chicago, for the loan of apparatus, and the use at times of their X-ray equipment and dark room, in connection with the more recent work. He is also indebted to Professor Charles A. Shull for assistance in preparation of the manuscript.

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