THE ESSENTIALITY OF CERTAIN ELEMENTS IN MINUTE QUANTITY FOR PLANTS WITH SPECIAL REFERENCE TO COPPER

D. I. Arnon and P. R. Stout

The recent discoveries of the importance of small amounts of boron, manganese, copper, and zinc (**1, 2, 4, 7, 13**) in the physiology of higher plants, and in a number of soil-plant problems of considerable agricultural importance, have added interest to studies on the rôle of these elements in plant nutrition. The most obvious and important question is whether or not these elements are indispensable to the growth of plants.

Recent investigations in this laboratory (**12**) on the importance of certain metals in minute quantity in the economy of higher plants, have resulted in the development of an experimental technique which makes possible a consistent and reproducible demonstration of the essentiality of copper, manganese, and zinc for the growth of higher plants. Although the details of the procedures will be described elsewhere (**12**) it is desired to present at this time some considerations and conclusions which may be of general interest.

It was deemed desirable to test experimentally the essentiality of each element according to the following criteria: an element is not considered essential unless (a) a deficiency of it makes it impossible for the plant to complete the vegetative or reproductive stage of its life cycle; (b) such deficiency is specific to the element in question, and can be prevented or corrected only by supplying this element; and (c) the element is directly involved in the nutrition of the plant quite apart from its possible effects in correcting some unfavorable microbiological or chemical condition of the soil or other culture medium. From that standpoint a favorable response from adding a given element to the culture medium does not constitute conclusive evidence of its indispensability in plant nutrition.

Conclusive proof of the indispensability of the so-called microelements, that is, elements required in minute amounts by plants, can be obtained by the use of artificial culture media (the water culture technique was used in this investigation), provided special procedures are employed to remove incidental impurities. The physiological importance of these contaminants is illustrated by the finding that young tomato plants grown in Pyrex glass containers with nutrient solutions deficient in zinc gave a measurable response to the addition of 1 gamma (0.001 mg.) of zinc to a plant (this gave a zinc concentration in the culture solution of 5 parts per billion). This response to a minute amount of zinc as well as similar responses to copper and manganese were consistently obtained only when redistilled water and purified chemicals were used. The metal content of ordinary distilled water was reduced by redistillation, using a Pyrex glass condenser, from 10 to 100 to consistently less than 0.2 part per billion. The metal impurity derived from salts was reduced, by using a modification of STEINBERG'S procedure (**11, 12**) from 15 to 150 to consistently less than 0.1 part per billion.

The experimental demonstration of the essentiality of microelements for plant life is frequently rendered difficult not only because plant needs may be satisfied by incidental contaminants, but also because different species vary greatly in their requirements. In this laboratory, for example, it was repeatedly found that a standard culture solution to which no manganese or boron was added produced excellent barley plants but gave severe boron and manganese deficiency symptoms in tomato plants. It may be advantageous therefore to select for experimental purposes plants having relatively high requirements for the microelement studied. For any one species the onset of deficiency symptoms may be hastened by reducing the reserve in the seed, either by using seed obtained from plants grown with a limited supply of microelements (**5**) or by early removal of the seed from the seedling (**10**). Thus the extent to which it is necessary to purify the culture medium in order to produce deficiency symptoms may be reduced through a suitable selection and preparation of experimental plants.

Since it cannot be assumed that any purification procedure has completely removed a given element from the nutrient medium, the conclusions from a negative result should best not be drawn with finality. When an element is omitted from a carefully prepared and purified artificial culture medium without seemingly hindering the growth and reproduction of plants, it need not be concluded that it plays no essential function in the physiology of plants. Regardless of the care exercised in the preparation of the culture medium, a minute, but physiologically significant, impurity may have persisted and needs to be considered as well as the reserves contained in the seed. It would seem best in such cases to determine analytically, whenever possible, the upper limit of impurity that may be present in the culture medium, and express the results of the experiment on a quantitative basis; that is, that with a certain upper limit of supply of a given element, no response is obtained with certain species of plants. It is possible that the attainment of a greater degree of purification of the culture medium by means of improved experimental procedures, the use of seeds having a low content of, or the selection of plants having a high requirement for, a particular element may give different results.

If these views are accepted, there can be no *a priori* objection to regarding almost every element in the periodic table, and particularly those most frequently encountered in plant products, as susceptible of being shown at some time to be essential for plants. (This is in accord with similar views expressed by HOPKINS (**3**).) What can be stated definitely at the present time is that a given element is essential or not, depending on whether the plant requires it in an amount greater than that present in the culture medium as a result of contamination. It was found, for example, that if the combined zinc, copper, lead, cadmium, and mercury content in the nutrient solutions was reduced to less than 0.1 of a gamma (0.0001 mg.) to a plant, as determined by analyzing the culture solution (**12**), very severe deficiency symptoms were obtained. In this case a definite response was obtained from supplying 2 gammas of copper and 2 gammas of zinc, but no further improvement was produced by supplying to a plant 0.5 of a gamma each of lead, cadmium, and mercury. These results, while showing the indispensability of zinc and copper for the growth of tomatoes in amounts larger than those left behind in the purified culture medium, were interpreted as permitting no final conclusion as to the rôle of cadmium, lead, and mercury in the nutrition of tomatoes. The possibility that these elements may be required in amounts smaller than the incidental impurities which could not be removed from the culture solution by the present technique, cannot be *a priori* excluded.

This quantitative approach to the problem of essentiality of microelements is regarded not as a mere theoretical generalization, but as a point of view conducive to a search for more refined analytical methods and procedures for growing plants, which would make it possible to investigate the rôle of a number of new elements in plant nutrition.

After experimentally producing the characteristic deficiency symptoms for an element, the next step was to show that it had a specific and direct effect on the physiology of the plant. The question of the essentiality of copper was a case in point. SOMMER, (**9**) and LIPMAN and MACKINNEY (**6**) have independently demonstrated the necessity of copper for the the vegetative growth and reproduction of higher plants. It was recently suggested, however, that the observed response to copper may have been due to an indirect influence, *i.e.,* its antagonistic or balancing effect on other elements (**8**). Attention was also called to the desirability of confirming the specificity of copper and excluding the possibility that some other element capable of existing in different valence states, could replace copper (**2**).

The specific and direct effect of copper on the growth of tomatoes was demonstrated as follows: Typical copper deficiency symptoms in tomatoes (very much stunted growth of shoots and exceedingly poor root development, dark bluish-green color of foliage, curling of leaves, and absence of flower formation) were produced by growing the plants in culture solution in two-liter Pyrex beakers, using purified salts and redistilled water (**12**). (Copper deficiency symptoms could be produced only if water redistilled over a Pyrex condenser was used. Ordinary distilled water contained enough copper to satisfy the needs of tomatoes, even when purified salts were used.) The development of the deficiency symptoms was prevented by supplying a plant with 2 gammas of copper as $CuSO_4$.

To test the possible antagonistic effect of copper on other elements, as distinguished from its direct effect on plant growth (9), plants were grown in solutions supplied in one case with only three microelements: boron, manganese, and zinc, and in another case with these plus 20 additional ones, including lead, mercury, arsenic, and selenium. It was found that the onset, type, and severity of copper deficiency symptoms were the same in both cases. The additional group of 20 elements included elements capable of existing in different valence states, such as molybdenum, vanadium, chromium, nickel, cobalt, titanium, and tungsten, none of which were found capable of replacing copper.

Leaves of young tomato plants showing copper deficiency symptoms were sprayed with a very dilute $CuSO_4$ solution (0.02 parts per million of copper). This brought about recovery and the resumption of normal growth of both shoots and roots. These findings, showing the direct effect of copper on the plant as distinguished from its possible effect on the root environment, were interpreted as providing a final link in the chain of evidence of the essentiality of copper for the growth of tomato plants.

The small quantitative requirement of copper and other elements for plant life need not detract from an appraisal of their importance in the physiology of higher plants. There is no choice between essential elements. In that sense the fraction of a milligram of copper is just as indispensable as several hundred milligrams of a "major" element like potassium, since in the absence of either of them a tomato plant will fail to complete its life cycle. It is for this reason that the term "minor elements" frequently used to designate the group of elements essential for plants in minute quantity, should not lead to the inference that these elements occupy a position of secondary importance in the nutrition of higher plants.

The fact that most plants are injured by relatively very low concentration of microelements (for example injury to tomatoes was obtained from 2.0 parts per million of copper in the culture solution) adds interest to the search for the explanation of their function in the plant.

College of Agriculture
University of California

LITERATURE CITED

1. Brenchley, Winifred E. The essential nature of certain minor elements for plant nutrition. Bot. Rev. **2**: 173–196. 1936.
2. Hoagland, D. R. Some aspects of the salt nutrition of higher plants. Bot. Rev. **3**: 307–334. 1937.

3. HOPKINS, E. F. Manganese an essential element for green plants. New York (Cornell) Agr. Exp. Sta. Memoir 151. 1934.
4. JACKS, G. V., AND SCHERBATOFF, H. Soil deficiencies and plant diseases. Technical Communication no. **31,** Imperial Bureau of Soil Science, Harpenden, England. 1935.
5. LIPMAN, C. B. Importance of silicon, aluminum, and chlorine for higher plants. Soil Sci. **45**: 189–198. 1938.
6. ———, AND MACKINNEY, G. Proof of the essential nature of copper for higher green plants. Plant Physiol. **6**: 593–599. 1931.
7. MAZÉ, P. The rôle of special elements (boron, copper, zinc, manganese, etc.) in plant nutrition. Ann. Rev. Biochem. **5**: 525–538. 1936.
8. RABER, O. L. Principles of plant physiology. Macmillan, New York. 1933.
9. SOMMER, A. L. Copper as an essential for plant growth. Plant Physiol. **6**: 339–345. 1931.
10. ———, AND LIPMAN, C. B. Evidence of the indispensable nature of zinc and boron for higher green plants. Plant Physiol. **1**: 231–249. 1926.
11. STEINBERG, R. A. Nutrient-solution purification for removal of heavy metals in deficiency investigations with *Aspergillus niger*. Jour. Agr. Res. **51**: 413–424. 1935.
12. STOUT, P. R., AND ARNON, D. I. Experimental methods for the study of the rôle of copper, manganese, and zinc in the nutrition of higher plants. Amer. Jour. Bot. **26**: 144–149. 1939.
13. YOUNG, R. S. Certain rarer elements in soils and fertilizers and their rôle in plant growth. New York (Cornell) Agr. Exp. Sta. Memoir 174. 1935.