THE INFLUENCE OF MANGANESE DEFICIENCY ON THE SYNTHESIS OF ASCORBIC ACID (VITAMIN C) IN FOLIAGE OF PLANTS

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EXPERIMENTAL PROCEDURE

The experimental work reported in this paper is part of a study to ascertain the influence of various minor element deficiencies on the vitamin content of the foliage of crops grown on organic soil. The effect of a deficiency of available manganese has been investigated in detail, because a considerable portion of the acreage of the organic soils which are devoted to the production of vegetable crops for commercial markets are deficient in this respect. This deficiency may result from an alkalinity produced by over-liming, burning or by the presence of alkaline spring water, of marl, or of other alkaline mineral oxides near the surface of the soil. Since application of manganese fertilizers to these soils produces a marked increase in crop yields, the influence of manganese on crop quality becomes a matter of considerable importance. The content of ascorbic acid can be considered as one measure of quality in any crop containing appreciable quantities of it.

In this study, samples of leaves were taken from crops growing on the part of the College Muck Soil Experimental Field which is deficient in available manganese. On these plots the deficiency was corrected in two ways, namely, by the application of commercial manganese sulfate (65% MnSO₄) each year and by making the soil acid with heavy applications of sulfur flour (3 pp. 32-41). Other samples were taken from plants grown in the greenhouse on similar soil deficient in available manganese. The plants produced in the greenhouse were used mainly to study seasonal differences.

Method of analysis.—Ascorbic acid was determined by the method described by Bessey and King (1). The method as adapted for this study are as follows:

Leaves collected from several plants were carefully free of foreign materials and then placed in a pile, one above the preceding one. A ribbon of the leaves was then taken from a cross-section of the middle of the pile of leaves. A sample of these ribbons was placed in a porcelain dish and 200 ml capacity. Twenty ml of the hot extraction solution containing 8.0% trichloroacetic acid (30%) metaphosphoric acid and about 3 grams of quartz sand were added to the sample in the manner described by Bessey and King (1). The sample was ground by carefully revolving the pestle and mortar, the leaf tissue was completely disintegrated. After grinding, the mixture was transferred to two 15-ml centrifuge tubes and made to volume with a 100-ml volumetric flask. The extraction was repeated on the liquid obtained from this extraction was added to the first. With some crops a third extraction was made. The solution in the flask was made alkaline and thoroughly mixed. Two samples of 30 ml each were taken for analysis; in the first the reduced ascorbic acid was determined and in the second the oxidized form of the vitamin was determined.

Reduced ascorbic acid was determined by direct titration with 2, 6, dichlorobenzeneindophenol. The indicator solution was made up so that each ml of solution was equivalent to one International Unit of vitamin C (0.06 gram in 500 ml distilled water). In the determination of total ascorbic acid, the second sample was transferred to a long narrow test tube; H₂S was passed through the liquid for 15 minutes, during which time the oxidized form of ascorbic acid was reduced. The excess of H₂S was washed out by passing a small amount of an inert gas, generally nitrogen, through the solution. The increase in the gas passed through until there was no discoloration of the paper moistened with lead acetate solution when held at the mouth of the test tube. The solution was then titrated with a standard solution of 2, 6, dichlorobenzeneindophenol.

Ascorbic acid was determined in leaves from oats, Sudan grass, and spinach, which showed a marked increase in crop yields when grown on the treated soil, a severely chlorotic condition of the foliage symptoms of manganese deficiency. The oxidized form of the vitamin was determined in the second sample. The oxidized form of ascorbic acid was determined by the method of Bessey and King (1).

RESULTS

The leaves of the plants growing on the plots which lack of available manganese had an increased content of ascorbic acid than those from the untreated plots. This increased content of ascorbic acid is not due to a deficiency of available nitrogen in the soil, because the content of ascorbic acid was not higher than pH 4.5. The content of ascorbic acid in leaves from plants grown on soil naturally deficient in available manganese was higher than the content of ascorbic acid in leaves from plants grown on soil naturally deficient in available manganese and which had received an application of a manganese salt, had more ascorbic acid than the fruit produced on the untreated soil. The recent work of Lyon and Beeson (6), however, failed to substantiate Hester's findings.

Since the degree of deficiency of available manganese in a soil can be estimated by the amount of chlorosis of the plants produced on it, it is evident that there must be some definite relationship between manganese supply and chlorophyll production. In fact, McHargue (7) has shown that manganese is essential to the formation of chlorophyll in plants. Since ascorbic acid is present in appreciable quantities in all plant tissue containing chlorophyll, it seemed reasonable to believe that the composition of the foliage of plants would reflect the influence of manganese deficiency on the synthesis of ascorbic acid, even to a much greater degree than will be true in fruits or other nonchlorophyllic plant tissue.