EFFECT OF INITIAL ACIDITY ON CALCIUM AND MAGNESIUM REQUIREMENTS OF TOBACCO IN ASEPTIC CULTURE [1]

By ROBERT A. STEINBERG

Physiologist, Division of Tobacco, Medicinal, and Special Crops, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

A great deal of investigational work has been done on the relation of acidity to the growth of plants. A summary by Russell [2] mentioned the fact that a slight degree of acidity is usually beneficial in solution culture. He continued with the statement: "In soils, on the other hand, plants make their best growth in neutral or nearly neutral conditions." Definite evidence is available, however, that this generalization is too broad and that acidity may or may not be beneficial in either growth medium.[3] A summary by Pettinger [4] seems to indicate that many soils suitable for good crop production are acid in character. These writers emphasized not acidity in itself, but its influence on availability of nutrients. Similar evidence was presented by Albrecht and Schroeder. [5] Arnon and Johnson [6] found in addition that an increase in calcium ions could compensate for decreased availability of calcium due to excessive acidity. Acidity of the growth medium, therefore, would seem to be only one of many factors influencing availability, and not the all-important factor it was first considered.

A brief study, therefore, has been made on the relation of acidity in the range pH 4 to 7 to the calcium and magnesium requirements of seedlings of Xanthi Turkish tobacco (*Nicotiana tabacum* L.) in aseptic culture under controlled environmental conditions. The absence of extraneous micro-organisms in such studies is not usually considered important, although no evidence for this assumption is known. The data obtained with increasing quantities of calcium and magnesium at several initial acidities of the nutrient solution are presented in the form of growth curves.

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[1] Received for publication January 27, 1947.

[2] RUSSELL, E. J. SOIL CONDITIONS AND PLANT GROWTH. Ed. 7, 655 pp., illus. 1937. (See p. 121.)

[3] HOAGLAND, D. R. LECTURES ON THE INORGANIC NUTRITION OF PLANTS. 226 pp., illus. 1944.

[4] PETTINGER, N. A. A USEFUL CHART FOR TEACHING THE RELATION OF SOIL REACTION TO THE AVAILABILITY OF PLANT NUTRIENTS TO CROPS. Va. Agr. Col. Ext. Bul. 136, 19 pp., illus. 1935.

[5] ALBRECHT, W. A., and SCHROEDER, R. A. PLANT NUTRITION AND THE HYDROGEN ION: I. PLANT NUTRIENTS USED MOST EFFECTIVELY IN THE PRESENCE OF A SIGNIFICANT CONCENTRATION OF HYDROGEN IONS. Soil Sci. 53: 313–327, illus. 1942.

[6] ARNON, D. I., and JOHNSON, C. M. INFLUENCE OF HYDROGEN ION CONCENTRATION ON THE GROWTH OF HIGHER PLANTS UNDER CONTROLLED CONDITIONS. Plant Physiol. 17: 525–539, illus. 1942.

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EXPERIMENTAL PROCEDURE

Xanthi Turkish tobacco seedlings were grown on 50 cc. of a mineral-salt solution in 200-cc. Pyrex Erlenmeyer flasks under aseptic conditions. The temperature used was 25° C., and light of about 500 foot-candles was furnished by 3,500° white fluorescent lamps. The growth period was 28 days.

The mineral-salt solution consisted of water, 1,000 cc.; $Ca(NO_3)_2 \cdot 4H_2O$, 1.444 gm.; $Mg.(NO_3)_2 \cdot 6H_2O$, 0.318 gm.; K_2HPO_4, 0.366 gm.; $KHSO_4$, 0.085 gm.; and NH_4Cl, 0.072 gm. Separate stock solutions of calcium nitrate, magnesium nitrate, and potassium phosphate plus potassium bisulfate plus ammonium chloride in 20× concentration were used in the preparation of the mineral-salt solution. In preparing cultures with varying quantities of calcium ion, calcium nitrate was replaced with 1.039 gm. of sodium nitrate ($NaNO_3$) and calcium was added as the chloride. Magnesium nitrate, similarly, was replaced with 0.211 gm. of sodium nitrate in the study of magnesium concentrations. The base solution, therefore, contained nitrogen, 225 mg.; potassium, 189 mg.; phosphorus, 65 mg.; magnesium, 30 mg.; calcium, 245 mg.; and sulfur, 20 mg. per liter. Micronutrients, except for boron (H_3BO_3), were added to this solution as the chlorides. The quantities used were iron, 15 mg.; zinc, 0.5 mg.; copper, 0.125 mg.; manganese, 1.0 mg.; and boron, 0.5 mg. per liter. Acidity was adjusted with 0.1 N hydrochloric acid.

The composition of the mineral-salt solution was equivalent to that used by McMurtrey[7] in solution-culture studies with tobacco. It differed only in that potassium was increased from 125 to 189 mg. per liter and in that potassium nitrate, monopotassium phosphate, and magnesium sulfate were replaced with dipotassium phosphate and potassium bisulfate, the other salts being readjusted in concentration.

The cultures in the magnesium series contained 57.1 mg. of sodium ion per liter and those in the calcium series 281.1 mg. in addition to all the essential elements. Addition of hydrochloric acid for pH adjustment totaled not more than 28 mg. of chloride ion per liter, as compared with the 31 mg. originally present as ammonium chloride. Use of the chlorides of magnesium and calcium further increased the chloride-ion content by a maximum of 29.2 and 442.3 mg. per liter, respectively, depending on the particular concentration of magnesium or calcium ion employed.

The tobacco seeds were germinated in sterilized petri dishes containing a layer of blotting paper and several layers of filter paper. Seeds sterilized by immersion in 1:1,000 silver nitrate solution for 15 minutes were washed several times with sterile distilled water and then poured as a water suspension into the petri dishes. With a flamed platinum needle the seedlings were transferred to sterile Erlenmeyer flasks containing the nutrient medium and deposited on a double layer of filter paper held in a plant holder. This holder consisted of a glass rod 4 mm. in diameter which passed through the absorbent cotton in the neck of the flask and had a glass ring fused to its lower end. The filter-paper disks were held in this ring by means of a loose inner glass ring as shown in figure 1. The edges of the paper

[7] McMurtrey, J. E., Jr. DISTINCTIVE EFFECTS OF THE DEFICIENCY OF CERTAIN ESSENTIAL ELEMENTS ON THE GROWTH OF TOBACCO PLANTS IN SOLUTION CULTURES. U. S. Dept. Agr. Tech. Bul. 340, 43 pp., illus. 1933.

disks were bent down and forced between the rings to keep them in position and were then perforated. The purpose of the holder was

FIGURE 1.—Three-week-old seedling of Xanthi Turkish tobacco growing under aseptic conditions. A glass rod with fused-on ring passes through the absorbent-cotton plug. Two filter-paper disks, which are perforated with a needle after insertion in holder, are held in place in the ring by means of a loose inner glass ring, the edges of the paper disks being forced between the two rings.

to prevent contact of all but the roots of the plant with the solution. All glassware and media were sterilized at 15 pounds' pressure for 30 minutes.

At harvest the seedlings were washed, dried in the oven at 103° to 105° C. for 4 hours, cooled in the desiccator, and then weighed.

Two of the four duplicate seedlings were weighed together as a unit in each determination. Statistical methods appeared inapplicable, since it was necessary to reject about 10 percent of the seedlings because of contaminations with micro-organisms and unintentional injuries during transfer to the flasks. Some injured seedlings did not grow out of the cotyledon stage.

INFLUENCE OF ACIDITY ON CALCIUM REQUIREMENT

The effects obtained by varying the calcium content on the growth of the seedlings at four levels of acidity are shown in figure 2. The initial acidity levels were pH 6.48, 5.98, 5.42, and 4.39. Each value for dry weight is the average for eight seedlings, or for four in each of two determinations. The acidities at harvest were obtained by mixing the four residual solutions in each run and averaging the pH values in both runs. The averages found in this manner are not true pH values, but the deviations for the small variations encountered are probably well within those of experimental error.

It will be observed that in the solution at pH 6.48 the calcium optimum for growth was about 100 mg. per liter and that residual acidity of the solution increased with calcium content. The increase in acidity persisted even with decreasing yields. At an acidity level of pH 5.98 the growth curve was much flatter because of the slightly increased yields at deficiency levels of 25 and 50 mg. of calcium per liter. A contributing factor was a moderate decrease in maximum yield. Acidities at harvest also increased at this initial pH, but less than at pH 6.48.

INFLUENCE OF ACIDITY ON MAGNESIUM REQUIREMENT

The relation of magnesium requirements to acidity of the nutrient solution is shown in figure 3. Determinations were made at three levels of initial acidity—pH 5.96, 5.31, and 4.34. The number of repetitions and the method of averaging values were the same as those in the calcium series. At pH 5.96 the maximum yield was obtained with 6 mg. of magnesium per liter. Increasing acidity decreased maximum yields only slightly and did not alter the optimum magnesium concentration. Increasing acidities also caused slight decreases in yield with suboptimum concentrations of magnesium. At harvest acidities were in all cases approximately the same and were influenced but little by the initial acidity of the nutrient solution.

DISCUSSION

Adjustments in hydrogen-ion concentration and magnesium or calcium content of the nutrient solution are of course not feasible without alterations in other constituents. Moreover, it seemed advisable in these experiments under aseptic conditions to follow the usual procedure of using sodium and chloride ions, that is, sodium nitrate, hydrochloric acid, and the chlorides of magnesium and calcium. The basis for this procedure is the apparent nonessentiality of sodium and chlorine for growth of green plants. Nevertheless, sodium and chloride ions cannot be assumed a priori to be without influence on growth. Similar series employing fluctuations in essential ions might also be

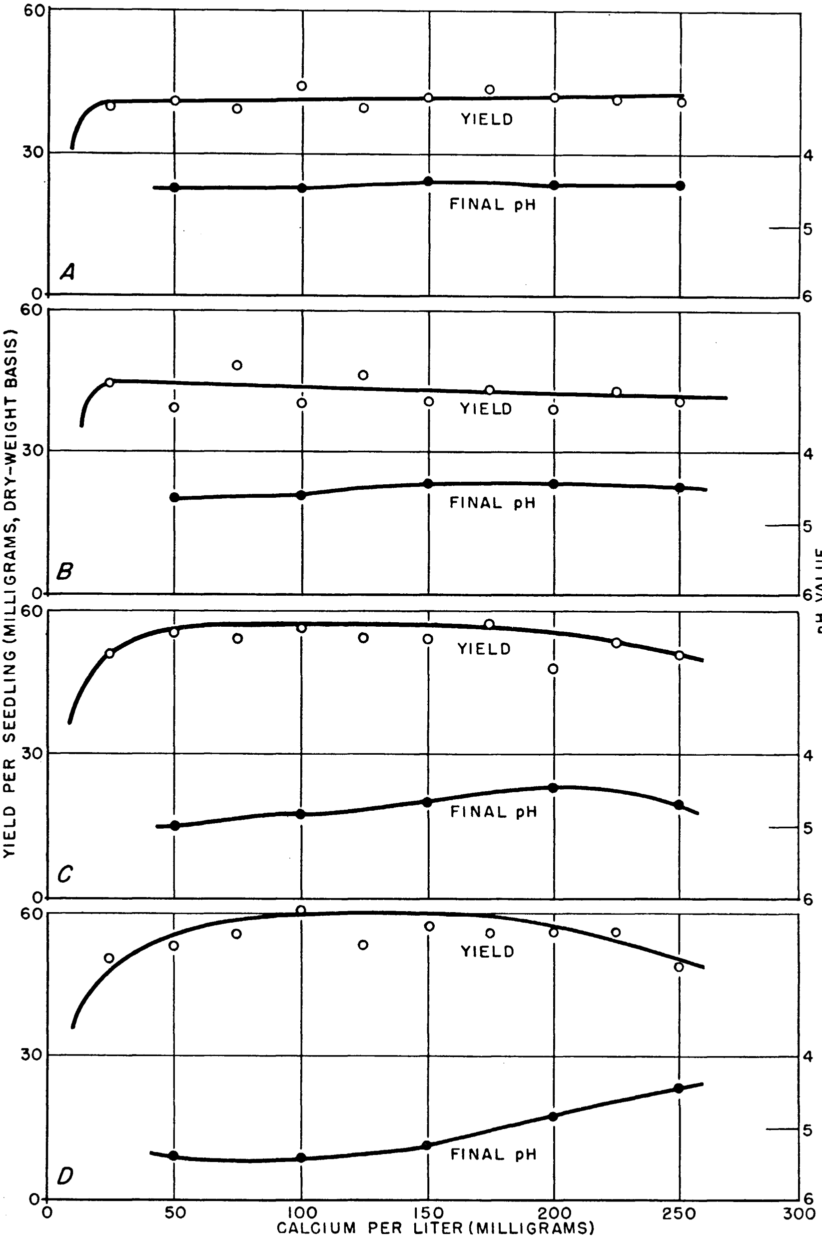

FIGURE 2.—Average acidities of solutions at harvest and average weights of Xanthi Turkish tobacco seedlings grown for 28 days with continuous illumination of 500 foot-candles in nutrient solutions containing different amounts of calcium and having different initial acidity levels: *A*, pH 4.39; *B*, pH 5.42; *C*, pH 5.98; and *D*, pH 6.48.

desirable for comparison, though it would be necessary to use initial excesses in order to avoid deficiencies.

The degree of influence of sodium and chloride ions in the calcium and magnesium series is indicated in several ways by the experimental

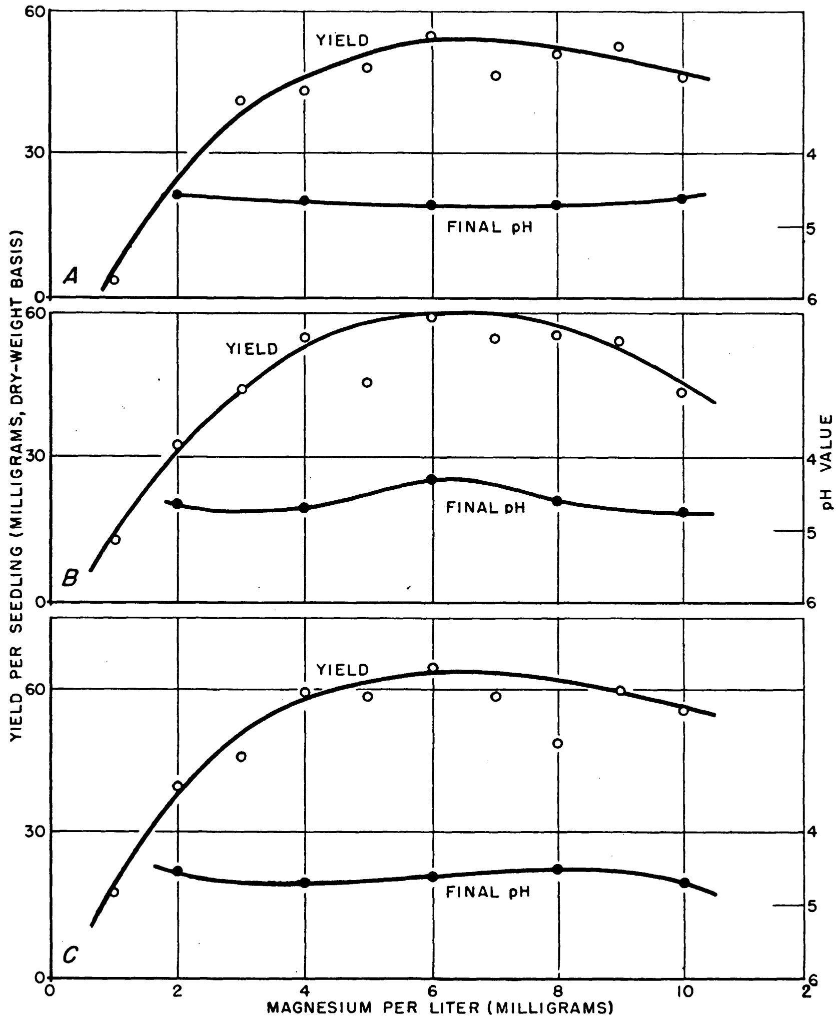

FIGURE 3.—Average acidities of solutions at harvest and average weights of Xanthi Turkish tobacco seedlings grown for 28 days with continuous illumination of 500 foot-candles in nutrient solutions containing different amounts of magnesium and having different initial acidity levels: *A*, pH 4.34; *B*, pH 5.31; *C*, pH 5.96.

data. The maximum yields in the two series were 63 mg. for magnesium and 60 mg. for calcium at the highest initial pH employed. The effects of a fivefold increase in sodium ion and of a sixfold increase in chloride ion as between the series are therefore rather small. Moreover, though the highest concentrations of chloride are concomitant with those of magnesium and calcium, the depressions in yield at high nutrient levels were greatest in the magnesium series containing only one-fifth the chloride content. Furthermore, no symptoms of injury attributable to sodium or chlorine could be detected in either series.

The effects of increasing acidities on requirements of Xanthi Turkish tobacco seedlings were not entirely uniform for calcium and magnesium. With magnesium maximum yield decreased with increasing acidities within an initial range of pH 5.96 to 4.34. Since the optimum concentration of magnesium for growth remained unaltered, the magnesium requirement was thereby increased slightly. Increasing acidity also decreased maximum yield with calcium concentrations and so also increased the calcium requirement for growth. Increased acidity, however, increased yields at suboptimum concentrations of calcium and thus brought about a *relative* decrease in calcium requirements at intermediate acidity levels in the more acid series. At an initial acidity of pH 6.48, for example, yields with 25, 50, 75, and 100 mg. of calcium per liter were 49.6, 52.8, 56.4, and 61.2 mg., respectively; whereas the corresponding yields for an initial acidity of pH 5.98 were 51.3, 54.4, 54.5, and 56.3 mg. There was also a slight increase in absolute values for yields in the more acid series at suboptimum levels as compared with the less acid series. Furthermore, it should be noted that maximum yield was attained with 75 mg. of calcium with an initial pH of 5.98, whereas 100 mg. of calcium was required at pH 6.48.

These data cannot, however, be considered as proof that growth responses to calcium and magnesium display an intrinsic qualitative difference. The ranges used were not identical; that for magnesium extended from 0 to 166.67 percent of the optimum, whereas that for calcium was 0 to 250 percent of the optimum. Moreover, it is not certain but that the concentrations of other macronutrients and of the micronutrients used in the nutrient solution form the basis for these qualitative differences.

These data seem to indicate that acidity is not necessarily beneficial in a solution culture but that its action is dependent on the composition of the nutrient solution. Maximum yields were obtainable without resorting to an increased acidity to increase availability of nutrient ions. On the other hand, if a stock solution of much lower calcium content had been used, it is evident that increased acidity would have proved beneficial in the calcium series and perhaps also in the magnesium series. These statements might be summarized by stating that acidity may prove harmful to growth if all nutrient ions are present in ample quantity, but beneficial if there is a deficiency of calcium and perhaps of other elements.

SUMMARY

Xanthi Turkish tobacco seedlings were grown aseptically on 50 cc. of a mineral-salt solution in 200-cc. Erlenmeyer flasks at 25° C. with 500 foot-candles of white fluorescent illumination. The calcium and magnesium optima for growth were determined at several levels of initial acidity within the range pH 4 to 7 (adjusted with hydrochloric acid). Increased acidity brought about increased calcium and magnesium (as chlorides) requirements by decreasing growth with identi-

cal supply of these elements. Growth decreases were greater with increased acidity in the calcium series than in the magnesium series. Moreover, although the concentration-yield curves for magnesium remained practically unaltered in form with varying acidity, the analogous calcium curves tended to become straight. That is, the optimum for magnesium remained unaltered whereas that for calcium decreased with acidity. The residual solutions at harvest were usually slightly more acid than the unused nutrient solutions and were rather uniform in acidity under varying conditions.

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