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

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 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. A summary by Pettinger 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. Arnon and Johnson 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.

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.

Journal of Agricultural Research, Washington, D. C.
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(NO₃)₂·4H₂O, 1.444 gm.; Mg(NO₃)₂·6H₂O, 0.318 gm.; K₂HPO₄, 0.366 gm.; KHSO₄, 0.085 gm.; and NH₄Cl, 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 (NaNO₃) 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 (H₃BO₃), 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 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

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

![Figure 3](image-url)

**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.
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 Turki-
ッシュ tobacco seedlings were not entirely uniform for calcium and mag-
nesium. 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 mag-
nesium requirement was thereby increased slightly. Increasing acidi-
ity also decreased maximum yield with calcium concentrations and
so also increased the calcium requirement for growth. Increased acidi-
ity, however, increased yields at suboptimum concentrations of cal-
cium and thus brought about a relative decrease in calcium require-
ments 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., respective;
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 ini-
tial 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 cer-
tain 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 mag-
nesium 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 mag-
nesium (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.
INFORMATION IN REGARD TO THE POLICY OF THE JOURNAL OF AGRICULTURAL RESEARCH AND SUGGESTIONS TO AUTHORS

1. The Journal accepts articles only from the United States Department of Agriculture and the State agricultural experiment stations.

2. Each article submitted must bear the formal approval of the chief of the department bureau or the director of the experiment station from which it emanates. The letter of transmittal must state that the manuscript has been read and approved by one or more persons (named) familiar with the subject, that the data as represented by the tables, graphs, summaries, and conclusions have been approved from the statistical viewpoint by someone (named) competent to judge, and that the computations have been verified.

3. Manuscripts originating at the State agricultural experiment stations should be forwarded to the chairman of the committee acting for the Association of Land-Grant Colleges and Universities, and those originating in the Department should be transmitted to the Division of Publications, which will forward them for approval to the committee, acting for the Department. Each manuscript is numbered and edited in the order received.


5. A recent copy of the Journal should be consulted and followed as to style, especially in regard to tables, illustrations, and literature citations.

6. Paper 8 x 10½ or 8½ x 11 inches, of good grade and medium weight, should be used.

7. All material except tables and quotations of more than three lines should be double-spaced. These may be single-spaced.

8. A table of contents properly indented to show the intended relationship between the different headings should accompany the manuscript.

9. Following the name of the author on the first page there should be given his official title and the name of the division, bureau, or station with which he is connected.

10. Each page of the manuscript should be numbered and should begin with a new paragraph; that is, no paragraph should carry over from one page to the next unless it is longer than one page.

11. Each footnote should be inserted in the text immediately after the line bearing the footnote reference.

12. Each table should be typed on a separate sheet, or on several if necessary. The page (or pages) carrying the table should immediately follow that containing the first reference to it. Each table should be referred to in the text and be numbered in the order of reference.

13. The illustrations in the Journal are usually shown as text figures, but to bring out fine detail plates may be used. Text figures and plates are each numbered in the order of reference. Each text-figure legend should be inserted in the text underneath the line carrying the first reference to it. Legends for plates should accompany the manuscript but should not be inserted in the text. All legends should be double-spaced and furnished in duplicate.

14. The major parts or units of illustrations are designated by capital italic letters; the subparts or subunits by lower-case italic letters. No final lettering on illustrations should be attempted, particularly on photographs. All lettering and necessary drafting will be done in the Section of Illustrations of the Division of Publications. Required letterings or markings should be indicated in the margins or lightly in pencil on the illustrations.

15. Graphs should be sent in final form, if possible, except for the lettering. If prepared in tentative form the curves and bars should be carefully indicated so that they may be accurately redrawn.

16. The plate or figure number and the title of the accompanying manuscript should be lightly written (not typed) on the back of each illustration. All photographs should be submitted unmounted, enclosed in an envelope.

17. Only references cited in the text should be listed in the literature citations. If there are seven or more they should be given at the end of the paper under the heading "Literature Cited." If fewer than seven they should be given as footnotes. All numbers referring to literature citations should be enclosed in parentheses in the text. The footnote reference to the first citation in the manuscript should be worded as follows: "Italic numbers in parentheses refer to Literature Cited, p. —." Material under Literature Cited should be double-spaced.

18. For further information consult Miscellaneous Publication No. 3 issued by the Joint Committee on Policy and Manuscripts. It may be obtained from the Division of Publications, United States Department of Agriculture.
BOOKS AND WORLD RECOVERY

The desperate and continued need for American publications to serve as tools of physical and intellectual reconstruction abroad has been made vividly apparent by appeals from scholars in many lands. The American Book Center for War Devastated Libraries has been urged to continue meeting this need at least through 1947. The Book Center is therefore making a renewed appeal for American books and periodicals—for technical and scholarly books and periodicals in all fields and particularly for publications of the past ten years. It will especially welcome complete or incomplete files of the Journal of Agricultural Research.

The generous support which has been given to the Book Center has made it possible to ship more than 700,000 volumes abroad in the past year. It is hoped to double this amount before the Book Center closes. The books and periodicals which your personal or institutional library can spare are urgently needed and will help in the reconstruction which must preface world understanding and peace.

Ship your contributions to the American Book Center, care of The Library of Congress, Washington 25, D. C., freight prepaid, or write to the Center for further information.