HYDROGEN ION CONCENTRATION IN RELATION TO ABSORPTION OF INORGANIC NUTRIENTS BY HIGHER PLANTS[1]

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(WITH TWO FIGURES)

The findings with regard to the influence of the external hydrogen ion concentration on growth (**2**), particularly the extreme effects observed at pH 3 and 9, have led to a search for a possible explanation of the observed responses in terms of the physiological activities of the plant. A further investigation of the relation of external hydrogen ion concentration to the absorption of inorganic nutrients, which was already elucidated for several ions by HOAGLAND and BROYER (**5**), seemed particularly pertinent. A series of experiments was therefore initiated to explore the effect of external reaction, within a pH range of 3 to 9, on the absorption of the nutrient ions: Ca^{++}, Mg^{++}, K^{+}, NO_3^{-} and $H_2PO_4^{-}$.

In order to differentiate between the direct effects of external reaction on absorption—which can best be explored by short-time experiments with uniform plants—and the more complex relation of hydrogen ion concentration to growth, a departure was made from the technique followed in the former investigation (**2**). Instead of growing young seedlings for extended periods of time in solutions of varying pH, the general arrangement of the experiments to be presently reported, provided for the use over short periods of time of relatively uniform, healthy plants with well-developed roots, which were grown prior to the absorption experiments in the same complete nutrient solution of a pH favorable to growth.

Methods

Tomato (*Lycopersicum esculentum* variety, Best of All), lettuce (*Lactuca sativa* variety, Imperial 847), and Bermuda grass (*Cynodon dactylon*) were germinated in sand in the greenhouse. Upon reaching a size convenient for transplanting they were transferred to corks in iron tanks (**2**), filled with a complete nutrient solution (table I, no. **1**). The plants were kept in the complete nutrient solution for 5 weeks. By allowing the nutrient solution to approach depletion at the end of this period the plants were put in a "low-salt" condition conducive to active absorption (**5**). At the end of this period they were selected for uniformity and placed for 3 days in a "minus-calcium" solution (table I, no. 2). Since no attempt was made to

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remove calcium contaminants, however, the "minus-calcium" solution could not be regarded as entirely free from calcium. The healthy appearance of the roots at the time of transfer and their subsequent capacity for absorption indicated that their immersion for 3 days in the "minus-calcium" solution did not result in injury. The purpose of keeping the plants in a "minus-calcium" solution just before the absorption test was to predispose them to calcium absorption. Preliminary experiments indicated that plants failed to absorb calcium at pH 3.

Nutrient solutions nos. 1 and 2 (table I) were each supplemented with 1 ml. of A5 solution (**1**) which supplied 0.5 p.p.m. each of boron and manganese, 0.05 p.p.m. of zinc, 0.02 p.p.m. of copper and 0.01 p.p.m. of molybdenum. Iron was added to nutrient solutions nos. 1 and 2 thrice weekly, at the rate of 0.7 ml./liter of 0.5 per cent. solution of ferrous sulphate.

At the end of the three-day period in nutrient solution no. 2, the plants were transferred for the duration of the absorption period, 96 hours, to individual tanks of about 40-liter capacity filled with nutrient solution no. 3 (table I), supplemented only with 0.5 p.p.m. of boron as H_3BO_3. The reaction of the nutrient solution in each tank was adjusted with either NaOH or H_2SO_4 to one of a series of pH values between pH 3 and 9, in gradations of 1 pH. The control of the initial pH was similar to that previously described (**2**), except that more frequent adjustments, three to six times daily, were resorted to.

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS

	NUMBER 1	NUMBER 2	NUMBER 3
	M.	*M.*	*M.*
KNO_3	0.005	0.005	0.005
$Ca(NO_3)_2$	0.0015	....	0.0005
$MgSO_4$	0.001	0.001	0.0005
$NH_4H_2PO_4$	0.001	....	....
KH_2PO_4	....	0.00006	0.00006

Na_2SO_4 was added to all solutions below pH 9 in amounts calculated to give the same concentration of sodium in all tanks. The sulphate concentration in the several tanks was allowed to vary, as this was the ion selected for the maintenance of the electro-ionic balance.

The concentrations of calcium, magnesium, potassium, phosphate and nitrate ions were analytically determined on a sample of the nutrient solution no. 3 (table I) at the beginning of the experiment and at the end of each 24-hour period. Potassium was determined by the volumetric cobaltinitrite method of HIBBARD and STOUT (**4**); magnesium, by the 8-hydroxy quinoline method described by SNELL and SNELL (**7**); phosphate, by a modification of the DENIGES method (**3**); nitrate, colorimetrically by the phenoldisulphonic acid method (**3**); and calcium by the standard oxalate-permanganate titration (**3**). The amounts absorbed were computed by difference and expressed in terms of milliequivalents or milligram-ions absorbed by a single plant. Positive values indicate a net absorption and negative values a loss from the plant to the nutrient solution.

Throughout the absorption period of 96 hours the same number of plants was used with 40 liters of nutrient solution for each pH value: 11 for Bermuda grass, 10 for lettuce, and 6 for tomato. The low phosphate concentration in the nutrient solution during the period of absorption, made it necessary to analyze from time to time for phosphate and to replenish it to its original concentration as needed.

All nutrient solutions were continuously aerated by means of porous carbon tubes (**2**).

Results

Within a short time—one hour or less—after the tomato and lettuce plants had been placed in a nutrient solution at pH 3, a distinct injury to the plants was invariably observed. The roots appeared damaged by showing a dull greyish color and a perceptible loss of turgidity. On clear warm days these symptoms of the roots were associated with prompt wilting of the shoots of the tomato and lettuce plants. Recovery from wilting usually occurred during the first night but the plants wilted again in the morning. Unlike the tomato and lettuce, the injury at pH 3 to the roots of Bermuda grass was relatively slight, and no wilting of the shoots was generally observed.

On the other extreme of the pH range, at pH 9, no visible damage to the plants was noted. The relation between the external hydrogen ion concentration and absorption will be discussed presently but so far as visible injury over short periods of time is concerned it was found to be confined to plants immersed in a nutrient solution at pH 3.

Figure 1 gives the results at the end of 96 hours, of absorption tests on tomato, lettuce, and Bermuda grass. Since an absorption period of 96 hours was conceivably long enough to obscure short time adjustments of plants to an unfavorable external reaction, additional time-absorption data at pH 3 are presented in table II.

An inspection of figure 1 and table II discloses at once the peculiar nature of absorption at pH 3. Common to all three plants at this hydrogen ion concentration is either a total failure or a great reduction in the absorption of calcium and phosphate. In the case of lettuce there is an actual loss of these nutrients from the plant to the nutrient medium. A similar loss was found for the tomato in the early periods of absorption (table II). Owing to the fact however, that these plants were grown for several weeks prior to the absorption experiment in a complete nutrient medium and hence could conceivably have had some calcium phosphate precipitated on the root surfaces, there is some doubt, particularly if the amounts involved are small, whether this "negative absorption" should be attributed to a withdrawal of calcium and phosphate from the roots or to a dissolving of the surface precipitate. Yet the prompt and profound injury of the lettuce and tomato roots observed at pH 3 render the former interpretation probable.

The injury to the roots of Bermuda grass at pH 3 in the absorption experiment was relatively slight, and no loss, in fact a gain, of phosphate

TABLE II

ABSORPTION AT pH 3.
MILLIEQUIVALENTS PER PLANT ABSORBED AT THE END OF EACH PERIOD

		Hours			
		24	48	72	96
Ca	Tomato	−0.33	0	0	0
	Lettuce	−0.28	−1.14	−0.85	−0.28
	Bermuda grass	0.20	0.20	0.20	....
PO_4*	Tomato	−0.08	−0.01	−0.01	0.03
	Lettuce	−0.03	−0.05	−0.08	−0.10
	Bermuda grass	0.02	0.06	0.18	0.29
Mg	Tomato	−0.38	1.15	0.82	0
	Lettuce	−0.19	0	0.42	0.33
	Bermuda grass	0.16	0.30	0.50	0.86
K	Tomato	0.17	1.35	−0.17	−0.51
	Lettuce	−1.31	1.75	−0.44	−0.43
	Bermuda grass	−0.41	0.92	2.25	3.38
NO_3	Tomato	2.87	2.77	5.23	4.70
	Lettuce	−1.38	−1.19	−1.29	1.01
	Bermuda grass	0.71	4.52	7.87	10.70

* Computations were made on the assumption that phosphate was absorbed as $H_2PO_4^-$.

was noted (table II). But the marked restriction in phosphate absorption at pH 3, in comparison with absorption at higher pH values, was apparent (fig. 2). With respect to calcium, however, Bermuda grass behaved more like tomato and lettuce: no further absorption occurred after the first 24 hours. In fact a loss of calcium at the end of 96 hours is indicated. It will be recalled in this connection that as with the other plants no growth of Bermuda grass took place at pH 3 (**2**). The conclusion then, that at pH 3 there is serious interference with the absorption of calcium and phosphate, seems warranted by all the evidence at hand.

As far as the other ions at pH 3 are concerned, Bermuda grass absorbed appreciable amounts of magnesium, potassium and nitrate (fig. 1, table II). The one negative value for the absorption of potassium by Bermuda grass at the end of the first 24 hours (table II), is followed by increasing absorp-

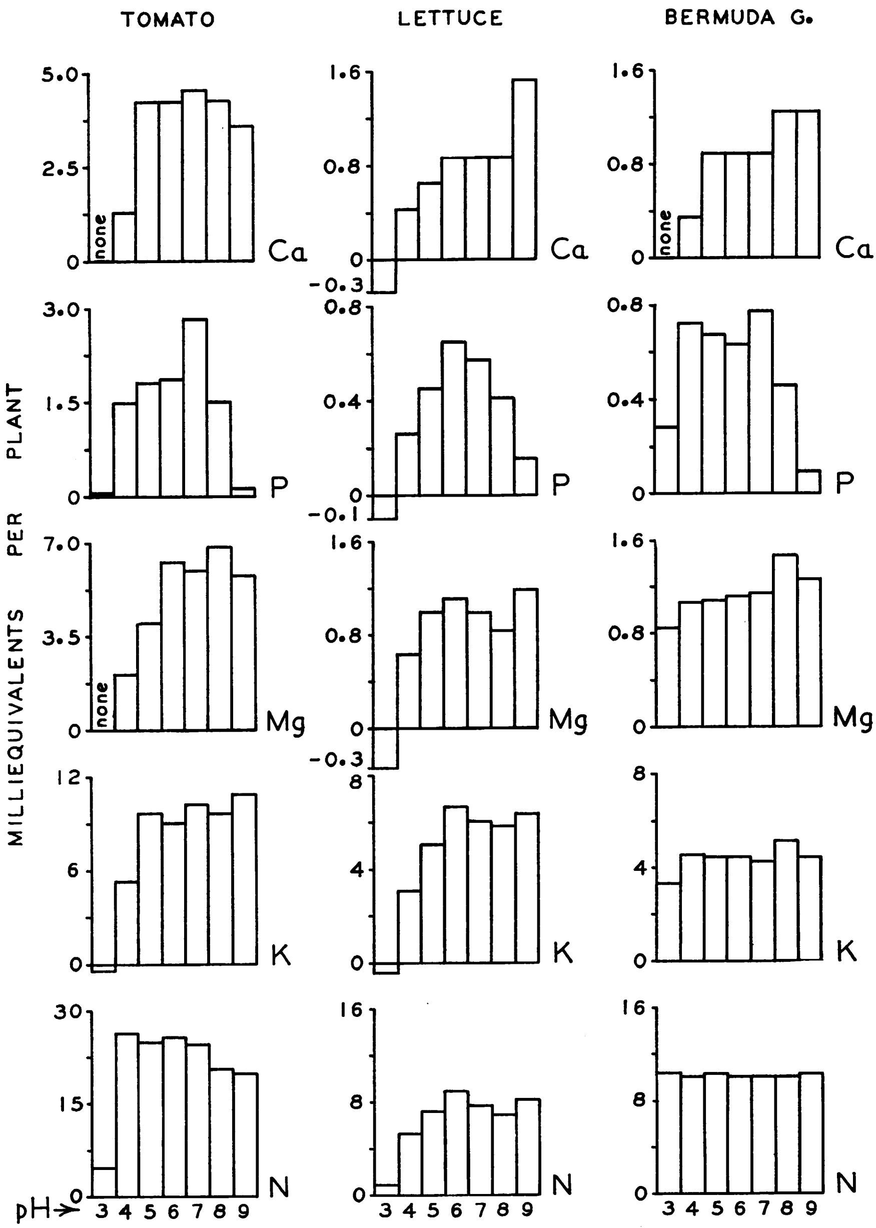

FIG. 1. Effect of pH on absorption of inorganic nutrients at the end of 96 hours by tomato, lettuce, and Bermuda grass. Phosphorus was assumed to have been absorbed as $H_2PO_4^-$.

tion at the end of each successive 24-hour period. Tomato and lettuce, however, exhibited at pH 3 no gain in magnesium and potassium at the end of 96 hours, despite indications of intermittent absorption. As for nitrate, a distinction can be drawn between tomato and lettuce. While the tomato plants absorbed measurable amounts of nitrate, though small by comparison with absorption at other pH values, the lettuce plants lost nitrate in the first 24 hours and showed a small gain only in the last 24 hours (table II).

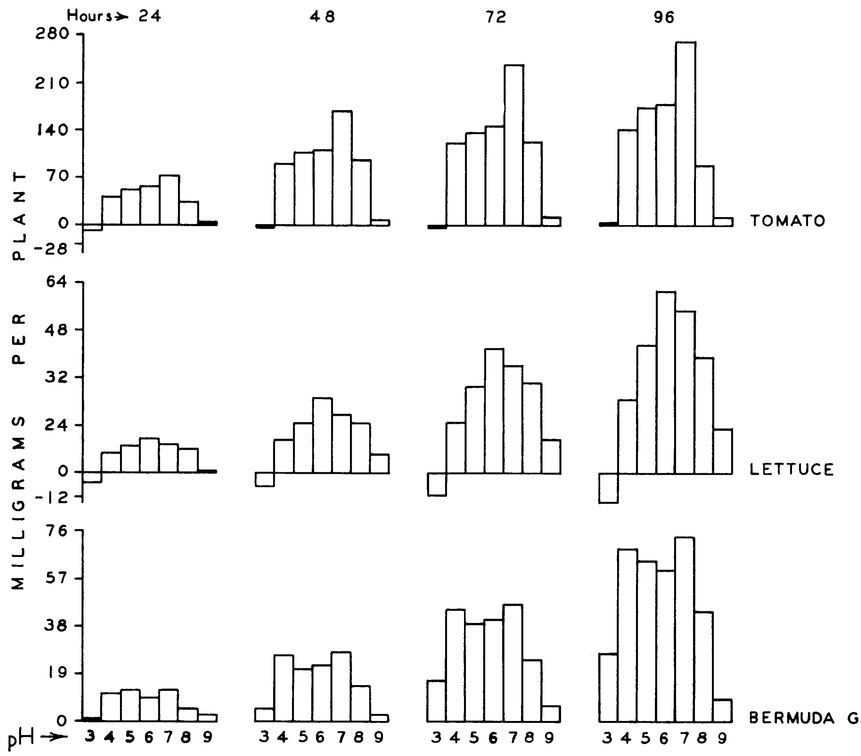

FIG. 2. Influence of pH of nutrient solution and time on the absorption of phosphate by tomato, lettuce, and Bermuda grass.

At the other extreme of the pH scale, at pH 9, the deleterious effect of external reaction on absorption was confined to phosphate. As indicated by the detailed data presented in figure 2, the decrease in phosphate absorption which became apparent above pH 7, was unmistakably clear at pH 9 at the end of each 24-hour period. Unlike pH 3 there is no indication that pH 9 represented a reaction unfavorable for the absorption of calcium, nor of the other nutrients investigated: nitrate, potassium, or magnesium (fig. 1).

The absorption of calcium from nutrient solutions maintained at pH values between 4 and 8 is of special interest. It was previously reported that at pH 4 and 5 increasing the concentration of calcium in the nutrient solution was conducive to increased growth of tomato and lettuce but that at pH 6 a higher calcium concentration in the nutrient medium was no longer associated with improved growth (**2**). The data given in table III

TABLE III

ABSORPTION OF Ca WITH TIME FROM ACID NUTRIENT SOLUTIONS. MILLIGRAMS PER PLANT ABSORBED AT THE END OF EACH PERIOD

	EXTERNAL pH	HOURS			
		24	48	72	96
		mg.	*mg.*	*mg.*	*mg.*
	3	-5.7	-22.8	-17.1	-5.7
Lettuce	4	0	-4.4	0	8.8
	5	0	-4.4	0	13.2
	6	4.4	4.4	13.2	17.6
	3	-6.6	0	0	0
Tomato	4	0	0	13.2	26.4
	5	13.2	33.0	59.4	85.6
	6	19.8	39.6	66.0	85.6

suggest that this effect of calcium on growth is related to the absorption of this element from acid nutrient solutions. Leaving aside the already discussed negative absorption at pH 3, relatively favorable absorption of calcium obtained only at pH 6, particularly for lettuce. At pH 4 and 5 a net absorption of calcium was indicated only at the end of 96 hours. At earlier intervals no calcium was absorbed. Although these relations were not as pronounced for tomato, yet for this plant as well as for lettuce, a decreasing acidity up to pH 6 was associated with an increasing absorption of calcium. A further reduction in hydrogen ion concentration, including pH 9, had no particular effect on calcium absorption.

A decreased absorption of calcium by Bermuda grass, similar to that of tomato and lettuce, was found only at pH 4 (fig. 1). At pH 5 and above no relation between the external hydrogen ion concentration and calcium absorption was indicated. Bermuda grass grew very well at pH 4 and 5 and showed no response at these reactions to an increased calcium concentration in the nutrient solution.

The absorption of nitrate, potassium, and magnesium between pH 5 and 9 was unaffected in any significant degree. At pH 4, however, a difference again was noted between lettuce and tomato on the one hand and Bermuda grass on the other. While there was no indication of a decreased absorption of these three nutrients at pH 4 by Bermuda grass this generally occurred for lettuce and tomato (fig. 1). It will be recalled in this connection that the growth of lettuce and tomato, but not that of Bermuda grass, was distinctly reduced at pH 4 (**2**).

Discussion

The results of this investigation offer a point of departure in interpreting the previously reported (**2**) effects of hydrogen ion concentration on growth. Of the five ions studied, the absorption of calcium and phosphate at various hydrogen ion concentrations emerges as particularly relevant to the observed growth responses. At pH 3 all three plants—tomato, lettuce, and Bermuda grass—consistently showed a serious curtailment in phosphate absorption and a complete stoppage, if not an actual reversal, of calcium absorption. Regardless of the particular mechanism of root injury which eventuated in these effects, they can in themselves account for the total failure of growth observed at pH 3. Likewise, at the other extreme of the pH scale, at pH 9, the marked reduction in the absorption of phosphate by all plants, can serve as an explanation for the reduced growth at this reaction. It was observed incidentally that tomato plants grown continuously at pH 9 exhibited symptoms of distress not unlike those encountered in phosphorus deficient plants.

The importance of calcium absorption for certain plants when grown at acid reactions is also illustrated by the data presented in table III. The previously observed poor growth of lettuce and tomato at pH 4 and 5 in nutrient solutions low in calcium (**2**), seems to be related to the reduced absorption of this nutrient at relatively high hydrogen ion concentration. At pH 6 and above large absorption of calcium was common to all plants. Whether the maxima in calcium absorption found at the most alkaline reactions for lettuce and Bermuda grass, but not for tomato (fig. 1), have any significance, cannot be answered on the basis of the present data.

Of special interest is the relation of external reaction to the absorption of phosphate; two distinct minima in the absorption of this nutrient were found at pH 3 and at pH 9. Had it been possible to prevent the intermittent shifts downward of the external reaction at pH 9 and to maintain it within as narrow limits as that at pH 3, it seems certain that an even lower phosphate absorption at pH 9 than that actually found could be demonstrated. In any event the influence of external reaction on phosphate absorption at both pH 9 and 3, was unmistakable (fig. 2).

These findings are not entirely consistent with the views of McGeorge (**6**) that phosphate can be absorbed by plants only in the form of $H_2PO_4^-$. It is certainly true that at pH 9 it is impossible to have much $H_2PO_4^-$ in solution since the predominant ionic form of phosphate at this reaction is $HPO_4^=$. $HPO_4^=$, however, is also the predominant phosphate ion at pH 8, and even at pH 7 its concentration exceeds that of HPO_4^-, without causing a drastic reduction in absorption.

But since the external pH determines the ionic dissociation of phosphate there is no opportunity to subject to an experimental test the relative absorption of different ionic forms of phosphate at varying hydrogen ion concentrations. Evidence on the effect of calcium and magnesium concentration in relation to phosphate absorption at high alkalinity and a better understanding of the influence of hydroxyl ions on phosphate absorption, promise to be of interest in this connection.

A distinct contrast, particularly for lettuce and tomato, between nutrient absorption at pH 3 and pH 9, is apparent. Whereas, at pH 9 only the absorption of phosphate was hindered, at pH 3, in addition to the already discussed failure of calcium and phosphate absorption, that of magnesium, potassium, and nitrate was also adversely affected. The question arises as to what extent these latter effects are related to the lack of absorption, or even to an actual loss, of calcium by the plant at pH 3. The influence of calcium on the permeability of cytoplasmic membranes and the absorption and retention of ions (**8**) and the rôle of calcium pectate as a structural component of the cell wall, are pertinent to the question. It should be noted that Bermuda grass, which unlike the other plants showed no initial loss of calcium at pH 3 (table II), was also the plant capable of absorbing other nutrients at this reaction. It is of course probable that even Bermuda grass, in view of its failure to grow at pH 3 (**2**), would cease to absorb with longer exposure to this reaction.

Our results on the effect of pH of the nutrient solution in relation to the absorption of Mg, K and NO_3 are in harmony with the data of HOAGLAND and BROYER (**5**), who have investigated the absorption of potassium, nitrate, and halide within a pH range of around 4 to 8. Their conclusion that within this range of hydrogen ion concentration there is no profound effect of pH on the absorption of these ions is borne out. This concordance of results is noteworthy in view of the differences in experimental technique: excised barley roots used by HOAGLAND and BROYER in an absorption experiment which lasted 9 hours as compared here with entire tomato, lettuce, and Bermuda grass plants studied over a period of 96 hours. The relatively lower absorption at pH 4 in our experiments can be attributed to possible injury as a result of longer exposure. The bearing that evidence of this kind has on suggested theories of absorption has been dealt with extensively by the previous authors.

Summary

As a part of a general investigation of the effects of hydrogen ion concentration on growth of higher plants, experiments were made on the relation of external pH to the absorption of calcium, magnesium, potassium, nitrate, and phosphate.

Five-week-old tomato, lettuce and Bermuda grass plants previously grown under favorable conditions, were placed for 96 hours in a series of nutrient solutions ranging, in gradations of 1 pH unit, from pH 3 to pH 9.

Injury to roots from the reaction of the medium was apparent only at pH 3. At this reaction, no absorption, in fact suggestions of loss, of calcium and phosphate, were in evidence. For tomato and lettuce this reaction was also wholly unsuited for the absorption of other ions.

With the exception of phosphate absorption which showed a marked decrease at pH 9, no other untoward effects were found at this reaction.

Evidence was obtained of lower calcium absorption, particularly by tomato and lettuce, from strongly acid solutions (pH 4 and 5) than at higher pH values.

No profound effects of external reaction between pH 4 and 9 on the absorption of magnesium, potassium, and nitrate were noted.

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