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## HYDROGEN-ION CONCENTRATION GRADIENT IN PLANTS

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In connection with some work on the hydrogen-ion concentration and immunity of certain bean varieties to bacterial blight, the writer noticed that there was a difference in the  $H^+$  concentration of the younger and older portions of the plants. This difference in itself was very interesting, and when viewed from the standpoint of the metabolism of the plant it might be considered of considerable importance.

As far as the writer is aware there has been only one reference made to a similar fact. Haas<sup>1</sup> states that the upper five inches of a blue-grass plant in the pollinating stage showed pH 6.11, while the lower 15 inches showed pH 5.92. The upper part of the stem of a sweet clover plant was found to be at pH 6.68, and that of the lower at pH 6.46. The data given by Haas are very much like those found by the writer in the bean plants.

Bush beans were unsatisfactory for any detailed investigation of this matter, and field corn was consequently used for further studies. This is an ideal plant for experiments of this type, because of its long single axis with leaves quite far apart and in a definite series, making it easy to determine the comparative age of the leaves.

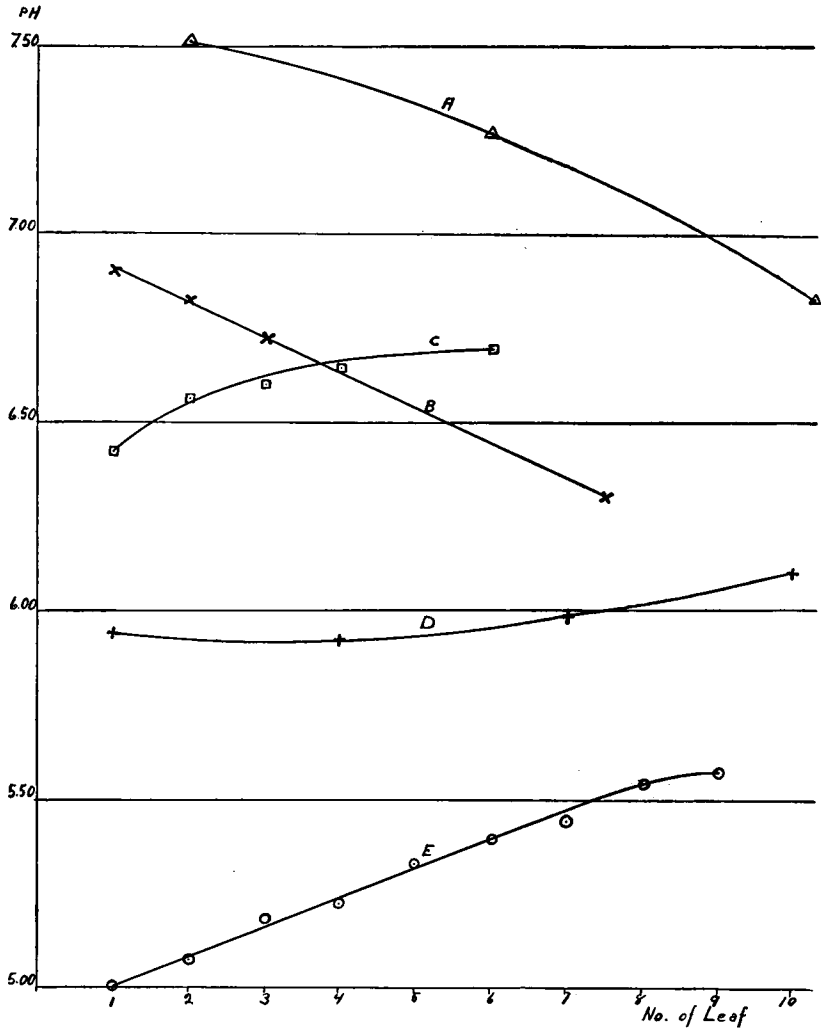
For the first experiments the plants were grown out of doors. When used the plants were a month and a half to two months old. At that time the plants were approximately five feet tall, having on an average 10 leaves. Most of them were in tassel and beginning to silk, though some older plants with nearly mature seeds were also used.

The first experiments were carried out with leaves. Usually about 12 plants were chosen as nearly alike as possible. The determinations began with the oldest leaves and progressed to the youngest, though the reverse order was also tried without any difference in the results. The leaf from the first node was taken from each plant. These oldest leaves were ground coarsely in a food-chopper and the juice was expressed by a hand press, using approximately the same pressure each time. The  $H^+$  concentration

<sup>1</sup> Haas, A. R. C. Studies on the reaction of plant juices. *Soil Sci.* 9: 341-369. 1920.

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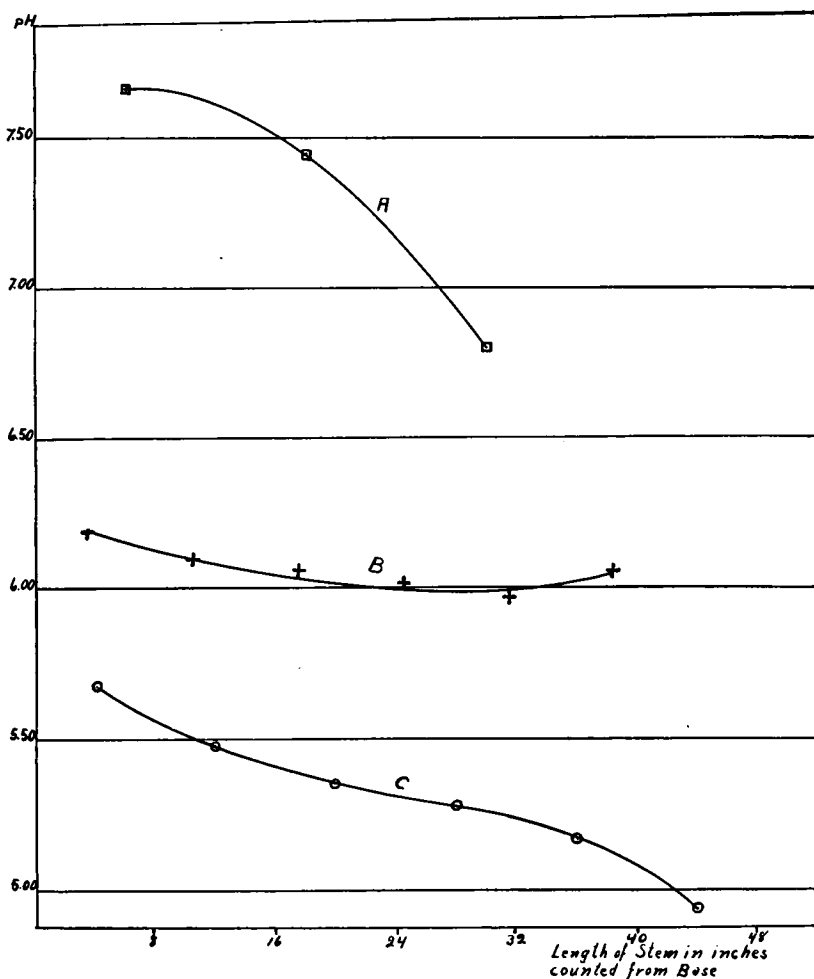
was determined at once as described below. Another set of leaves was being prepared in the same way, while the  $H^+$  determination was being made on the preceding lot.



TEXT FIG. 1. Curves showing the  $H^+$  concentration gradient in leaves. *A* represents pumpkin, *B* sunflower, *C* squash, *D* pole bean, and *E* corn.

The  $H^+$  concentration was determined by means of a potentiometer, with an open-type electrode. A closed Clark electrode was used in preliminary experiments, but as no difference in results was noticed it was abandoned on account of its greater complexity. About 10 cc. of juice was used for each determination. The readings were taken 5 minutes after the

electrode was placed in the solution; during this time hydrogen was continually bubbling over the electrode. It had been previously found that this was sufficient time for the electrode to come into equilibrium with the juice. Before and after an experiment the electrode was checked up against a standard buffer solution to see that it was in proper working order.



TEXT FIG. 2. Curves showing the  $H^+$  concentration gradient in stems. A represents pumpkin, B sunflower, and C corn.

From the work on the leaves of corn it was found that, as one proceeds from the lowermost leaves to the uppermost, there is a decrease in  $H^+$  concentration, *i.e.*, an increase in the pH value. It was found that the pH increased with every leaf from base to tip, though not always by the same increment. The lowermost limit was about pH 5.00 while the upper limit was about pH 5.60.

The stems were cut into 20-cm. pieces, usually 6 in all. Four or five stems gave sufficient juice to make the determinations. The lowermost portion was determined first and the others were determined in sequence. The stems gave rather unexpected results. The oldest parts of the stems, unlike the oldest leaves which were attached to those portions of the stem, were found to have a higher pH value than the younger stem portions. In other words, the oldest part of the stem and the youngest leaves had about the same  $H^+$  concentration, while the youngest part of the stem and the oldest leaves were approximately alike. It is rather surprising to find this divergence in parts so intimately connected as a leaf and the stem to which it is attached.

These experiments on corn have been repeated a number of times, with plants grown out of doors in the summer and plants grown in the greenhouse in the winter. There has been only one exception to the above-cited results. That was after several days of heavy rain, when the ground in which the plants were growing became very soggy. For two days in succession experiments were run on the stems, and in all experiments there was no general gradient. The leaves behaved in the usual fashion throughout.

When plants mature the gradient still persists, but there is very much less difference between leaves at two adjacent nodes, especially in the older portions of the plant, and the curve flattens out.

To find whether a gradient in the  $H^+$  concentration was present in other plants than corn, experiments have also been made with squash, pumpkin, pole bean, and sunflower plants. The leaves of squash and pole beans behaved essentially like those of corn, *i.e.*, the older leaves had a higher  $H^+$  concentration than the younger leaves. In the case of the squash the juice was very near the neutral point. The highest that was noticed for squash was pH 6.40, as compared with pH 5.00 for corn. The pole bean is intermediate, at about pH 6.00.

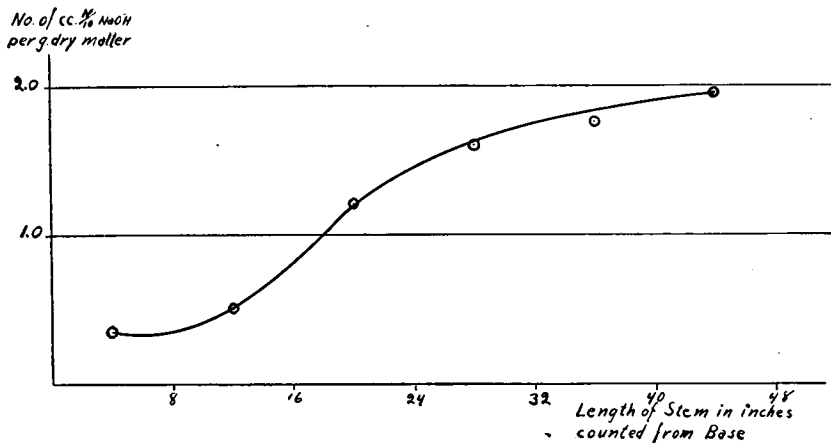
Pumpkin and sunflower leaves gave results the opposite of those above mentioned. In both cases the older leaves had a lower  $H^+$  concentration than the younger. The sunflower leaves ranged from pH 6.90 to pH 6.30. The older leaves of pumpkin are the only leaves so far found that had a juice on the alkaline side of neutrality. In the one experiment that has so far been made the range was from pH 7.50 to pH 6.80.

The stems of the pumpkin and of the sunflower are the only stems that have been tested besides those of corn. In both these the gradient of the stem agrees with their leaves, and also with the corn stem which is the reverse of its leaves.

To what is this difference in  $H^+$  concentration due? It is not probable that it is due to unequal amounts of  $CO_2$  in these parts, because hydrogen was allowed to bubble through the plant juice until there was no further change, indicating that  $CO_2$  equilibrium had been attained. It can not be due to different degrees of dilution of the substances in the cell, as the older

portions always have more dilute juice in them than do the younger and still immature parts, yet these older parts sometimes have a higher  $H^+$  concentration.

The total amount of water-soluble acids has been determined in dried corn stems. The results agree fairly closely with the  $H^+$  concentration of the stems. The lower parts of the stem had very much less total acid than did the upper parts of the stem. The two curves are not identical, and undoubtedly part of the difference is due to some other factor.



TEXT FIG. 3. Curve showing the total water-soluble acidity of dried corn stems at different levels in the stem.

Whether the unequal amount of acid-reacting substances is due to storage, to unequal metabolism, to unequal absorption of cations, or to some other factors is at present impossible to state. When we consider the case of corn it seems unlikely that any one factor would operate alone.

The disappearance of the gradient in the stems and not in the leaves of corn after the heavy rain is probably due to a dilution of the juice with consequent change in the  $H^+$  concentration. As has been shown by Bauer and Haas,<sup>2</sup> and also by Hurd,<sup>3</sup> the stem has a very much lower buffer concentration than the leaves. The excessive amount of water in the soil, the humid conditions following a rain in warm weather, and the cloudy conditions caused the stems to contain more water than usual with the consequent dilution of the weak buffer solution and the upsetting of the  $H^+$  gradient.

<sup>2</sup> Bauer, F. C., and Haas, A. R. C. The effect of lime, leaching, form of phosphate and nitrogen salt on plant and soil acidity, and the relation of these to the feeding power of the plant. *Soil Sci.* 13 : 461-477. 1922.

<sup>3</sup> From paper given at the Boston meeting of the A. A. A. S. Cited with Miss Hurd's permission.

## SUMMARY

The data submitted show that there is a  $H^+$  concentration gradient in corn, squash, pole bean, pumpkin, and sunflower plants.

This gradient is not always in the same direction in different species, or in the leaves and stems of the same species (*e.g.*, corn). In corn, squash, and pole bean the older leaves had a higher  $H^+$  concentration than the younger leaves, while in pumpkin and sunflower the reverse was the case.

The bases of the stems of corn, sunflower, and pumpkin had a lower  $H^+$  concentration than the tops.

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