HYDROGEN-ION CONCENTRATION AND THE INITIATION OF GROWTH

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Experiments have been carried out in the past on the effect of hydrogen-ion concentration on the initiation of growth, using the spores of many fungi and the pollen grains of a few angiosperms. Likewise, work has been reported, using fungus spores, potato tuber tissue, fungus mycelium, root tips, and succulent cactus tissue, which indicates that the protoplasm of plant tissue behaves as though it were a protein with a definite isoelectric point. This behavior has been determined on the basis of water absorption, the behavior of the tissue in relation to staining with acid and basic dyes, and by the effect of the tissue upon the reaction of solutions in which it is immersed. The literature dealing with these points will be considered briefly in connection with the discussion of the results of the present experiments.

The experiments reported in this paper are concerned with the effect of hydrogen-ion concentration upon the germination of the spores of *Rhizopus suinus* Niels. and upon the sprouting of the tubers of the potato, *Solanum tuberosum* L.

EXPERIMENTAL

I. The influence of hydrogen-ion concentration upon the germination of the spores of *Rhizopus suinus* Niels.

The buffer solutions used for the germination tests were made up after the method of Webb (14). Phosphoric acid and potassium hydroxide were made up in m/5 concentrations, both solutions containing mannitol in a concentration of m/5. The two solutions were combined in various proportions to give a wide range of hydrogen-ion concentrations and were sterilized in the autoclave for 15 minutes at 15 pounds pressure.

The spore suspensions were prepared after the method of Tilford (11). Thirteen different buffer solutions, each with a volume of 15 cubic centimeters, were used to remove the spores from an equal number of agar slant cultures of the fungus. The hydrogen-ion concentrations of the solutions were redetermined after the spores were taken up in order to be sure that there was no change in pH as a result of absorption of materials from the agar slants. The values given in the tables for the hydrogen-ion concentrations of the different solutions are these redetermined values.

TABLE I

THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE GERMINATION OF THE SPORES OF *Rhizopus suinus*

Tube Number	pH	Percentage of Spore Germination
1	1.2	0.0
2	2.2	22.3
3	3.3	36.6
4	4.0	67.6
5	5.0	17.6
6	5.7	90.0
7	6.2	80.0
8	7.0	50.0
9	7.6	27.8
10	8.0	16.5
11	8.8	12.6
12	9.3	5.8
13	10.0	0.0

TABLE II

THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE GERMINATION OF THE SPORES OF *Rhizopus suinus*. SECOND SERIES.

Tube Number	pH	Percentage of Spore Germination
1	1.1	0.0
2	2.4	21.0
3	3.1	30.0
4	4.2	61.1
5	4.6	43.0
6	5.6	94.8
7	6.5	58.1
8	7.2	31.0
9	7.8	23.1
10	8.6	11.0
11	9.0	5.3
12	10.0	0.0

Hanging drop cultures were made from each tube. Evaporation from the drop was prevented by placing a small quantity of the non-inoculated buffer of the same pH in the bottom of the cell and by sealing the cover glass to the cell with vaseline.

After twenty-four hours the spore germination counts were made. Five fields were counted from each cell, and three cells were used for each pH value. In all, a total of about five hundred spores at each pH were counted, and the percentage of germination was calculated. The production of germ tubes was taken as the sole criterion of germination, those spores which merely showed swelling being disregarded. The results of the first series are contained in Table I.

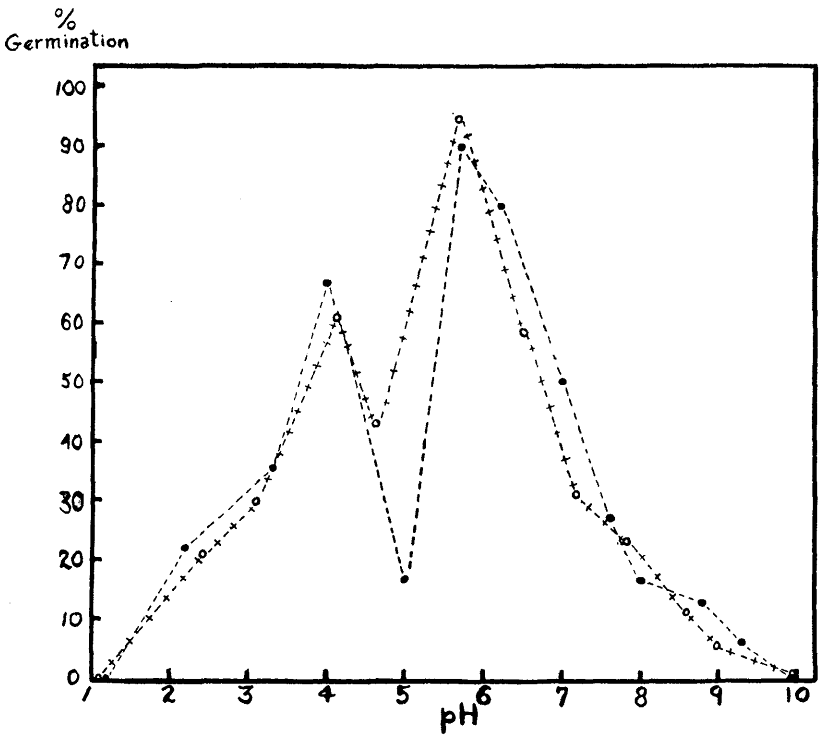

Figure 1. The effect of hydrogen-ion concentration upon the germination of the spores of *Rhizopus suinus*. Series one is represented by the broken line with closed circles; series two by the crossed line with open circles.

In a second series of experiments, which were carried out exactly as described above, except that the hydrogen-ion concentrations of the different solutions varied a little from the values used in the first series, very similar results were obtained. These results are given in Table II.

In figure 1 the percentage of spore germination is plotted against hydrogen-ion concentration expressed as pH. The results of both curves are used. Although the two curves are apparently somewhat different, it may be seen that it is possible to obtain from the two curves a single smoothed curve passing through the important points of both experimental curves.

II. The effect of hydrogen-ion concentration on the sprouting of tubers of *Solanum tuberosum* L.

The further influence of the concentration of hydrogen-ions on the initiation of growth was demonstrated through the use of potato tubers. The experiments were carried out in the late spring, in order to eliminate from consideration the factor of dormancy.

The buffers used in these experiments were prepared from an m/5 solution of monobasic potassium phosphate and an m/10 solution of citric acid. These solutions were mixed in varying proportions to give a series of fifteen buffer mixtures, ranging in hydrogen-ion concentration from that equal to pH 2.0 to that equal to pH 9.3.

TABLE III

THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE GERMINATION OF POTATO TUBERS

Container Number	pH	Average Sprout Length in mm.	
		Two Weeks	Three Weeks
1	2.0	0.0	0.0
2	2.3	0.0	0.0
3	2.5	0.0	0.0
4	3.0	0.0	0.0
5	3.5	0.0	0.0
6	4.0	0.0	0.0
7	4.5	13.0	17.0
8	5.0	8.0	12.0
9	5.5	8.0	24.0
10	6.1	7.0	25.0
11	6.5	9.0	15.0
12	7.0	4.0	10.0
13	7.9	9.0	13.0
14	8.5	10.5	24.0
15	9.3	8.0	14.0

Potato tubers of the Wisconsin Rural variety were cut in half, sterilized in a 0.1 per cent solution of bichloride of mercury, rinsed, and three half-tubers were immersed in each of the buffer solutions. After the twenty-four hour immersion period was completed, the tubers were removed from the buffers, resterilized in the bichloride solution, and air dried for 48 hours to promote suberization of the cut surfaces and thus to prevent rotting. These half-tubers were then placed in moist sphagnum in containers fashioned from two large pot-saucers. At the end of a two-week period, and again at the end of a three-week period, the sprouts which had developed were measured. Measurements of the apical sprouts were not taken, as these in general tend to be somewhat erratic in their behavior. The results, expressed as an average of the sprout lengths of the three half-tubers used at each hydrogen-ion concentration are given in Table III and are shown in figure 2. A second series gave similar results.

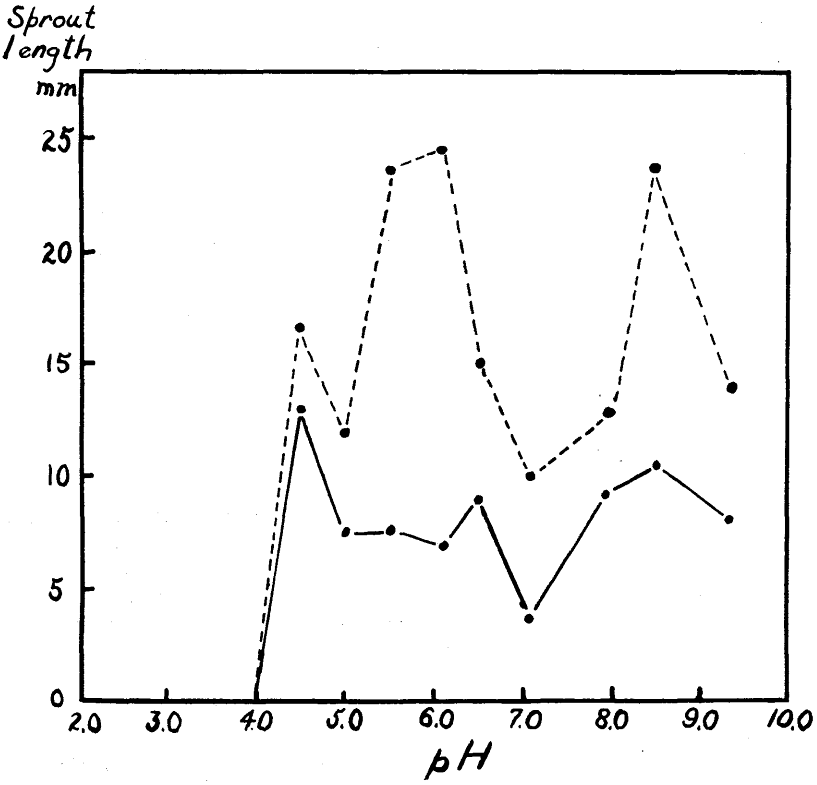

Figure 2. The effect of hydrogen-ion concentration on the sprouting of potato tubers. The solid line represents two weeks of growth; the broken line represents three weeks of growth.

DISCUSSION

There is established by the experimental work herein reported a curve for the germination of the spores of *Rhizopus suinus* as a function of hydrogen-ion concentration which is bimodal in nature. The two maxima come at about pH 4.0 and at about pH 5.7. The point at which minimum spore germination occurs lies between these, at about pH 5.0. This minimum point has been demonstrated by other authors. Robbins (5) showed by experiments on the absorption of acid and basic dyes that the mycelium of *Rhizopus nigricans* responded much like a protein with an isoelectric point between pH 4.8 and pH 5.1, while the mycelium of *Fusarium lycopersici* responded like a protein with an isoelectric point in the vicinity of pH 5.5. Robbins and Scott (7) determined, by means of the effect of the tissue on the reaction of solutions that the minimum point (isoelectric point) for *Fusarium oxysporum* was at pH 4.9–5.0 and that for *Gibberella saubinetii* was near pH 6.2. Scott (10) showed that *Fusarium lycopersici* exhibited a minimum in the spore germination curve at pH 5.2. Webb (13, 14) showed that there was a minimum in the spore germination curve of *Penicillium cyclopium* at pH 7.0, and a *Fusarium* sp. had a minimum point in the curve at pH 6.2. On the other hand, this same author found a minimum point in the curves for *Aspergillus niger*, *Botrytis cinerea*, *Lenzites saepiaria*, *Penicillium italicum* and *Puccinia graminis* only under certain temperature conditions. Webb was apparently the first to call attention to the phenomenon of the double maximum. Tilford (11) found that *Sclerotinia fructicola* gave a minimum of spore germination at pH 6.0, the two maxima being obtained at about pH 2.4 and about pH 6.6. Webb and Fellows (15) found a minimum in the growth curve for *Ophiobolus graminis* at pH 8.3, with maxima at pH 5.2 and pH 9.6. The criterion used for growth in this case was the diameter of colonies produced on corn meal agar. Scott (9) found for *Fusarium lycopersici* a minimum in the growth curve at pH 5.2, using as a criterion of growth the dry weight of mycelium produced by the fungus in 24 days. This minimum value is identical with that obtained (10) by spore germination tests.

The occurrence of the double maximum is not confined to fungus tissue. Pfeiffer (2) tried experiments on the effect of hydrogen-ion concentration on the germination of pollen grains. He found a minimum in the curve for the pollen of *Alopecurus pratensis* at pH 4.4, while for that of *Phaseolus multiflorus*, *Urtica dioica*, *Betula verrucosa*, and *Melandryum album* the minimum came at pH 5.2. Robbins (4) further showed that there was a minimum in the water absorption of potato tuber tissue at about pH 6.0. Robbins and Scott (7) showed an isoelectric point for soy bean root tips at pH 6.2–6.4. Ulehla (12) demonstrated that sections of the succulent tissues of *Opuntia* sp. behaved like an ampholyte with an equilibrium point in the neighborhood of pH 5.6. Salter and McIlvaine (8) found a minimum in the growth curve of wheat seedlings at pH 6.15. In the experimental work of this paper on the sprouting of potato tubers in relation to hydrogen-ion concentration, the minimum in the curve is found at pH 7.0, with the maxima at pH 6.3 and pH 8.5.

In all of these phenomena which are concerned with the initiation of growth, it is found that when the initiation of growth is considered as a function of the hydrogen-ion concentration of the medium surrounding the particular plant organ concerned, a bimodal curve is established. The exact location of the two maximum points and of the minimum point depends upon the nature of the organ concerned, the particular buffer system used, the nutrition of the growing parts, the method of determining the response to the various levels of hydrogen-ion concentrations, and finally, to the various environmental conditions under which the experiments are carried out. Of these, temperature is of special importance.

Growth, which in its initial stages at least involves merely an increase in size, is entirely dependent upon the uptake of water. Loeb (1) showed that the physical properties, such as viscosity, osmotic pressure, conductivity, and *total swelling* of such a protein as gelatin are at a minimum at the isoelectric point of pH 4.7. The point at which minimum swelling, and hence minimum growth, takes place in complicated materials such as plant tissues must be a resultant of the isoelectric points of the biocolloids of the protoplasm of the tissue. This conception is not new, having been advanced by Robbins (3, 6), Robbins and Scott (7), Scott (10), and Tilford (11).

From the experimental data reported in this paper, it is evident that the relative toxicity of the hydrogen- and hydroxyl-ion varies greatly with different tissues. In the case of the sprouting of the potato tubers, good growth took place from about pH 4.5 to pH 6.5 and from about pH 7.9 to pH 9.3, the upper limit of hydroxyl-ion concentration used. The minimum point in the curve (the isoelectric point) was determined to be at pH 7.0. On the other hand, with the spores of *Rhizopus suinus*, almost the entire curve lies below pH 7.0. Germination values above fifty per cent are obtained only between the narrow pH ranges of about pH 3.5 to pH 4.5 and about pH 5.5 to pH 7.0. The minimum point in the curve is located at pH 5.0, where the concentration of hydrogen-ions is one hundred times that at the minimum point for the potato sprouting.

SUMMARY

1. Data for the effect of hydrogen ion concentration on the initiation of growth are given for two different types of asexual reproductive units—mold spores and potato tubers.

2. For both types of organs investigated, a bimodal curve for the effect of hydrogen-ion concentration on the initiation of growth is obtained. For the spores of *Rhizopus suinus* the minimum in the curve is at about pH 5.0. For the sprouting of the potato tubers the minimum in the curve is at about pH 7.0.

3. Hydrogen-ions are relatively more toxic to potato tissue than to the spores of *Rhizopus suinus;* hydroxyl-ions are relatively more toxic to the spores of *Rhizopus suinus* than to the potato tissue.

4. The effect of the hydrogen-ion concentration on the initiation of growth is considered to be directly related to the effect of hydrogen-ion concentration upon the hydration of the biocolloids of the protoplasm of the plant tissues concerned.

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