



ORGANIC IRON AND HYDROGEN-ION CONCENTRATION AS ASSOCIATED FACTORS AFFECTING THE RATE OF REPRODUCTION OF LEMNA MAJOR

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Lemna major (*Spirodela polyrhiza*) was grown in Clark's nutrient solution (1) modified to use ferric citrate instead of ferric chloride as the source of iron. The concentrations of iron and the pH of the solutions were varied and the plants were grown in an apparatus designed by the author to give a light intensity of 300 candle power at the leaf surface and to maintain a constant temperature of 25°C. Solutions were changed every few days and the number of fronds counted and reduced each time. Both sterile and non-sterile cultures were used. The duration of the experiments were usually from fifteen to twenty days; the rate of reproduction of Lemna was such that good results were obtained in this length of time.

PRELIMINARY EXPERIMENTS

Some of the reactions of iron citrate in pure aqueous and culture solutions were studied. Since different ways of preparing this salt do not yield the same product, stock materials were always carefully analyzed. Pure aqueous solutions of ferric citrate when autoclaved 20 minutes at 15 pounds pressure, or when exposed to a light of 400 candle power intensity for several days, darkened but did not form a precipitate. However, when various concentrations of this salt were added to Clark's culture solution and exposed for five days to light, or autoclaved, almost complete precipitation of the iron was found when tested with sulfocyanate. Analyses showed that the amount of soluble iron after these treatments was uniformly 1 p.p.m., although the original concentrations of iron varied from 16 to 80 p.p.m. and the original pH values varied from 4.8 to 8.8 by 0.5 pH intervals. A typical analysis of 125 cc, of culture solution, in which plants had been grown in the light for five days, showed only 0.07 milligrams of iron in solution. The precipitate contained 10.5 milligrams or 98 per cent of the total iron added, thus leaving 0.13 milligrams or 1.23 per cent to be accounted for by plant growth and experimental error.

Plants would not grow on the filtered solutions, nor would they grow well in solutions to which freshly precipitated ferric hydroxide or ferric phosphate had been added as the source of iron. That the plants grew well on the autoclaved solutions suggested their ability to utilize the low concentration of ferric ions. Apparently the shift of equilibrium thus produced between precipitated or colloidal iron and ionic iron was rapid enough to keep the plants supplied. Another possibility in non-sterilized media was that during the stages of reduction and oxidation, preceding precipitation of the iron, the plant stored enough to last through subsequent periods of growth.

REPRODUCTION RATE OF LEMNA AS AFFECTED BY CHANGES IN IRON AND HYDROGEN-ION CONCENTRATION

To expedite the work of adjusting the pH of a large number of solutions every two or three days the buffering power of ferric citrate in Clark's nutrient solution was determined and plotted as shown in Figure 1. The necessary amount of 0.005 N KOH to adjust the solution to the desired pH was determined by inspection for each concentration of iron used. These values were frequently rechecked against a quinhydrone electrode and found to be quite constant.

Studies were made to determine whether or not the solutions remained at the original pH during the subsequent growth of plants and the precipitation of iron in the light. Some of the results of this study are shown in Figure 2. The plants were grown in a light of 300 candle power intensity for five days and the pH of the solution determined by using a quinhydrone electrode. The original pH values of the solutions are indicated.

In every case the change was toward the more alkaline side. The greatest increase was in the solution having the lowest original pH. Also the increase in pH decreased uniformly as the original pH was increased. The relationship of increases in pH to original iron concentrations show that the highest concentration of iron has the greatest effect on increase of pH after five days growth of plants. Also, the increase of pH decreased uniformly as the original concentrations of iron were decreased. These data showed that to maintain a constant pH during the growth of plants in Clark's culture solution (1) was impossible when using organic iron, unless the original pH was very close to neutrality. Hopkins (3) claimed to have maintained a constant pH in his culture solutions when using ferric citrate. This may be explained by the fact that the original pH of his solution was 7.2.

In these studies growth was used as synonymous with rate of reproduction since generally the size of the plants correlated so well with rate of reproduction that the measurements were made on the basis of the latter. As Lemna major reproduces by budding, the number of fronds were counted and the rate of reproduction K determined for the equation $\log_{10} N - \log_{10} N_0 = K(t - t_0)$. The plants were grown in 500 cc., cotton

plugged, Erlenmeyer flasks placed in an apparatus which maintained 25°C. in the culture solution and an intensity of 300 candle power at the surface of the plants. Both sterile and non sterile cultures with pH values varying from 4.8 to 8.8 by 0.5 pH intervals were used. The iron concentrations varied from 0.5 to 80 p.p.m., making in all 90 combinations which were tried. Some results of this experiment are shown in Figure 3. The values indicated for pH and iron concentration were those obtained for the freshly prepared solutions.

These data show that the rate of reproduction increased with increasing iron concentration up to 32 p.p.m. at all pH values studied. Growth rate in solutions containing less than 8 p.p.m. original iron concentration are not shown above pH 7.3 as the plants were unable to maintain growth in the more alkaline media. The most important thing about these data (shown by the broken line) was that for every concentration of iron there was a definite pH for optimum growth and that this value was proportionately higher for each increase in the original iron concentration. Values which were optimum for reproduction rates were also optimum for size, depth of color and general healthfulness of plants.

Although autoclaving the culture solutions caused precipitation of most of the iron, the growth rate and general appearance of non-sterile

plants grown in these were similar to results obtained using non-auto-claved solutions. Thus it seemed to matter very little whether the iron precipitated before or during plant growth as far as response to original pH and iron concentration was concerned.

Data in Figure 3 suggests that higher concentrations of iron than 32 p.p.m. should have higher pH values for optimum growth. Data in Figure 4 show this was not the case. Comparisons of the growth rate of Lemna in solutions containing 32 p.p.m. of iron with similar values for 48, 64 and 80 p.p.m. of iron, show that there is very little increase in growth over 32 p.p.m. for pH values up to 7.8. Plants grown in solutions having a pH above 7.8 decreased in rate of reproduction and became small, chlorotic and lost roots. A concentration of 32 p.p.m. was best for growth

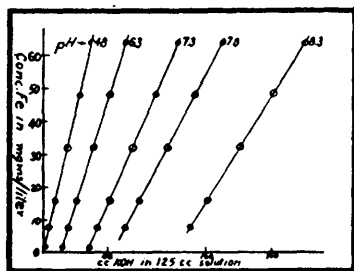


Fig. 1. Buffering power of ferric citrate

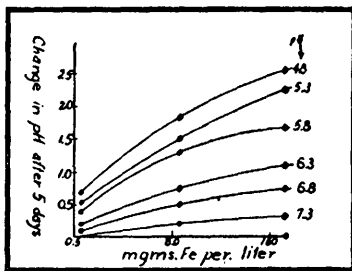


Fig. 2. Effect of plant growth on pH of nutrient solutions

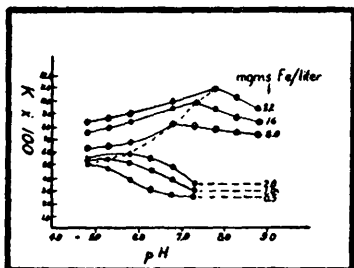


Fig. 3. Growth of Lemna in solutions containing organic iron

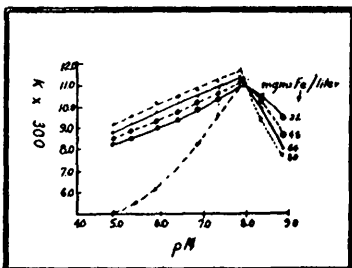


Fig. 4. (Same as fig. 3, except K x 300 is used)

above pH 7.8 but no plants grew as well as in more acid solutions. A pH of 7.8 appeared therefore to be optimum for the growth of Lemna when iron concentration was not a limiting factor. That higher concentrations of iron might produce increased growth at a pH of 7.8 may be inferred; however, this has not been investigated. The optimum pH values for all concentrations of iron, as shown in Figure 4 by the broken line, rise logarithmically from pH 4.8 to pH 7.8 after which further increases in pH are toxic. The rapid increase in growth rate of plants with increasing iron concentration of nutrient solution was noted by Hopkins (3) using solutions of pH 7.2. The data presented here showed that there was an optimum pH for each concentration of iron and that this value became proportionately larger as the concentration of iron increased.

The plants were healthy in appearance in all solutions having a hydrogen ion concentration equal to or less than the optimum. The generation time of Lemna was thus varied from 2.5 to 6.0 days without

any appreciable effect upon the general healthfulness of the plants. In the more acid solutions containing 48, 64 and 80 p.p.m. of iron, stunted, yellowish and brownish spotted plants were produced. Stunted chlorotic plants, which were unable to retain their roots, appeared as the alkalinity of the culture solutions was increased above the optimum for any given concentration of iron. Also, the degree of chlorosis increased with increasing alkalinity.

CONCLUSIONS AND SUMMARY

1. When the availability of iron was not the limiting factor, as when an organic source of this element was used, *Lemna major* made better growth in a neutral or slightly alkaline media.
2. The reproduction rate of *Lemna* may be stabilized at any desired rational figure, without appreciably affecting the appearance of the plant, by regulating the iron concentration and the pH of the nutrient solution.

LITERATURE CITED

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