THE GROWTH OF DUCKWEEDS IN MINERAL NUTRIENT SOLUTIONS WITH AND WITHOUT ORGANIC EXTRACTS.

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In recent years doubt has been thrown upon the assertion that green plants are able to obtain all their raw materials from the atmosphere and inorganic matter of the soil. Experiments that have been reported within the last 10 years suggest that possibly still another factor in nutrition, besides the carbon dioxide in the atmosphere and inorganic salts in the soil solution, is necessary for the complete growth of green plants. This factor is believed to be in the form of organic substances. Bottomley (2–4) believed minute amounts of organic matter to be essential to the continued growth of green plants and gave them the name “auximones.”

Bottomley grew *Lemna minor* (5–7) in Detmer’s mineral nutrient solution with and without extracts of a specially treated peat which he called “bacterized peat” (1, 3). From his results he concluded that *Lemna minor* cannot grow for any length of time in a pure mineral culture solution, and that the presence of soluble organic matter (auximones) was essential for complete growth.

Miss Mockeridge (13), working with Bottomley, studied the effect of extracts of leaf mold, soil, fresh and well rotted stable manure, and of bacterized peat on the growth of *Lemna major*, using Knop’s as the basal solution. The addition of the extracts caused an increase in number of plants produced, and in dry weight per plant, over the controls. The controls (Knop’s solution) decreased in dry weight per plant. Her results were in substantial agreement with Bottomley’s.

Mendiola (12), studying clonal variations in *Lemna minor*, stated that his plants would grow in a modified Pfeffer’s solution, and disagreed with Bottomley’s statement.

Duggar (10), in noting the effect of excision of cotyledons upon the growth of peas, believed that the cotyledons might contain a “vitamine requisite” for the vigorous development of the plant.
Robbins (14) grew excised root tips under sterile conditions, and found that the addition of small amounts of peptone or of autolyzed yeast to a Pfeffer's solution plus glucose considerably lengthened the period of growth of the tips. It is stated that the results would seem to be best explained by assuming that the seedling root contains some substance or substances derived from the grain which are not supplied by the mineral salts, glucose, free oxygen, and water.

Recently Clark and Roller (8, 9) have published results of experiments with *Lemna major*. They do not believe auximones are essential for the growth and reproduction of green plants.

**Methods.**

Since Bottomley and Mockeridge claimed that green plants require both mineral nutrients and organic accessory food for normal growth, it was decided first to attempt an experiment similar to the ones they performed. Their chief work was done with *Lemna major* and *Lemna minor*. Since these species were not available at the time it was decided to use another of the Lemnaceae, *Spirodela polyrhiza* (L.) Schleiden (*Lemna polyrhiza* L.).

In general the methods employed by Bottomley and Mockeridge in growing *Lemna* were followed in preparing the cultures of *Spirodela*. 225 cc. of the nutrient solution were placed in cleaned tumblers of about 250 cc. capacity. At first the tumblers were wrapped separately with black paper, to cut off illumination from the sides. This was an inconvenience during washing, so a box containing as many compartments as desired, usually fifteen, was constructed of black cardboard. The compartments were just large enough to hold the tumblers, and exactly as deep as their height. This arrangement is believed to be efficient in shading from light, and has the advantage of allowing the tumblers to be cleaned rapidly without removal and replacement of paper. In all experiments the plants were protected from falling dust by placing a sheet of glass about 3 cm. above the solutions. Ten plants were placed in each culture at the beginning of an experiment. The plants were washed in distilled water and transferred to fresh solutions at intervals ranging usually from 4 to 7 days, at which time the plants were counted. Whenever dead leaves were found their number was recorded. As the number of plants increased so that the cultures became crowded, either half the number, or else all except a certain number were removed. Such
discarded plants were used to obtain dry or green weights. The total number of plants that would have been produced, had none been discarded, was calculated. The cultures were kept in the laboratory, near windows exposed to the south or east. Since ordinary distilled water was found to be less favorable for growth, redistilled or "conductivity" water was used in preparing the nutrient solutions and extracts. This water was prepared by distilling from acidified potassium permanganate, then from alkaline potassium permanganate, using a Pyrex glass still. The water so prepared was nearly neutral in reaction.

The mineral nutrient solutions were prepared as follows:

<table>
<thead>
<tr>
<th>Delmer's Solution</th>
<th>Knop's Solution (Modified)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ \text{KNO}_2 ]</td>
<td>[ \text{KNO}_3 ]</td>
</tr>
<tr>
<td>[ 23.3 \text{ gm.} ]</td>
<td>[ 3.0 \text{ gm.} ]</td>
</tr>
<tr>
<td>[ \text{K}_2\text{HPO}_4 ]</td>
<td>[ \text{K}_2\text{PO}_4 ]</td>
</tr>
<tr>
<td>[ 5.0 \text{ gm.} ]</td>
<td>[ 3.0 \text{ gm.} ]</td>
</tr>
<tr>
<td>[ \text{MgSO}_4 \cdot 7\text{H}_2\text{O} ]</td>
<td>[ \text{MgSO}_4 \cdot 7\text{H}_2\text{O} ]</td>
</tr>
<tr>
<td>[ 5.0 \text{ gm.} ]</td>
<td>[ 3.0 \text{ gm.} ]</td>
</tr>
<tr>
<td>[ \text{NaCl} ]</td>
<td>[ \text{Ca(NO}_3)_2 \cdot 4\text{H}_2\text{O} ]</td>
</tr>
<tr>
<td>[ 5.0 \text{ gm.} ]</td>
<td>[ 9.0 \text{ gm.} ]</td>
</tr>
<tr>
<td>[ \text{CaSO}_4 \cdot 2\text{H}_2\text{O} ]</td>
<td>[ \text{FeCl}_3 ]</td>
</tr>
<tr>
<td>[ 16.7 \text{ gm.} ]</td>
<td>[ 0.72 \text{ gm.} ]</td>
</tr>
<tr>
<td>[ \text{FeCl}_3 ]</td>
<td>[ \text{Redistilled water} ]</td>
</tr>
<tr>
<td>[ \text{trace.} ]</td>
<td>[ \ldots \ldots 18 \text{ liters} ]</td>
</tr>
<tr>
<td>[ \text{Redistilled water} ]</td>
<td>[ \ldots \ldots 10 \text{ liters} ]</td>
</tr>
</tbody>
</table>

Knop's was prepared from three stock solutions, one containing the first three salts, the second the calcium nitrate, and the third the ferric chloride. The salts used were Merck's Blue Label, Baker's Analyzed, or salts of similar quality.

The organic extracts were prepared in the following manner. *NaHCO₃ Extract of Raw Peat, or Bottomley's "Nucleic Acid Derivative of Raw Peat"* (7).—A weighed quantity of raw peat was extracted with successive portions of a 1 per cent solution of NaHCO₃ until the extract was almost colorless. The combined extracts were neutralized with HCl, and a few drops of chloroform were added to prevent bacterial growth. Before use the chloroform was removed by heating over a water bath. The peat used was black and well decomposed.

*Water Extract of Autolyzed Yeast.*—5 gm. of autolyzed yeast (Fleischmann's) and 250 cc. of distilled water were heated to the boiling point, then filtered through the same filter paper until clear. The extract was preserved with chloroform.
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Spirodela polyrhiza Grown in Dilute Knop’s Solution and in Dilute Detmer’s Solution.

Experiment 1.—Preliminary experiments indicated that both Detmer’s and the modified Knop’s solutions were unfavorable to the growth of Spirodela polyrhiza. To determine what effect reducing the concentration of salts in these solutions might have, Knop’s solution was diluted to 5, 10, 50, and 150 times its volume with distilled water. Three cultures of ten plants each were set up for each dilution. The dilutions of Knop’s solution are hereafter abbreviated as follows: Knop/5, Knop/10, Knop/50, and Knop/150. The plants were transferred to fresh solutions about once a week, the experiment extending over a period of 33 days (July 7 to Aug. 9, 1922).

TABLE I.

Spirodela Growing in Varying Dilutions of Knop’s and Detmer’s Solutions.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Original No. of plants</th>
<th>No. of plants at end of experiment</th>
<th>No. of dead leaves</th>
<th>Dry weight of all plants (mg.)</th>
<th>Dry weight per 100 plants (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knop’s</td>
<td>30</td>
<td>90</td>
<td>263</td>
<td>9.2</td>
<td>10.2</td>
</tr>
<tr>
<td>Knop/5</td>
<td>30</td>
<td>95</td>
<td>123</td>
<td>45.0</td>
<td>47.4</td>
</tr>
<tr>
<td>Knop/10</td>
<td>30</td>
<td>98</td>
<td>111</td>
<td>53.9</td>
<td>56.0</td>
</tr>
<tr>
<td>Knop/50</td>
<td>30</td>
<td>105</td>
<td>189</td>
<td>28.2</td>
<td>26.8</td>
</tr>
<tr>
<td>Knop/150</td>
<td>30</td>
<td>69</td>
<td>220</td>
<td>8.9</td>
<td>12.9</td>
</tr>
<tr>
<td>Detmer’s</td>
<td>30</td>
<td>64</td>
<td>123</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Detmer/5</td>
<td>30</td>
<td>73</td>
<td>81</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Detmer/10</td>
<td>30</td>
<td>78</td>
<td>74</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Detmer/50</td>
<td>30</td>
<td>66</td>
<td>69</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Detmer/150</td>
<td>30</td>
<td>61</td>
<td>68</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

A similar experiment, using varying dilutions of Detmer’s solution, but extending over a period of only 16 days, was carried out at the same time. The data are given in Table I.

Results.—In the Knop’s solution series the greatest amount of growth, as measured by dry weight, occurred in Knop/10. The greatest number of individual plants occurred in the Knop/50, but the average size of these plants was below that of the plants growing in Knop/10.

The results for the Detmer’s solution series indicate again the favorable effect of dilution, since more plants were produced in the Detmer/10 than in any other of the dilutions.
In both sets of solutions it is evident that too great dilution is unfavorable, the conditions of the plants approaching that of plants grown in distilled water.

_Spirodela polyrhiza Grown in Dilute Knop's Solution and in Pond Water, with and without the Addition of Organic Extracts._

**Experiment 2.**—Although Experiment 1 described above had not been completed at the time this one was begun, the indications pointed to Knop’s solution diluted to 10 times its volume (Knop/10) as the most favorable one in the series for growth of _Spirodela_. For this reason a set of cultures was prepared to determine the effect of extracts of autolyzed yeast and of peat upon _Spirodela_ growing in Knop/10. For comparison a similar series was prepared, using the pond water of the plant’s natural habitat as the basal solution. The following cultures were therefore prepared after the usual manner.

Nos. 1, 2, 3. Knop/10 only.

Nos. 4, 5, 6. Knop/10, every liter of which contained 20 cc. distilled water having the soluble material of 0.4 gm. autolyzed yeast, obtained by boiling the autolyzed yeast with 50 times its weight of distilled water and filtering.

Nos. 7, 8, 9. Knop/10, every liter of which contained 66 cc. water having the soluble material of 2.5 gm. raw peat, obtained by extracting with 1 per cent NaHCO₃ solution, as outlined under Methods.

Nos. 10, 11, 12. Filtered pond water.

Nos. 13, 14, 15. Filtered pond water, containing a similar and like amount of autolyzed yeast extract as Nos. 4, 5, and 6.

Nos. 16, 17, 18. Filtered pond water, containing a similar and like amount of peat extract as Nos. 7, 8, and 9.
The condensed data are presented in Table II. Counts were made and the solutions were renewed after 4, 8, 12, 16, and 23 days, after which the dry weights were obtained. The period of the experiment was July 18 to Aug. 10, 1922.

Results.—The addition of small quantities of autolysed yeast extract and of peat extract to both dilute Knop's solution and pond water caused an increase in the number of plants of from 1.4 to 3.4 times.

The addition of these extracts increased the total dry weight of all the plants in both dilute Knop's solution and in pond water from 1.3 to 2.6 times.

The average dry weight per plant, however, was increased by the addition of these extracts only in the case of the dilute Knop's solution.

Growth of Spirodela polyrhiza and of Lemna valdiviana in Mineral Nutrient Solution without Organic Extracts.

Experiment 3.—On November 4, 1922, ten plants of Spirodela polyrhiza were placed in the dilute Knop's solution used in Experiment 2 (Knop/10). The plants available apparently had completed their season's growth. Vegetative growth seemed to have ceased, and dormant winter fronds were being produced in abundance. After 2 weeks in the nutrient solution all plants had begun to grow actively, and after 4 weeks the production of winter fronds had practically ceased.

The plants were kept in battery jar aquaria or in tumblers, shaded on the sides by black paper. They were transferred to new solutions at intervals usually of 1 or 2 weeks. As the plants increased in number the surplus was discarded. Data on weight of plants or percentage increases in number of plants were not obtained for the entire period of the experiment, but data collected over a period from Mar. 31 to Aug. 4, 1923, showed that the rate of increase in number of plants per week ranged from 9 per cent during the 1st week of April to over 100 per cent during June and July.

The plants of this same culture have now been kept in the dilute Knop's solution for a period of over 26 months (Jan. 4, 1925), without the addition of any organic matter except that which might have been introduced through the chemicals used, or through the dust particles from the air falling into the solutions.

On Mar. 31, 1923, ten plants of Lemna valdiviana Phillipi were placed in the dilute Knop's solution (Knop/10). These have been grown successfully in this

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1 By October vegetative reproduction in Spirodela practically ceases under conditions of the local natural habitat, and the plants then produce an abundance of small dormant winter fronds. These later become detached, sink to the bottom of the pond, rise again to the surface the following spring, and germinate.
solution for over 21 months (Jan., 1925) without any apparent reduction in size or rate of growth.

Results.—Even though no comparison has been made with the growth of similar plants in pond water, the experiment shows that the two species mentioned are able to grow and multiply normally over long periods of time in a mineral nutrient solution devoid of organic extracts.

DISCUSSION.

Since none of the species of Lemnaceae used in this investigation was the same as those used by Bottomley (5) or by Miss Mockeridge (13), strict comparison with their results cannot be made. But attention may be called to the cases where the results obtained with the nearly related species, *Spirodea polyrhiza* and *Lemna valdiviana*, agree or disagree with the conclusions of these authors.

The solutions employed in preliminary experiments, Detmer’s and a modified Knop’s, were unfavorable to the growth of *Spirodea polyrhiza*. Of the two, Detmer’s solution was the more toxic. When a small quantity of an extract of autolyzed yeast was added to Knop’s solution it was considerably improved, resulting in growth far in excess of the mineral solution alone, and also in excess of the growth in pond water obtained from the plant’s natural habitat. The question arises as to how the autolyzed yeast can improve an unfavorable mineral culture medium. According to Bottomley and Mockeridge the small quantities of organic matter furnished auximones, which supplied a deficiency in the mineral solution. However, as pointed out recently by Clark and Roller (9) the reason for poor growth may have been the unsuitability of the mineral solution. In order to produce a suitable solution they followed the general methods of Shive (15) and others (11). The author used a simpler method with success, that of diluting the unfavorable Knop’s solution with distilled water. Such a dilute Knop’s solution has supported growth of *Spirodea polyrhiza* for a period of 26 months, and of *Lemna valdiviana* for a period of over 21 months, without resulting in any permanent decrease in size of plants or in rate of reproduction. In fact the *Spirodea* plants at the end of 23 months appeared larger and more healthy in the mineral solution than similar
plants growing in the pond from which the original plants for the experiment were obtained. The reason for this improvement of Knop's solution by dilution is a subject under further investigation. The important point is the fact that a purely mineral solution can be prepared which will support growth and reproduction of species of Lemnaceae over a long period of time. The necessity of organic accessory foods or auximones for the continued growth of green plants, Lemnaceae at least, cannot be accepted as an established fact.²

The question now arises as to the manner in which small quantities of autolyzed yeast extract improved the unfavorable Knop's solution. As pointed out by Robbins (14), who used a similar extract in prolonging the growth of excised root tips, the autolyzed yeast may supply substances which balance the solution. It is known that yeast cells often have a high ash content (Fleischmann's 8.74 per cent of dry weight), and it is possible that the benefit derived from the addition of the yeast extract may be due to the balancing effect of some substance or substances which it contains.³ The view might also be taken that the yeast furnishes an additional food supply which is not necessarily required by the plant but which, when added, results in a stimulation of growth and reproduction. This view is held by Clark (8), who suggests that the auximones may be of the same nature as bios in the reproduction of yeast. However, the benefit derived from the addition of extracts of organic matter to unfavorable mineral solutions may be due to another cause or to a combination of other factors.

Experiments 1 and 2, and especially Experiment 3, seem to show that the dilute Knop's solution alone is a favorable culture medium for the growth of Spirodela and Lemna. However, Experiment 2 shows that the growth of Spirodela in this favorable solution can also

² The above conclusion is open to this criticism: auximones might have been added to the mineral solution as a contamination of the chemicals used, they might have entered as particles of dust from the atmosphere, or they might have been supplied by the bacteria always present in such cultures. The frequent transfer of the plants to fresh solution, however, prevented excessive bacterial contamination.

³ A later experiment has shown that the water-soluble material of 0.4 gm. autolyzed yeast plus a liter of distilled water cannot support growth of Spirodela for much more than 4 weeks.
be stimulated by the addition of yeast or of peat extracts, although not to such an extent as in the more concentrated solutions. Whether a purely mineral solution can be prepared which is not improved by the addition of extracts of organic matter cannot as yet be definitely stated. A number of preliminary experiments seem to indicate that at least one species of Lemnaceae is retarded in growth by the addition of a yeast extract, and also that the stimulating effect of a peat extract on *Spirodea* is diminished after a number of months, resulting in an actual retardation of growth as compared with controls in a mineral solution.

**SUMMARY.**

1. Detmer's solution and a modified Knop's solution are unfavorable culture media for the growth of *Spirodea polyrhiza*.

2. When the modified Knop's solution was diluted to 10 times its volume, *Spirodea polyrhiza* and *Lemna valdiviana* grew and reproduced for periods of 26 months and 21 months, respectively.

3. Growth in the dilute Knop's solution, which alone can support the growth of *Spirodea* indefinitely, was considerably stimulated over a period of 23 days by adding to every liter the water-soluble material of 0.4 gm. autolyzed yeast, or the material of 2.5 gm. peat soluble in a 1 per cent solution of NaHCO₃.

4. The nature of the stimulus or of the protection afforded by the organic material is as yet unknown.

5. The necessity of organic accessory foods (auximones) in the nutrition of green plants cannot be accepted as an established fact.

The experimental work reported here was done in part in the Department of Botany of the University of Missouri, Columbia, Mo., and in part in the Department of Biology of Junior College of Kansas City, Kansas City, Mo.

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