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ONO, HAYASHI
Biological Laboratory, General Education Department, Kyushu University

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THE EFFECT OF GROWTH SUBSTANCES AND SOME PHYSIOLOGICAL FACTORS ON THE GROWTH OF ROOTS OF LEMNACEOUS PLANTS

HAYASHI ONO

(Biological Laboratory, General Education Department, Kyushu University)

1. Preface.

Many reports relating to the physiological studies of Lemnaceous plants have been published; especially the reports dealing with the effect of growth substances, the nutritional relation of organic or inorganic substances, and ecological factors, namely, light, concentration of hydrogen ions and the like, have been innumerably published. But few of these reports substantially dealed with the growth of roots of Lemnaceous plants, but except H. L. White. The root of Lemnaceous plants is sure to differ slightly from that of the other plants, but, nevertheless, it is never insignificant thing to investigate it and it will be contribute to the studies of common roots. The author therefore, mainly in vestigated the effect of physiological and ecological factors, namely, growth substances, vitamin B_1, concentration of hydrogen ions, light and the like, on the growth of roots. The author profoundly thanks to Prof. Dr. H. Kojima for his helpful suggestions and criticisms to the experiments.

2. Experimental method and its results.

Spirodela polyrhiza and *Lemna* sp. were used as materials. These plants were cultured in the medium prepared according to F. Yoshimura's conscription, like follows;

KH_2PO_4	0.025 g	$MgSO_4$	0.05 g	$NaNO_3$	0.15 g
KCl	0.025 g	$CaCl_2 \cdot 2H_2O$	0.04 g	Dis. Water	1000. cc

As Lemnaceous plants were generally constituted of three or four individuals chaining one another, these individuals were separated and

theire roots were cut off before experiments. Groups, each of which contained twenty five individuals, were cultured in petri dishes or test tubes, placed in the light or dark rooms at the temperature from 20°C to 25°C, without sterilizing. After seven or ten days, the growth of individuals, the increase or decrease of the number of individuals, or the number and length of roots were examined.

A). The effect of growth substances.

α-Naphthalen acetic acid, Heteroxin (made by Sankyo) and 2.4-Dichlorophenoxy acetic acid were used for growth substances and plants were cultured in the above mentioned medium, containing these substances of varying concnetrations. The resuts of this experiment is shown in Figs. 1 to 3. From these figures, the growth of plants was inhibited at higher concentrations. The individuals were shrinked, and in generals, the formation and the growth of roots were worse in

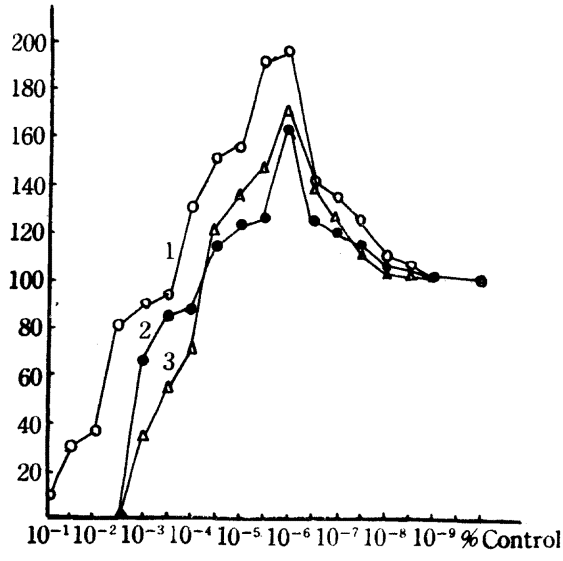

Fig. 1. The effect of α-Naphthalene acetic acid on the growth of *Lemna* sp.
Axis of abscissa: concentration.
Axis of ordinate: rates of growth; compared with those in the control represented 100 %.
1. Number of individuals.
2. Number of formed roots.
3. Length of roots.

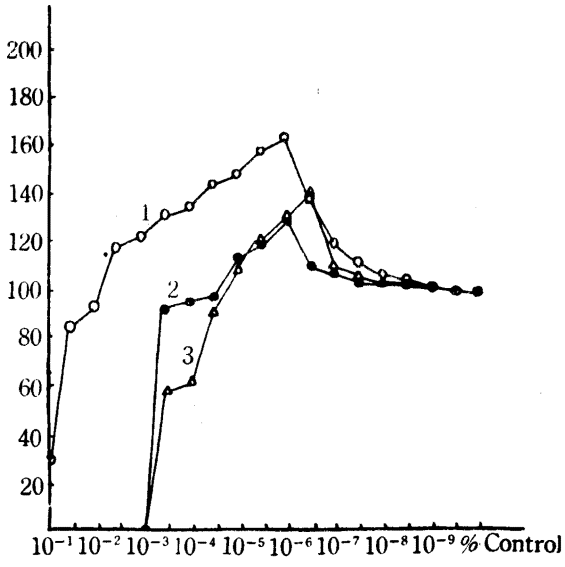

Fig. 2. The effect of Heteroxin on the growth of *Lemna* sp.
Axis of abscissa: concentration.
Axis of ordinate: rates of growth, compared with those in the control represented 100 %.
1. Number of individuals.
2. Number of formed roots.
3. Length of roots.

comparison with growth of individuals. At the concentrations of 10^{-6}% on there about of α-Naphthalene acetic acid and Heteroxin, the increase of individuals and the formation and the growth of roots were more exceedingly promoted, but at the lower concentraions less than 10^{-8} % almost the same in comparison with those in the control and consequently the effect of α-Naphthalene acetic acid or Heteroxin was hardly recognized at the lower concentrations.

As for the effect of 2.4-D (Fig. 3), the author previously, reported, that at the concentration show in this figure, the growth of individuals and the formation or the growth of roots were inferior to these in the control, but would be promoted at the concentrations lower than them. In all cases the rate of growth of roots was inferior to that of increase of individuals, the roots were more sensitive to these growth substances than individuals and the higher the concentration, the growth of roots was checked.

B). The effect of light.

In order to observe the effect of light, Lemna sp. were cultured in the dark room or in brown glass bell jar. When vitamin B_1 was not contained but as carbon sources the glucose or fructose was contained in the medium, then the growth of individuals and that of roots of Lemna sp. were not seen. When they were cultured in the period from two weeks to three monthes, they showed more remarkable invalid signs in the proportion to prolonging of the period and at last got chlorotic, but when these chlorotic plant were exposed to sunlight, they again began to grow rapidly. In the medium containing 10^{-6}% of vitamin B_1 and not any carbon sources, the action of vitamin B_1 was not recognized clearly, but, nevertheless, the invalid signs of cultured plants in it appeared more slowly than in mediums containing no vitamin B_1.

The individuals, illuminated only from the under surface, showed

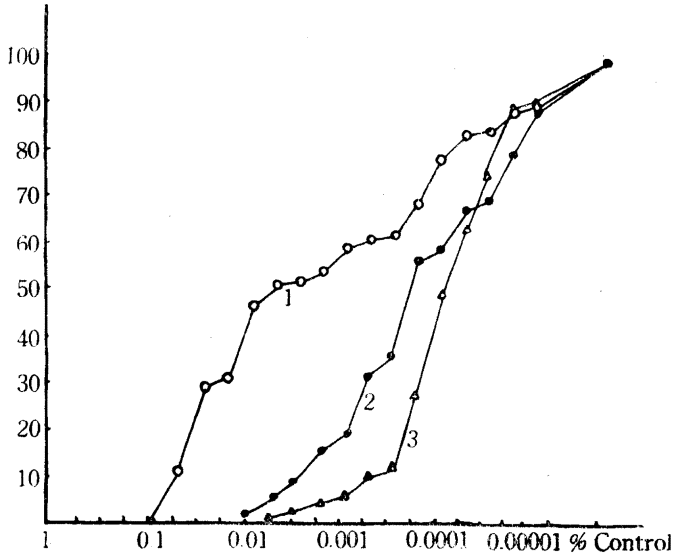

Fig. 3. The effect of 2.4-D on the growth of *Lemna* sp.
Axis of abscissa: concentration.
Axis of ordinate: rates of growth compared with those in the control represented 100 %.
1. Number of individuals.
2. Number of formed roots.
3. Length of roots.

the same signs as when cultured in the dark place, and naturally the formation of roots were not recognized because the under surface of individuals could not feel the stimulus of light, Illuminated from only the one side, their roots also apparently showed negative phototropism at the early stage of growth. In the case of several individuals sticking each other and forming a group, their roots, under natural condition, grew freely in all directions, but in the case of each individual separated from others its roots grew in all direction till each length attained to three or four mm and thenceforth their tip curved inwardly without jutting out of the shadow made by the same individual.

C). The effect of concentration of hydrogen ions.

From the experiments concernings the effect of concentration of hydrogen ions resulted Fig. 4. According to this figure, the P^H was

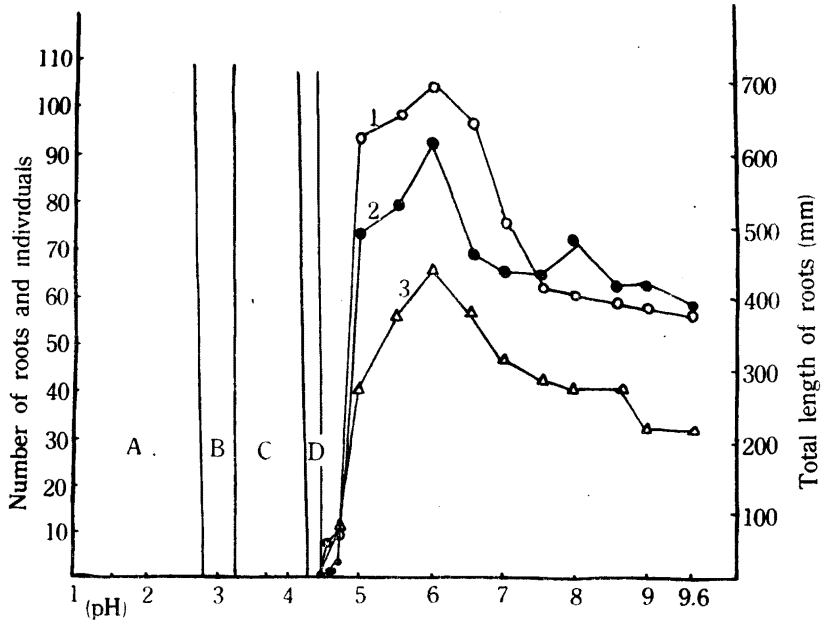

Fig. 4. The effect of concentration of hydrogen ions on the growth of roots.

A. Die after three days.
B. Die after four days.
C. Die after seven days.
D. Die after ten days.

1. Number of roots.
2. Total length of roots.
3. Number of individuals.

adjusted between 1 and 4.4, the formation of roots was not recognized and each individual was dead with chlorosis. The optimum P^H for the growth of *Lemna* sp. was 6.0 or these about, which is equal to the value of P^H of ponds or rice fields where Lemnaceous plants grew luxuriously. The increase of individuals and the formation and the growth of root were vigorously at the P^H of 6.0 with these abouts and especially the growth of roots was more remarkable in alkaline medium than their formation and concequently the average length of roots longer at higher values than at lower values of P^H.

D). The effect of carbon and nitrogen ratio on the growth of roots.

As a carbon source the glucose of varying concentrations and as a nitrogen source KNO_3 and $NaNO_3$ were used; Ammonium salts, however, were not used, for they strengthen the acidity of the medium;

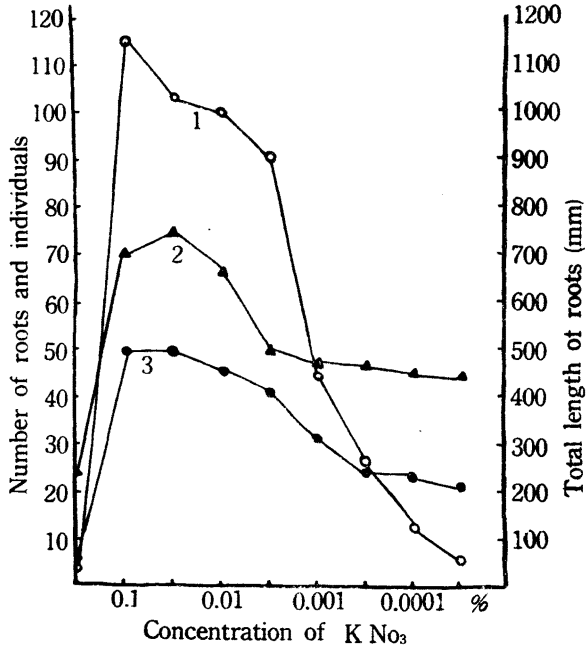

Fig. 5. Cultured in light room.
1. Total length of roots.
2. Number of individuals.
3. Number of roots.

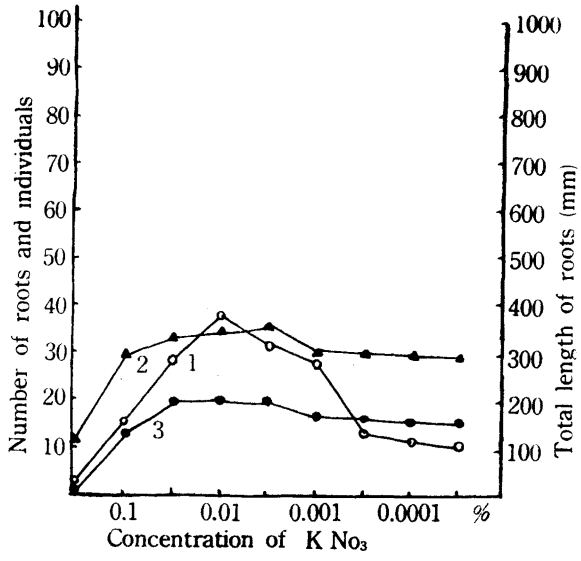

Fig. 6. Cultured in dark room.
1. Total length of roots.
2. Number of individuals.
3. Number of roots.

vitamin B_1 was used only in the occassion of the dark room culture. In the case to change the concentrations of glucose, was used 0.5% of KNO_3 or $NaNO_3$ as a nitrogen sources and to change nitrogen sources, 0.4% of glucose was used as a carbon source. These results are shown in Figs. 5 to 8, which show the effect of varying concentration of nitrogen sources on the growth of *Lemna* sp. and Fig. 9 show the effect of glucose of varying concentration on it. In all cases, these figures show the total number and length of formed roots and the increase or decrease of individuals. Figs. 5 and 7 show them in the case of the light room culture. The C/N ratio went up higher from left to right, the curve indicate, of formation and growth of roots went down lower than that of individuals, and at the concentration below 0.01% or 0.001% the former two went suddenly down but the latter did not. From experiments in the dark rooms resulted Figs. 6 and 8. In comparison between the dark room culture and the light, the growth of roots and that of individuals were generally worse in the dark room culture than

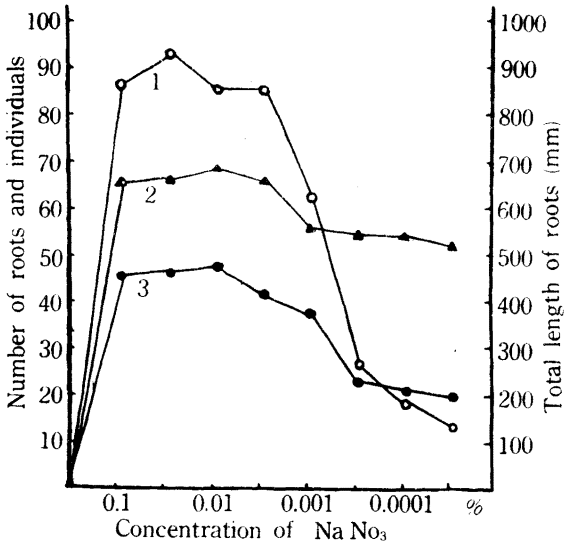

Fig. 7. Cultured in light room.
1. Total length of roots.
2. Number of individuals.
3. Number of roots.

in the light room but the shapes of their curves were almost resembled. From these four figures it is clearly known that the roots never grew better in proportion to the increase of C/N ratio, but on the contrary, were mach controlled by the quantity of nitrogen sources. At the concentration below 0.1% (Fig. 5, Fig. 7) or 0.01% (Fig. 6, Fig. 8), the poorer the quantity of nitrogen became, the weaker became the growth of roots, which it was more influenced by the quantitative change of nitrogen sources than at individual bodies. Fig. 9 shows both results in the light and the dark room culture. In the light room culture, the curve of the growth of roots and that of the growth of individuals were parallel, so I knew that C/N ratio gave no effect to their growth, but only the carbon sources did. The growth of roots and individuals was more promoted in solutions containing 0.1% to 0.0001% glucose than in the control, and in the 0.01% solution was seen their maximum growth. In the dark room culture, their growth in general was slightly different from in the light room culture, compared with the growth of roots, that of individuals was

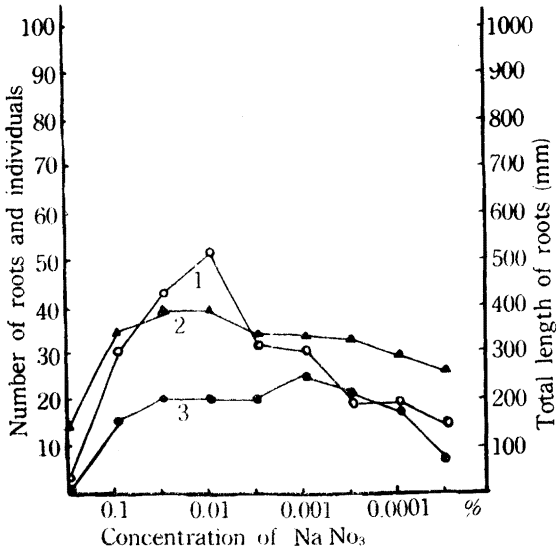

Fig. 8. Cultured in dark room.
1. Total length of roots.
2. Number or individuals.
3. Number of roots.

very weak and the denser the concentration of glucose, the better their growth.

3. Consideration and conclution.

N. A. Clark's experiments on the effect of Indol-3-acetic acid, Phenyl acetic acid, Phenyl propionic acid and P. R. Gorham's on the effect of 3-Indol butylic acid and α-Napthalen acetic acid on the growth of Lemnaceous plant were already reported and their reports were almost the same as the results of my experiments, but they did not detailed particulars of the effect of these growth substances on the growth of roots. The roots of plants, in general, are more sensitive to these growth substances than the other parts. These substances positively hinder the growth of root in their solution of high concentration, but at lower concentration promote it. These tendencies were apparently recognized in the case of the Lemnaceous plants. F. Yoshimura has reported the Lemnaceous plants could be cultured in dark room in the case when vitamin B_1 existed with such a carbon sources

as glucose or fructose in the same medium. It is sure that plants are cultured in dark room, the vitamin B_1 and the growth substances has no effect in that case the carbon sources has not contained in the same medium. But in the medium containing vitamin B_1, though they may not be promot their growth, it was more slowly get chlorotic than in the medium containing no vitamin B_1. From that it can be naturally thought that vitamin B_1 have some close relation with metabolism of carbon which reserved in plant bodies or be consumed as carbon sources. In light room culture, neither vitamin B_1 nor any carbon sources need not be mixed in the medium. The weak and chlorotic bodies that were cultured in the dark room for a long period, was exposed to the light again, begin to grow rapidly, i. e. individuals begin to increase in number and roots be formed and grow.

According to H. L. White's reports, the growth of roots is influenced by C/N ratio in leaves and promoted in proportion to the

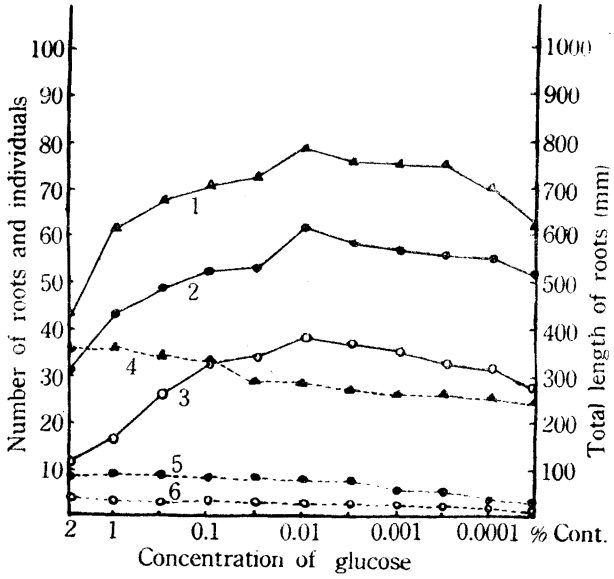

Fig. 9. Both the results in the light and dark room cultured.

1. Number of individuals. (light).
2. Number of roots. (light).
3. Total length of roots. (light).
4. Number of individuals. (dark).
5. Number of roots. (dark).
6. Total length of roots. (dark).

increase of C/N ratio; at the higher C/N ratio roots are formed, but at the lower C/N ratio carbohydrates produced by means of carbonassimilation are consumed for the increase of individuals and few roots formed. But I guess the relation between C/N ratio and the growth of roots is not so important as White has said and is to be considered only under special conditions, in accordance with the so called "Law of minimum" either nitrogen or carbon act as a limiting factor and then the growth of individuals begin and the growth of roots follows it. When growth substances were contained in the medium, the roots were more sensitive than in other parts of bodies, and roots were not effected by C/N ratio but it were controlled by direct action of these substances. The effect of concentration of hydrogen ions on cultured plant are like the fact above: It makes individuals grow at the optimum P^H and secondarily help the formation and growth of root has be seen. The roots of Lemnaceous plant, shows negative phototropism at the early stage of growth and never jut out of the shadow made by each individual, which teaches us the roots evade the light. It is interesting that they are insensible to the light from under surface and showed the same reactions as in the dark place.

4. Summary.

1. Especially the effect of growth substances, such as α-Naphthalene acetic acid, Heteroxin, 2.4-Dichlorophnoxy acetic acid, and vitamin B_1, light, concentration of hydrogen ions, and carbon and nitrogen ratio, on the growth of roots of the Lemnaceous plant were investigated.

2. The roots were more sensitive to growth substances than other parts of bodies. At the higher concentrations the growth of roots were inferior to that of individuals, but at the concentration of $10^{-6}\%$ to $10^{-7}\%$ both of them were more strongly promoted by these substances than those in control.

3. At the early period of growth, the roots were sensitive to light and showed negative phototropism.

4. The under surface of individuals was insensitive to the light, so they, when illuminated from under surface could not grow as in the dark place, grew weak and was dead with chlorosis.

5. In the case of culturing plants in dark rooms was absolutely necessary a carbon source such as glucoseor fructose, and vitamin B_1, and when the plants were cultured with out them in the medium

the growth substances has no effects, but vitamin B_1 seemed to have some relation with carbon metabolism.

6. The root formation and growth were not effected by the C/N ratio in the medium, but controlled by the quantity of nitrogen or carbon sources as in so called "Law of minimum", and carbon or nitrogen acted as limiting factors.

7. The optimum P^H for the growth of Lemnaceous plant was 6.0 or these about, and these P^H as same as a natural pond or rice fields their thriving luxuriously. In alkali side the growth of roots more thriving than formation of roots, but acide side, less than P^H 4.6, the rapidly chlorosis and weaker body appear, and then became die.

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