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THE INFLUENCE OF THE PHYTOCHROME REACTION ON THE GROWTH OF *LEMNA MINOR* L.

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INTRODUCTION

Multiplication rate and frond-growth of *Lemna minor* is very sensitive to low light intensities. HILLMAN (1957) found that a ten minutes period of red illumination given every three days could maintain heterotrophic growth in a culture otherwise kept in darkness. This effect of red light could be annihilated by a subsequent irradiation with far red, which was taken as an indication that the light acts via the phytochrome system. ROMBACH (1961) found that kinetin considerably enhances the sensitivity to red light.

The present paper deals with data concerning the quantitative relationship between radiation dose and its effect on growth and multiplication rate, and tries to connect these data with the phytochrome concept of BUTLER, HENDRICKS and SIEGELMAN (1964).

The existence of phytochrome was postulated by BORTHWICK c.s. (1952), BUTLER c.s. (1959) have extracted a protein complex which in vitro shows the required photochemical properties. The photochemical conversion approximates a first order reaction. It is usually represented by the equation:

$$P_{660} \underset{h\nu\lambda 730}{\overset{h\nu\lambda 660}{\rightleftharpoons}} P_{730}$$

P_{660} is the red absorbing form of the pigment, with a absorption maximum at λ 660 mμ. By illumination it is converted into the far red absorbing form P_{730} with a absorption maximum at λ 730 mμ. P_{730} is thought to be the physiologically active form, and is reconverted into P_{660} by absorption of far red. P_{730} disappears more or less slowly after its generation by the red illumination (DE LINT and SPRUIT, 1963; BUTLER and LANE 1965).

MATERIAL AND TECHNIQUES

Lemna minor is grown in sterile culture in glass tubes of 2,5 cm width, at 22°C, on a medium with mineral salts, and 15 g saccharose, 0.2 g casein hydrolysate, 0.005 g tryptophane and 0.0008 g (3.10^{-6} M) kinetin per liter. The adult fronds contained plenty of starch, but there was only a trace of chlorophyll in them, the illumination periods being so short that chlorophyll formation did not take place.

Red light was obtained from the beam of a 500 W projector, passing an interference filter with maximum transmittancy at λ 658 mμ and 12 mμ half bandwidth. Far red was obtained with an interference filter of maximum transmittancy at λ 740 mμ, half bandwidth 13 mμ. During the experiment the cultures were handled in darkness.

The multiplication rate is given by the equation: $R = (\log n_t - \log n_0)/t$ in which R is the multiplication rate, $1/R$ being the time in days necessary for a tenfold increase in frond number; n_0 is the number of fronds at the beginning of the experiment; n_t is the number of fronds at the time of harvest; t is the time in days from start to harvest.

The growth rate of the fronds could be measured from enlarged shadowprints. For this purpose two photographs were taken against a background of blue light with a 4 days interval towards the end of an experiment.

DESCRIPTION OF THE EXPERIMENTS

The experiments were designed in such a way that it would be possible to relate the light effect to the amount of P_{730} generated by illumination. In order to minimize effects of light on other processes the illumination periods were kept as short as possible. During the course of the experiment (14–24 days) the cultures were exposed once in 24 hours to different energy doses of red light. These energy doses were obtained by variation of intensity and duration of the illumination, as is indicated in table I, column 1 and 2.

Because the plants were illuminated once in 24 hours, some phytochrome P_{730} might still be present at the onset of a subsequent illumination. So the necessity was felt to make sure that the plants were in a definitely low P_{730} condition before the treatment with red light. In order to effectuate this the plants were irradiated with a saturating dose of far red before each illumination.

The dose relation of the reversing effect of far red is tested in the same way; in this case irradiation with different doses of far red was preceded by a saturating red illumination. The results of both treatments are recorded in table I.

In column 4 of table I, the increase in growth rate above the growth rate in continuous darkness is recorded as red light effect, and its relative value is plotted in fig. 1 against the quantity of irradiation expressed in quanta/cm^2.

Table I and fig. 1 show that the multiplication rate rises to a saturation level with increasing energy dose of red light. The values of the annihilating effect of far red after a saturating dose of red light show that no complete reversal of the red light effect can be obtained. This is not due to a growth promoting effect of

TABLE I. Multiplication rate (R) of *Lemna minor* grown 15 days at the conditions indicated. The cultures were exposed once in 24 hours to various doses of red light ($\lambda = 658$ mμ), preceded by 60 sec far red (158×10^{15} quanta/cm^2.24h) or to various doses of far red ($\lambda = 740$ mμ), preceded by 180 sec red (210×10^{16} quanta/cm^2.24h). Each value is the mean of data from 8 cultures

1	2			3	4	
	Irradiation dose			Multiplication rate	Effect of radiation	
Kind of treatment	erg/cm^2. sec	sec	quanta/ cm^2.24h $\times 10^{15}$	R (log base 10) $\pm$ standard error of the mean	R light minus R darkness	Percentage of maximum value
Red	200	5	0.32	0.035	0.009	24
at	200	20	1.30	0.042 $\pm$ 0.002	0.016	43 $\pm$ 5.5
indicated	680	10	2.21	0.045	0.020	54
quantities,	680	30	6.75	0.055 $\pm$ 0.002	0.029	78 $\pm$ 5.4
preceded by	3500	30	35.0	0.063	0.037	100
far red	3500	180	210.0	0.063	0.037	100
Far red	1280	5	2	0.064	0.038	100
at	1280	15	7	0.063	0.037	97
indicated	1280	30	14	0.061 $\pm$ 0.0006	0.035	92 $\pm$ 1.6
quantities,	7050	30	79	0.048	0.022	58
preceded by	7050	60	158	0.040 $\pm$ 0.002	0.014	37 $\pm$ 5.4
red	7050	720	1900	0.033	0.007	18
Far red only	7050	60	158	0.026		
No radiation				0.026		

far red because growth with far red alone does not differ from growth in continuous darkness.

The growth response, expressed in fig. 1, is the ultimate effect of the photochemical phytochrome conversion; the kinetics of this reaction are implied in the curves. HENDRICKS, BORTHWICK and DOWNS (1956) have described a method to derive the relation between light energy and the photochemical conversion of phytochrome from experimental results as given by the curves of fig. 1. Applicability of this method depends on the fulfillment of three requirements:

1. the conversion of phytochrome behaves as a monomolecular reversible photochemical reaction in both directions;
2. a definite physiological response belongs to each P_{730} level, irrespective of the way this level is reached, with red illumination or with far red illumination after a red light period;
3. the total phytochrome concentration is the same in plants treated with different doses of radiation.

About requirement 1. nothing can be said by lack of knowledge about the chemistry of phytochrome; usually the reaction behaves as if monomolecular. From the fact that reversion by far red is not complete, it must be concluded that requirement 2. is not fulfilled, at least not at the lowest P_{730} levels. Requirement 3. has been tested by photospectrometric phytochrome determination by C. J. P. SPRUIT with plants treated once in 24 hours during a period of 10 days with

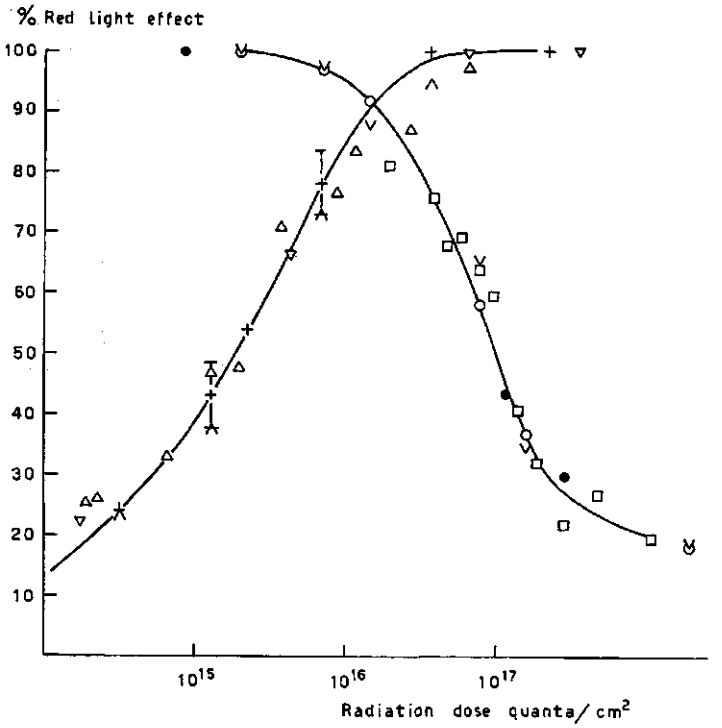

FIG. 1. The red light effect (percentages of the maximum value) on multiplication rate and frond expansion of *Lemna minor* plotted against the multiplication rate. The values from 3 experiments are indicated with different marks. The plants received once in 24 hours the indicated dose of red light (λ 658 mμ), preceded by 210 × 10^{15} quanta/cm² far red (λ 740 mμ) (△, +, ▽ and Λ) or the indicated dose of far red (λ 740 mμ), predeced by 210 × 10^{15} quanta/cm² red light (λ 658 mμ) (□, ○, ● and V); Λ and V are marks for the frond expansion data, the other marks indicate the multiplication rate.

a. red radiation at 3 dose levels,
b. a saturating dose of far red after a high dose of red light,
c. a saturating dose of far red alone,
d. continuous darkness during the 10 days preceding the measurement.

The results are given in fig. 2.

Figure 2 shows that there is a large difference in phytochrome content between plants of the different treatments; so the calculation method of HENDRICKS c.s. can not be directly applied to the data of fig. 1. However, the phytochrome data allow an estimate as to what the growth would have been if the phytochrome content under all conditions would have been the same: the velocity of the photochemical conversion is higher as the pigment concentration is higher, so with a high phytochrome level less energy is needed to attain a definite P_{730} level than with a low phytochrome level. An estimation of the value of the

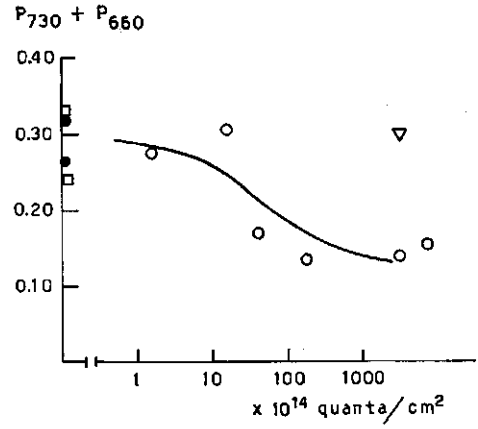

FIG. 2. Relative phytochrome contents of *Lemna minor* plants measured with difference photospectrometry in vivo.
○ : Cultures were exposed during 10 days once in 24 hours to doses of red radiation indicated on the abscissa.
● : Cultures in darkness.
□ : Cultures obtained once in 24 hours a high dose of far red.
▽ : Cultures obtained once in 24 hours a high dose of red light, followed by a high dose of far red.

correction can be made with aid of the formula $\frac{P}{P - P_{730}} = e^{KE}$. P is total phytochrome concentration, E energy dose, K reaction constant, e base of the logarithm. Now, if disregarding the lack of fulfillment of requirement 2 is not too serious, the following calculation can be made.

According to the notation BUTLER (1961) used, the relation between light absorption and phytochrome conversion is

$$\frac{dF_{730}}{dt} = E\varepsilon_r \varphi_r F_{660} - E\varepsilon_i \varphi_i F_{730} \qquad (1)$$

F_{730} is the fraction of phytochrome in the 730 mμ absorbing form, F_{660} is the fraction of phytochrome in the 660 mμ absorbing form; the sum of the fractions $F_{730} + F_{660} = 1$;
ε_r and ε_i are the extinction coefficients of P_{660} and P_{730} respectively; φ_r and φ_i are the photochemical efficiency coefficients (mole/einstein) for the conversion of $P_{660} \rightarrow P_{730}$ and $P_{730} \rightarrow P_{660}$ respectively; t is the time of irradiation in seconds; E is the energy of radiation. Integration of (1) gives:

$$E\varepsilon_r \varphi_r t = \frac{\varepsilon_r \varphi_r}{\varepsilon_r \varphi_r + \varepsilon_i \varphi_i} \ln\left(1 - \frac{\varepsilon_r \varphi_r + \varepsilon_i \varphi_i}{\varepsilon_r \varphi_r} F_{730}\right) + C \qquad (2)$$

in which C is an integration constant.
In case $t = 0$

$$C = \frac{\varepsilon_r \varphi_r}{\varepsilon_r \varphi_r + \varepsilon_i \varphi_i} \ln \left(1 - \frac{\varepsilon_r \varphi_r + \varepsilon_i \varphi_i}{\varepsilon_r \varphi_r} F_{730}0\right)$$

In case $t = \infty$

$$\frac{\varepsilon_r \varphi_r + \varepsilon_i \varphi_i}{\varepsilon_r \varphi_r} F_{730\,\infty} = 1$$

$$F_{730\,\infty} = \frac{\varepsilon_r \varphi_r}{\varepsilon_r \varphi_r + \varepsilon_i \varphi_i} \tag{3}$$

$F_{730\,\infty}$ is the fraction of phytochrome in the far red absorbing form after a saturating dose of red light energy,
$F_{730}0$ is the fraction of phytochrome in the far red absorbing form at the start of an irradiation.
Substitution of (3) in (2) gives

$$Et \frac{\varepsilon_r \varphi_r}{F_{730\,\infty}} = \ln \frac{F_{730\,\infty} - F_{730}0}{F_{730\,\infty} - F_{730}} \tag{4}$$

The values of $P_{730\,\infty}$ and $P_{730}0$ depend on the spectral quality of the radiation. Because the absorption spectra of P_{660} and P_{730} show some overlap it is not possible to get all phytochrome in the P_{730} form by radiation with λ660 mμ. According to BUTLER (1964) the equilibrium between P_{660} and P_{730} after a saturating λ660 mμ red illumination is at 81 % P_{730} and 19 % P_{660}, so $F_{730\,\infty}$ with red light is 0.81. In the case the radiation is with far red, $F_{730\,\infty}$ is about 0.01 according to BUTLER. At the onset of red radiation $F_{730}0$ is assumed to be 0.01 and at that of far red radiation 0.81.

A definite multiplication rate of the Lemna fronds, and also a definite F_{730} value, to each radiation dose plotted in fig. 1 belongs. Which F_{730} value belongs to a given radiation dose can be calculated. For this purpose two values of growth rate are chosen, I and II which belong to the light doses in red $E_r t$ I and $E_r t$ II, and the irradiation doses in far red $E_i t$ I and $E_i t$ II. These data are substituted in equation 4, yielding the following relations:

$$\frac{E_r t\,\mathrm{I}}{E_r t\,\mathrm{II}} = \frac{\ln \dfrac{0.81 - 0.01}{0.81 - F_{730}\mathrm{I}}}{\ln \dfrac{0.81 - 0.01}{0.81 - F_{730}\mathrm{II}}} \tag{5 and}$$

$$\frac{E_i t\,\mathrm{I}}{E_i t\,\mathrm{II}} = \frac{\ln \dfrac{0.01 - 0.81}{0.01 - F_{730}\mathrm{I}}}{\ln \dfrac{0.01 - 0.81}{0.01 - F_{730}\mathrm{II}}} \tag{6}$$

With several pairs of values in fig. 1, this calculation has been made with and without correction for inequality in phytochrome content. These calculations yield the values of $\varepsilon_r \varphi_r$ and $\varepsilon_i \varphi_i$ given in table II.

TABLE II. Values of the effectiveness $\varepsilon_r \varphi_r$ of red radiation (λ 658 mμ) in phytochrome conversion $P_{660} \rightarrow P_{730}$, and of the effectiveness $\varepsilon_i \varphi_i$ of far red radiation (λ 740 mμ) in phytochrome conversion $P_{730} \rightarrow P_{660}$. The values are calculated from experimental observations with or without correction for inequality in phytochrome content. The values of the product $\varepsilon \varphi$ are expressed in cm^2/einstein, log base e

Combinations of effect levels which serve as calculation basis	$\varepsilon_{\lambda 658} \varphi_{\lambda 658} \times 10^7$	$\varepsilon_{\lambda 740} \varphi_{\lambda 740} \times 10^7$
Values of fig. 1 not corrected		
85% red effect and 70% red effect	4.41	1.32
85% red effect and 55% red effect	5.17	1.07
85% red effect and 40% red effect	5.96	0.88
Values of fig. 1 with correction		
85% red effect and 70% red effect	4.10	1.44
85% red effect and 55% red effect	3.13	1.91
85% red effect and 40% red effect	3.09	1.94

The values of $\varepsilon_r \varphi_r$ and $\varepsilon_i \varphi_i$ obtained from the non-corrected data show, as expected, a variation which is counteracted by the correction. The mean values from the corrected data are $\varepsilon_{\lambda 658} \varphi_{\lambda 658} = 3.4 \times 10^7$ cm^2/einstein and $\varepsilon_{\lambda 740} \varphi_{\lambda 740} = 1.8 \times 10^7$ cm^2/einstein. These values are not far from those obtained by BUTTLER (1964) from in vitro measurements; viz., $\varepsilon_{\lambda 660} \varphi_{\lambda 660} = 3.9 \times 10^7$ and $\varepsilon_{\lambda 728} \varphi_{\lambda 728} = 1.6 \times 10^7$.

The relation between light dose and $P_{660} \rightarrow P_{730}$ conversion, expressed by formula 4, viz.,

$$E\,t = \frac{F_{730\,\infty}}{\varepsilon_r \varphi_r} \ln \frac{F_{730\,\infty} - F_{730}0}{F_{730\,\infty} - F_{730}}$$

can be evaluated for radiation with λ 658 mμ ($\varepsilon_{\lambda 658} \cdot \varphi_{\lambda 658} = 3.4 \times 10^7$ cm^2/einstein). The F_{730} values obtained are fractions of total phytochrome, irrespective of the actual concentration of the pigment. The true P_{730} levels depend on the total phytochrome concentration, which according to fig. 2, differs according to the experimental treatment. The total phytochrome concentration in a given situation is estimated from fig. 2.

The same can be done for the relation between radiation dose and $P_{730} \rightarrow P_{660}$ conversion by far red radiation. This relation is expressed by the formula:

$$E\,t = \frac{1 - F_{730\,\infty}}{\varepsilon_i \varphi_i} \ln \frac{F_{730}0 - F_{730\,\infty}}{F_{730} - F_{730\,\infty}}$$

In case of radiation with λ 740 mμ $\varepsilon_{\lambda 740}$ $\varphi_{\lambda 740} = 1.8 \times 10^7$ cm^2/einstein. So, for each radiation dose both growth rate and P_{730} level are known, and it is possible to plot the growth stimulus against the P_{730} level, as is done in fig. 3. The values obtained by far red radiation do not fully cover those obtained by red irradiation, since the red light effect is not completely reversible by far red radiation.

Not only multiplication rate, but also frond expansion in *Lemna minor* is controlled by the phytochrome reaction. In table III, the daily increase in length of the fronds is given, determined from the increase over 5 days towards the end of the experiment. The rate of frond expansion is almost constant from

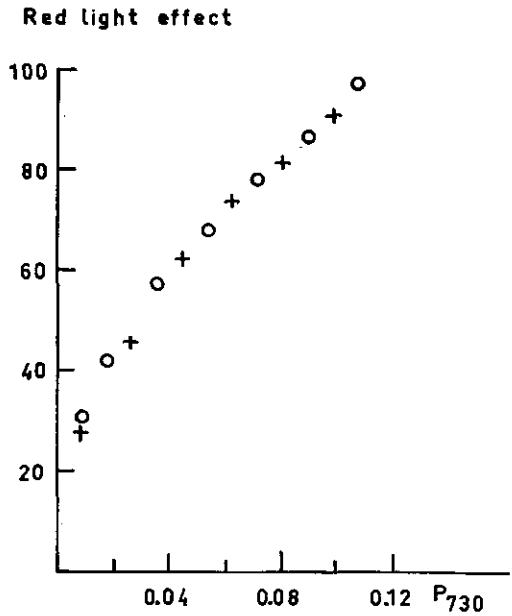

FIG. 3. Relation between phytochrome in the 730 mμ absorbing form and the red light effect on the multiplication rate (percentages of the maximum value). The points are derived from the dose effect curve of red light (+) and from the dose effect curve of far red radiation (○). The phytochrome concentration scale is made correspondent to the scale in fig. 2, but the values are calculated with the method of HENDRICKS, BORTHWICK and DOWNS.

the appearance of a frond outside the reproductive pocket untill the end of elongation, and shows the same relation to irradiation as does the multiplication rate. The final length of the fronds, given in the 5th column of table III, is affected by radiation to a much smaller degree. However, the final length recorded for fronds grown in darkness or in cultures treated with far red only is probably too high, because a growth period of 15 days is too short to obtain mature fronds initiated under these conditions.

TABLE III. Rate of frond expansion and ultimate size of fronds of *Lemna minor* grown 15 days at the conditions indicated. The cultures were exposed once in 24 hours to various doses of red (λ = 658 mμ), preceded by 60 sec far red (λ = 740 mμ, 158 × 10^{15} quanta/cm^2.24h) or to various doses of far red (λ = 740 mμ), preceded by 180 sec red (λ = 658 mμ, 210 × 10^{15} quanta/cm^2.24h). The values of rate of expansion are averages of 20–50 measurements, the highest standard deviation of the mean (5%) was with cultures treated with far red only. (Same experiment as recorded in table I)

1	2	3	4		5
Kind of treatment	Irradiation dose quanta/cm^2 × 10^{15}	Increase in length mm/day	Effect of irradiation mm/day	%	Final length of fronds mm
Red	0.32	0.29	0.09	24	5.0
at	1.30	0.34	0.14	38	5.1
indicated	2.21	0.40	0.20	54	5.0
quantities,	6.75	0.47	0.27	73	5.3
preceded by	35.0	0.55	0.35	95	5.4
far red	210.0	0.57	0.37	100	5.5
Far red	2.3	0.58	0.38	103	5.4
at	7.0	0.57	0.37	100	5.3
indicated	14.0	0.53	0.33	91	5.4
quantities,	79	0.45	0.25	69	5.0
preceded by	158	0.33	0.13	35	4.7
red	1900	0.27	0.07	18	4.4
Far red only	158	0.20	0	0	4.6
not irradiated					3.8

GENERAL DUSCUSSION

An average higher plant has a stem with leaves, and sprouts in the axils of the leaves. The sprouts grow out and again form stems with leaves. The growth pattern of *Lemna minor* is not far from this picture, only both stem and leave are present in the frond. Every frond bears two reproductive pockets, which are comparable to the axils of the leaves of the typical plant. From these pockets new fronds are successively pushed out, the meristem being a sprout. Red light stimulates both the production of fronds from a reproductive pocket, and the expansion of the fronds. If this situation is compared with the influence of red light on *Alaska peas*, as investigated by THOMSON and MILLER (1961), it comes out that in *Pisum* leaf expansion behaves in a similar way, but that the rate of leaf production in red light is only slightly higher than in darkness. In this respect, *Lemna polyrhiza* behaves much in the same way as *Pisum* by the formation of nearly as many fronds in darkness as in light, but with a much smaller frond area. (Unpublished experiments of the author).

The agreement between the outcome of the calculation of $\varepsilon_r \varphi_r$ and $\varepsilon_i \varphi_i$ with the values found by BUTLER c.s. (1964) strengthens the confidence in the applicability of the calculation method of HENDRICKS c.s. (1956). Of course, the transparency of the used plant material is a condition for the accordance. The

values found by HENDRICKS c.s. (1956) with this method for seed germination and for the inhibition of the elongation of a bean internode showed much variation, dependent on the kind of plant material and the experimental treatment. This may be due to the optical properties of the plant material, but it is also possible that the values found by BUTLER c.s. (1964) are valid only for phytochrome in vitro, and that the accordance with the values obtained from the experiments reported here is incidental. The recent communications of BUTLER, LINSCHITZ, SIEGELMAN and NORRIS (1965) and of BRIGGS and FORK (1965), and the observations of SPRUIT (1965) show that the phytochrome photochemistry is much more complicated than first was supposed; it is very well possible that the system differs from plant to plant and from one condition to another.

The relation between the relative concentration of P_{730} and the light effect on growth shows that under all conditions phytochrome was the limiting factor. Nevertheless, in continuous light of high intensity, the multiplication rate is three times the value found with a saturating dose given once in 24 hours, whereas the mean phytochrome concentration is probably higher in the last case. So it seems that either the physiological activity of P_{730} in light is manyfold increased, or that in continuous light growth is not controlled by phytochrome. This problem needs a closer examination for its solution.

Another question, not yet answered, is whether the spectrophotometrically determined phytochrome really is identical with the physiologically active phytochrome. The improvement in the agreement between $\varepsilon_r \varphi_r$ and $\varepsilon_i \varphi_i$ determinations from different radiation levels by a correction indicated by the spectrometrical phytochrome determination speaks in favour of this identity, but on the other hand, experiments on reversibility of the red light effect by far red at different intervals of time after a red illumination show that there is no accordance between the decrease in physiological reversibility and the decrease of the spectrometrically determined phytochrome concentration (SPRUIT, DE LINT and ROMBACH (in preparation)).

SUMMARY

Multiplication rate and frond expansion of *Lemna minor*, grown on a sucrose medium, is stimulated by illuminations of short duration. This effect is mediated by phytochrome. Photospectrometrical measurements in vivo show that the phytochrome content is lower in plants treated with a light dose of high energy content than in plants treated with a light dose of low energy content or plants kept in darkness. The relation between energy dose and growth rate is investigated with narrow bands of red and far red radiation, the photochemical data derived from this physiological reaction were found to be in close accordance with the in vitro measurements of BUTLER, HENDRICKS and SIEGELMAN. The relation between growth and P_{730} showed that in the conditions of the experiment P_{730} was at all levels the limiting factor for growth.

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LITERATURE

BORTHWICK, H. A., S. B. HENDRICKS, M. W. PARKER, E. H. TOOLE, V. K. TOOLE, Proc. Nat. Ac. Sci. U.S. **38**, 662–666 (1952).

BRIGGS, W. R., D. C. FORK, Plant Physiol. **40**, Suppl. liii (1965).

BUTLER, W. L., in Progress in Photobiology (B. Christensen and B. Buchmann Eds) p. 569–571, Elsevier Amsterdam, 1961.

BUTLER, W. L., S. B. HENDRICKS, H. W. SIEGELMAN, Photochem. Photobiol. 3, 521–528 (1964).

BUTLER, W. L., H. C. LANE, Plant Physiol. **40**, 13–17 (1965).

BUTLER, W. L., H. LINSCHITZ, H. W. SIEGELMAN, K. H. NORRIS, Plant Physiol. **40** Suppl. liii (1965).

BUTLER, W. L., K. H. NORRIS, H. W. SIEGELMAN, S. B. HENDRICKS, Proc. Nat. Ac. Sci. U.S. **45**, 1703–1708 (1959).

HENDRICKS, S. B., H. A. BORTHWICK, R. J. DOWNS, Proc. Nat. Ac. Sci. U.S. **42**, 19–26 (1956).

HILLMAN, W. S., Science **126**, 165–166 (1957).

LINT, P. J. A. L. DE, C. J. P. SPRUIT, Meded. Landbouwhogeschool Wageningen **63**, (14) 1–7 (1963).

ROMBACH, J., in Progress in Photobiology (B. Christensen and B. Buchmann Eds) p. 379–380, Elsevier Amsterdam, 1961.

SPRUIT, C. J. P., in Currents in Photosynthesis (J. B. Thomas and J. C. Goedheer Eds) 1965, in press.

SPRUIT, C. J. P., Biochim. Biophys. Acta, in press.

SPRUIT, C. J. P., P. J. A. L. DE LINT, J ROMBACH, in preparation.

THOMSON, B. F., P. M. MILLER, Amer. Jour. Bot. **48**, 256–261 (1961).