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FORMATIVE EFFECTS OF VARIOUS SPECTRAL REGIONS OF LIGHT ON *LEMNA TRISULCA* L.

by

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INTRODUCTION

Recently, numerous investigations concerning the influence of narrow spectral regions on the development of plants have been undertaken (for review see WASSINK and STOLWIJK 1956). The formative effects of coloured light have been studied mostly on higher land plants. In the frame of research in this field at this laboratory, we have tried to investigate some photomorphotic effects of light on the water plant *Lemna trisulca* L. There are several reasons for choosing such material, *viz.*,

a. the specific morphology of *Lemna* species, which is rather different from other higher plants (ASHBY *et al.* 1948),
b. the simplicity of culture and exactness of measurement of the frond shape and dimensions,
c. the possibility to obtain a pure culture of this plant, which permits to give supplementary carbohydrate nutrition in case the quality or the intensity of the light does not permit adequate photosynthesis.

For our investigation the equipment for high and low light intensity available in this laboratory was used.

METHOD

The plants were collected from a pond in the vicinity of Wageningen in October 1956. For sterilisation the method described by GORHAM (1945) and LINDEMAN (1952) was used. The single fronds were put into a 0.1 % solution of $HgCl_2$, rinsed quickly in sterile water, then put into 50 % alcohol, rinsed twice in sterile water and finally put into a sterile mineral nutrient solution according to CLARK, containing 0.5 % glucose. It was found that the best time for the baths in sublimat and alcohol is 5 or 10 seconds. Longer treatment causes death or abnormal growth in a very high percentage of the plants. Infections with bacteria or algae were seen in the first two weeks after sterilisation. For obtaining good results, the choice of the right stage of development of the fronds used for sterilisation is of great importance. The best results gave the

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fronds containing a young daughter one. This young frond must be as large as possible but should still remain in the pocket at the mother frond. The old leaves usually die after sterilisation, but young ones start to grow quickly.

The plants were cultivated in 300 ml ERLENMEYER flasks containing 100 ml of the nutrient solution. In the course of preliminary experiments it has been found that the best nutrient solution for *Lemna trisulca* is that according to HOAGLAND, containing: $CaNO_3$, 4 H_2O 1.180 g; Mg SO_4, 7 H_2O 0.493 g; KNO_3 0.506 g; KH_2PO_4 0.136 g; ferritartrate 0.005 g per liter, with the following addition of microelements: H_3BO_4 286 mg; $MnCl_2$ 1.81 mg; $ZnSO_4$, 7 H_2O 0.22 mg; $CuSO_4$, 5 H_2O 0.08 mg; Na_2MoO_4 0.07 mg. The pH of the solution was adjusted to 5.8–6.0 by adding KOH. For stock cultures and low light intensity experiments 1.5 % saccharose was added. Usually 30–50 fronds were used for inoculation. Stock cultures were maintained in a light cabinet illuminated by two daylight fluorescent tubes giving a light intensity of 8000–8500 ergs/cm² sec. Temperature in the cabinet was about 24–26 °C.

In low light intensity experiments *Lemna* plants were cultivated in a solution with carbohydrate during 2 months. The plants were continuously illuminated in the coloured light cabinets described elsewhere (WASSINK and VAN DER SCHEER 1950, STOLWIJK 1954), The optical centres of the coloured light were as follows: violet 400, blue 460, green 550, yellow 589, red 660, infrared 760 mμ. For a control culture, white light of the same intensity from a single 40 W daylight fluorescent tube without filter was used. The light intensity as measured with a calibrated thermopile in each cabinet was 1000 ergs/cm² sec, the temperature was 20 °C.

In the second series of low light intensity experiments, mineral nutrition without carbohydrates was used. Because the intensity 1000 ergs is too low to produce adequate photosynthesis for effective growth, a basic illumination period in strong white light was used; the flasks with plants were exposed during 8 hours per day in a white light cabinet at 8000 ergs/cm² sec. and 26°, and during 16 hours remained in the colored light cabinets mentioned above at 1000 ergs/cm² sec. and 20°.

For strong light experiments in narrow spectral regions *Lemna* plants were cultivated in pure mineral nutrition in the light cabinets described by WASSINK and STOLWIJK (1952) in blue, green, yellow, red, and red+infrared light. The optical centres of the coloured light, and the light intensities as measured with the spherical photometer (WASSINK and VAN DER SCHEER, 1951), were as follows: blue 460 mμ, 25000 ergs/sec. cm² ⌀ sph., green 540 mμ, 46000 ergs/sec. cm² ⌀ sphere, yellow 589 mμ, 58000 ergs/sec. cm² ⌀ sphere, red 660 mμ, 80000 ergs/sec. cm² ⌀ sphere. In the last cabinet, red radiation with supplementary infrared illumination at the intensity of about 40000 ergs/cm² sec. was used. The infrared, given only from the top of the cabinets, was measured by a flat photometer. The period of illumination was 16 hours in the 24 hours cycle; temperature 20 °C.

At the end of the experiments the fronds were placed in water between two glass slides, put into the photographic enlarging apparatus, and their outlines, magnified 10 times, were drawn on the paper. From these drawings the measurements of frond dimensions were taken, with the precision of about 1 mm, which corresponds to a maximal error of 0.1 mm in the original dimensions. For statistical description of the frond shape three dimensions were measured: breadth (a) and length (b) of the lamina and length of the petiole (c), and two

shape coefficients were calculated: $\frac{b}{a}$ and $\frac{c}{b}$. For each kind of light 50 fronds from 2–4 flasks were used.

Expecially in low light intensity experiments, where the growth is very slow, care must be taken to choose the right fronds for measurements. Old fronds of the inoculum, and too young, still growing ones must be eliminated. The first ones are easy to be found because the shape of fronds in the stock culture in quite different. For demonstration in which stage of development the growth is finished, and the shape does no more change, a series of successive photographs of a rapidly growing culture was taken (fig. 1). It has been stated that when a

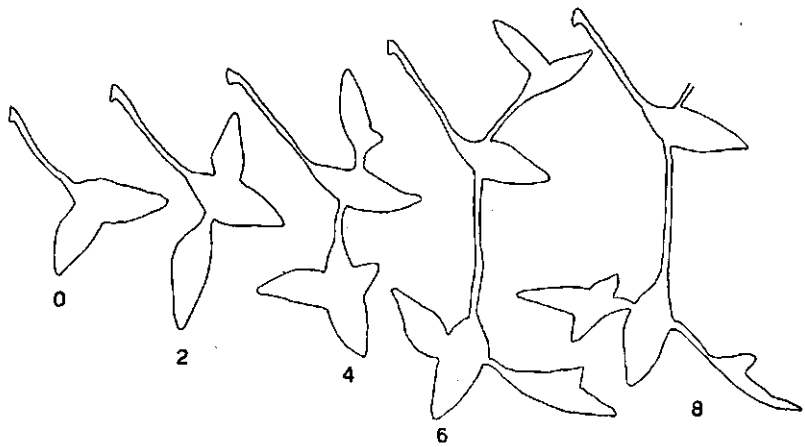

FIG. 1. Successive stages of the growing of *Lemna trisulca* fronds. CLARK solution, 1 % saccharose. 26°C. Age in days

frond produces a young one, it still may grow, while just after the development of the fronds of 2nd order its shape and dimensions do not change any more. Therefore, for measurements of frond shape only fronds were chosen which had produced at least one frond of the 2nd order.

RESULTS

The reaction of *Lemna trisulca* on different conditions points to a great sensitivity and responsiveness of this plant in comparison with other *Lemna* species. This fact finds its expression in a harmful action of too long a time of sterilisation (optimum 5–10 sec., in comparison with 45 sec. for *Lemna minor* (LINDEMAN (1952)) and in easy degeneration and abnormal growth under inadequate culture conditions (too high temperature, or too high pH). But the sensitivity in morphotic response makes *Lemna trisulca* a very good object for the study of morphotic effects of different culture conditions, in spite of the technical difficulties of its culture. Fig. 2 gives an example of different morphotic reactions obtained on *Lemna* plants growing in green light of 1000 ergs/cm² sec. in a solution with carbohydrates under continuous illumination, and at 46000 ergs/sec. cm² ⌀ sphere in mineral nutrition and illumination during 16 hours per day. The differences are clear even without any statistical

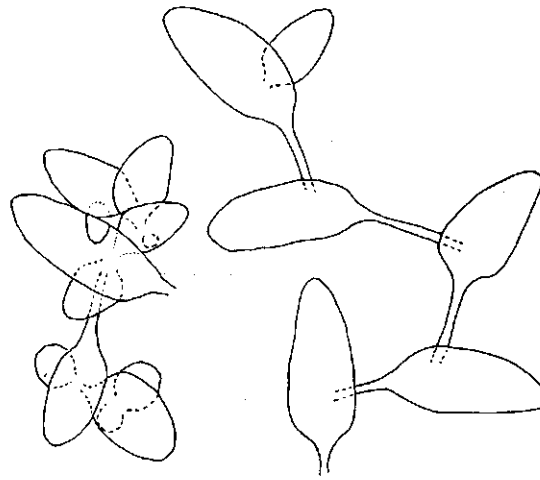

FIG. 2. Shape of the fronds growing in green light. Left: nutrition with carbohydrate, low light intensity continuous illumination; right: mineral nutrition, high light intensity during 16 hours in a day.

treatment. They are in the frond dimensions and shape, in petiole length and in the number of daughter fronds growing out from one mother frond. On the other hand, in a series of cultures started from the same stock culture and growing under the same conditions, variations are not very great.

The low light intensity experiment with the addition of sugar under con-

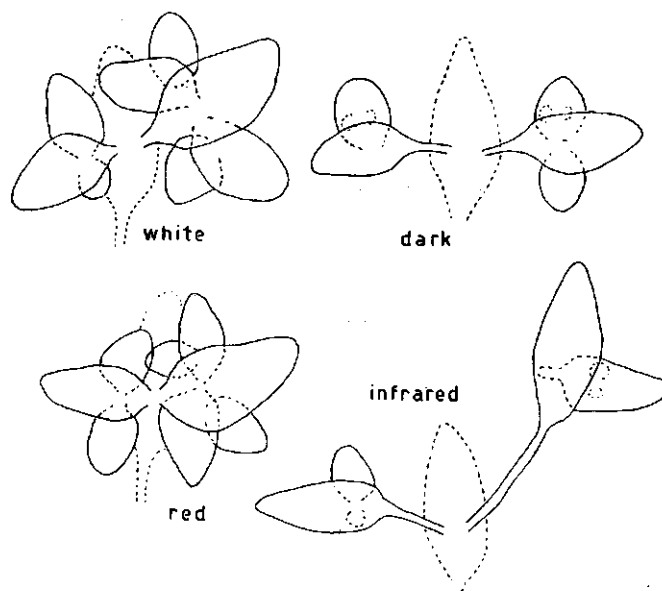

FIG. 3. Shape of the fronds growing in continuous low light intensity on mineral nutrition with carbohydrate.

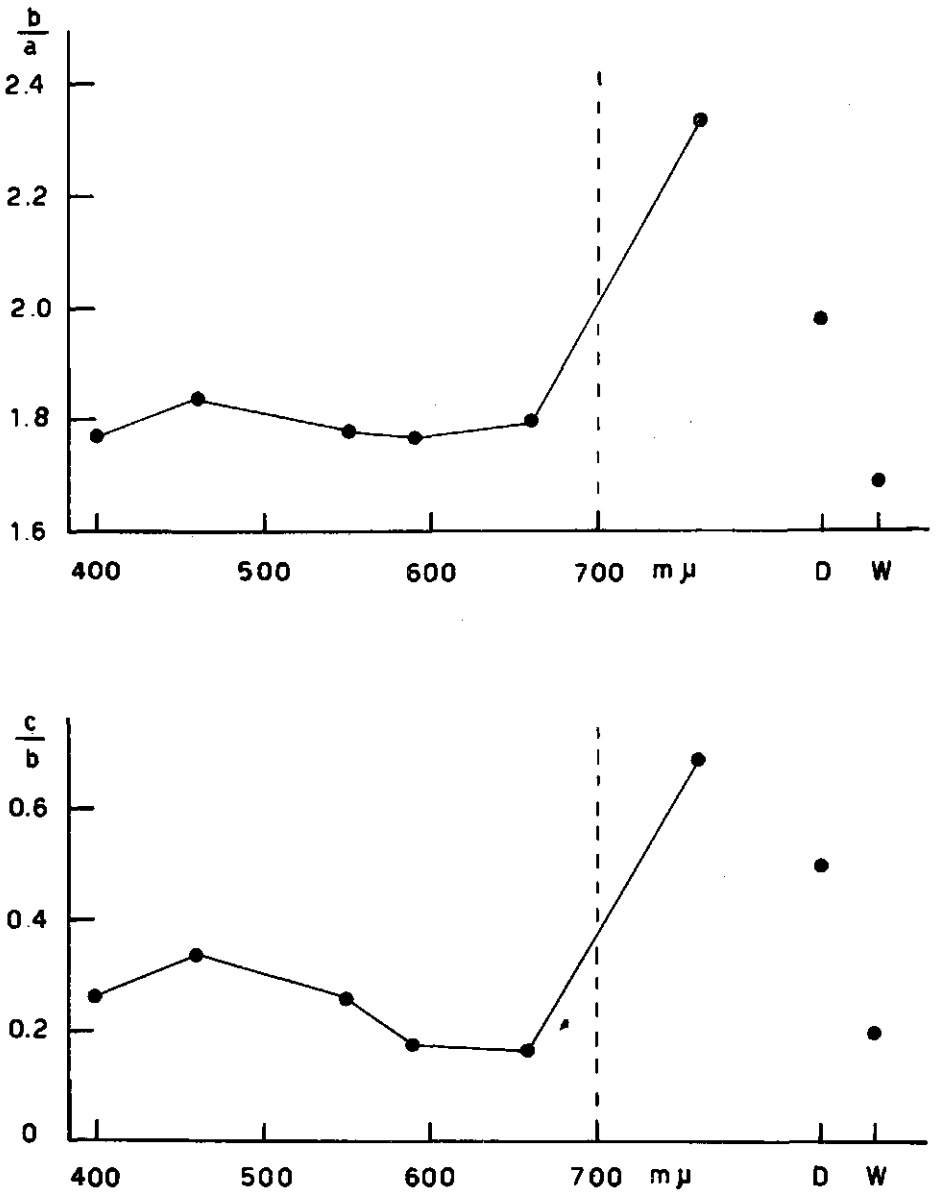

FIG. 4. Shape coefficients of the fronds of *Lemna trisulca* growing in coloured light at an intensity of ∾ 1000 ergs/cm²sec. in mineral nutrition supplied with carbohydrate. Continuous illumination.

tinuous illumination was started 31.1.'57, and measurements were made after 8 weeks (between 25 and 28.3.'57). Although the nutrient solution contained carbohydrate, a very low growth rate was observed. The exact determination of this rate is difficult because the amounts of inoculum in all flasks were not

exactly equal but approximately 100 % increase in dry weight was observed. The best growth appeared in red light. Fig. 3 gives some examples of plants growing in light of different spectral regions. Measurements of frond blade dimensions and petiole length are given in Table I; in fig. 4 the shape coefficient has been plotted against the wave length of light. In the range of visible light there is no marked difference in frond shape, except of a very small elongation in the blue. A very significant elongation has been found in (near) infrared. It is much greater than the elongation in etiolated dark cultures. The coloured light exerts a similar effect on the petiole length. The petioles are slightly longer in the violet-green range than in yellow, red and white light and show a marked elongation in infrared.

Very characteristic for this culture condition (low intensity coloured light + sugar) is the production of a greater number (2–5) of young fronds by one old mother frond. This, and the often observable shortness of the petioles make the shape of the plants strange for this species and make it resemble *Lemna minor* In fig. 5 the frequency distribution of the number of young fronds growing out from one old frond is plotted. The number 2 or 3 is most common in all types of light. In contrast to the other spectral regions, in infrared and dark the number one does not occur at all, and in infrared as well as in blue the number 3 occurs especially often.

The low light intensity experiments with mineral nutrition and a basic high intensity white light period were started 20.2.'57 (series I) and 11.3.'57 (series II) and finished 14–17.5.'57. The results are summarized in Table II and in figs. 6 and 7. As in the previous case there is no significant difference in the whole range of visible light. Infrared has a distinctly elongating effect, as well as darkness. The shape coefficient of the lamina in infrared has a higher value than in dark, the petiole coefficient has nearly the same value. It is clear that the morphotic reactions of *Lemna* under these conditions are quantitatively the same as with continuous illumination and organic nutrition. The differences are less obvious in the case of a basic white light period and mineral nutrition. The absolute values of the shape coefficients also show some differences. When plants are growing exclusively in low light intensity with sugar the fronds are brighter and the petioles shorter than in the series which received a high intensity basic white light period, and only mineral nutrition.

Great difference are observed in the number distribution of young fronds growing from one mother frond (fig. 7). Contrary to cultures with carbohydrate, in pure mineral nutrition with high intensity white light as basic illumination, the number 1 is most often in all kinds of supplementary light while the number 3 is rather rare. The frequency distribution for different colours of light does not show marked differences except that in red and infrared the number 1 is still more frequent than in other spectral regions.

Experiments in exclusive strong light of narrow spectral regions were started on 7.2.'57 (series I) and 6.3.'57 (series II), and measurements of both series were made between 29.4 and 4.5.'57. The results of measurements of frond dimensions are summarized in Table III and fig. 8. It is evident that, though the light intensities in different cabinets were not exactly the same, the shape of the fronds is nearly the same in all ranges of visible light except in red and red + infrared. Red light in both series of experiments produced great elongation of the frond blade and petiole. In red, supplied by infrared, elongation has also been observed, but markedly less than in pure red.

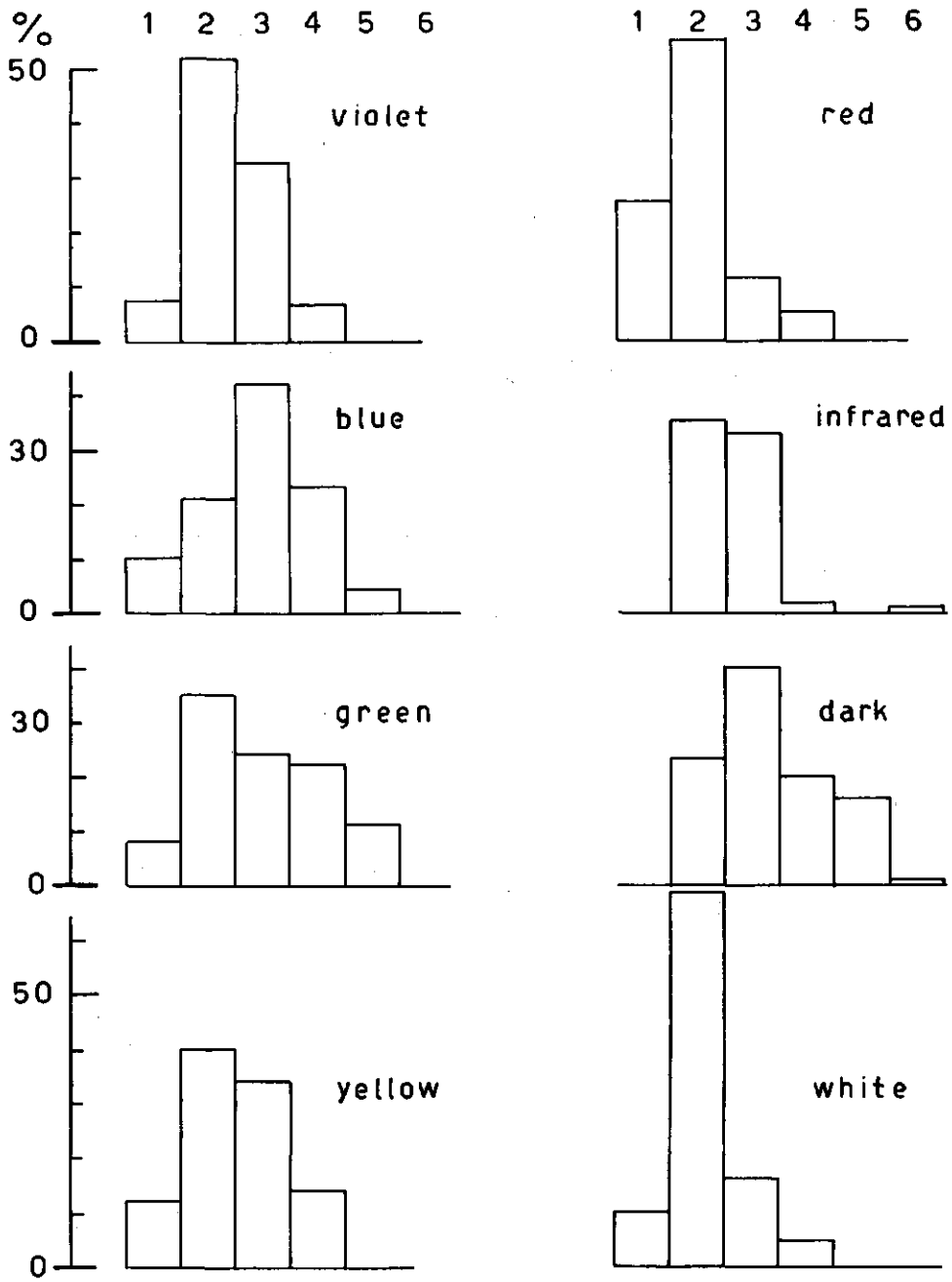

FIG. 5. Frequency distribution of the number of young fronds growing out from one mother frond. Mineral nutrition with carbohydrate, continuous illumination, coloured light at ∞ 1000 ergs/cm²sec.

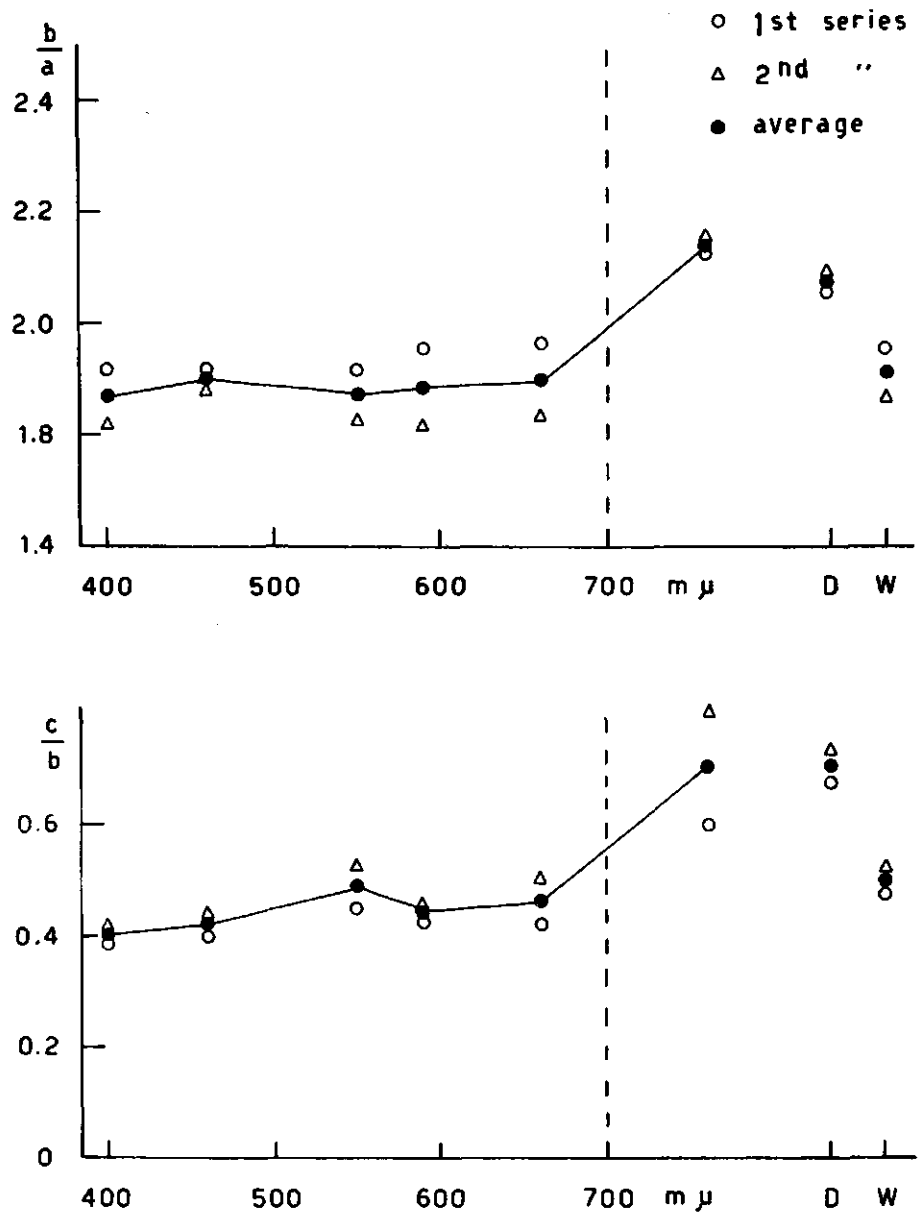

FIG. 6. Shape coefficients of the fronds of *Lemna trisulca* growing in mineral nutrition. Basic illumination with white light, 8000 ergs/cm²sec. during 8 hours a day, supplementary illumination with coloured light 1000 ergs/cm²sec. during 16 hours in a day. Average (●) of 2 experiments (○ and △)

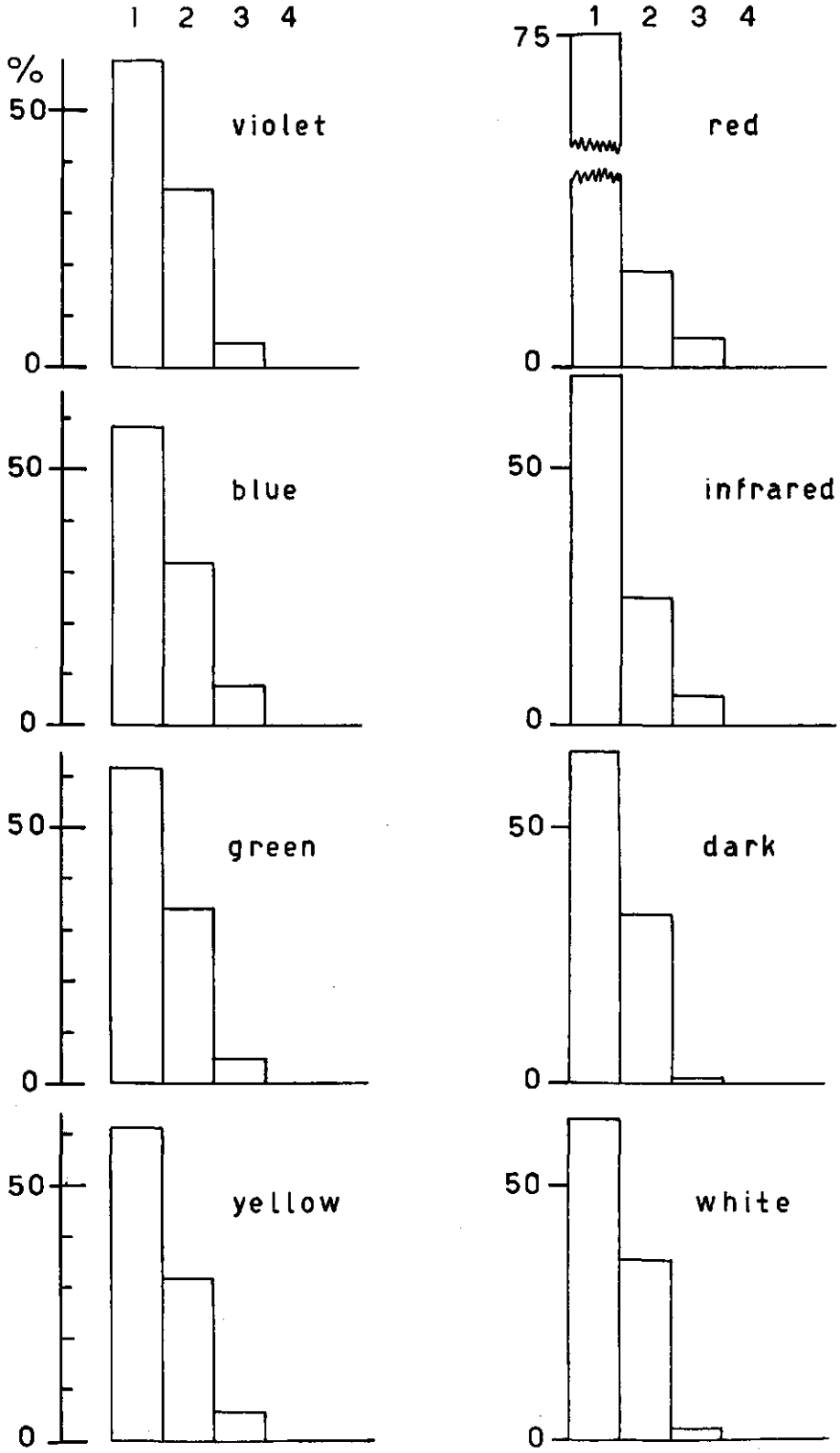

FIG. 7. Frequency distribution of the number of young fronds growing out from one mother frond. Mineral nutrition, supplementary coloured illumination. Short day in strong white light (fluorescent tubes). No dark period.

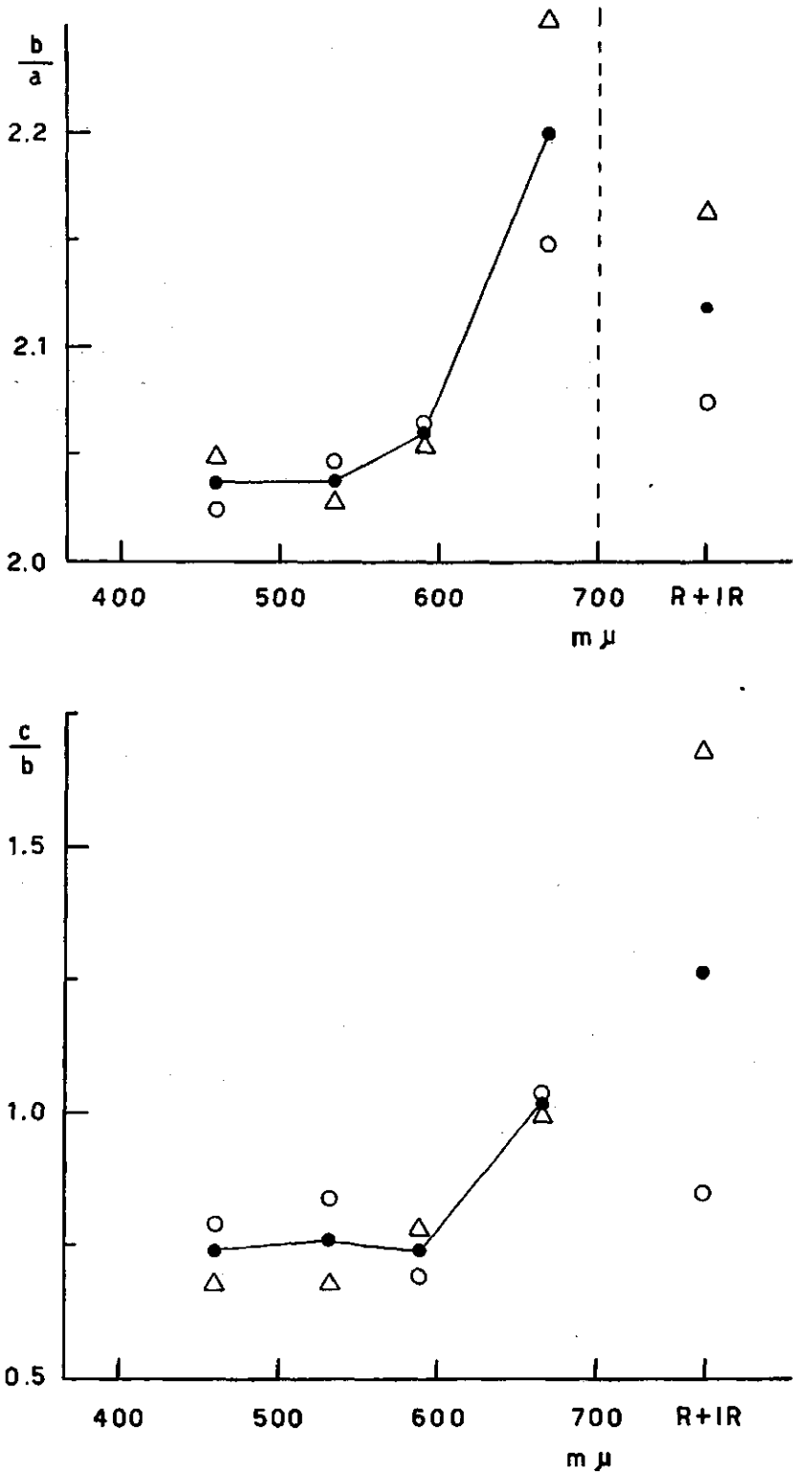

FIG. 8. Shape coefficients of the fronds of *Lema trisulca*, growing in mineral nutrition. Illumination 16 hours in a day in high light intensity cabinets (∾ 10000 ergs/cm sec., spectral regions). Average (●) of 2 experiments (○ and △)

The frequency distribution of young fronds (fig. 9) shows a greater frequency of the numbers 1 and 2; 3 is very rare, and no higher numbers occur. In blue, green and yellow 1 is predominant, in red and red + infrared the frequencies of 1 and 2 are nearly equal.

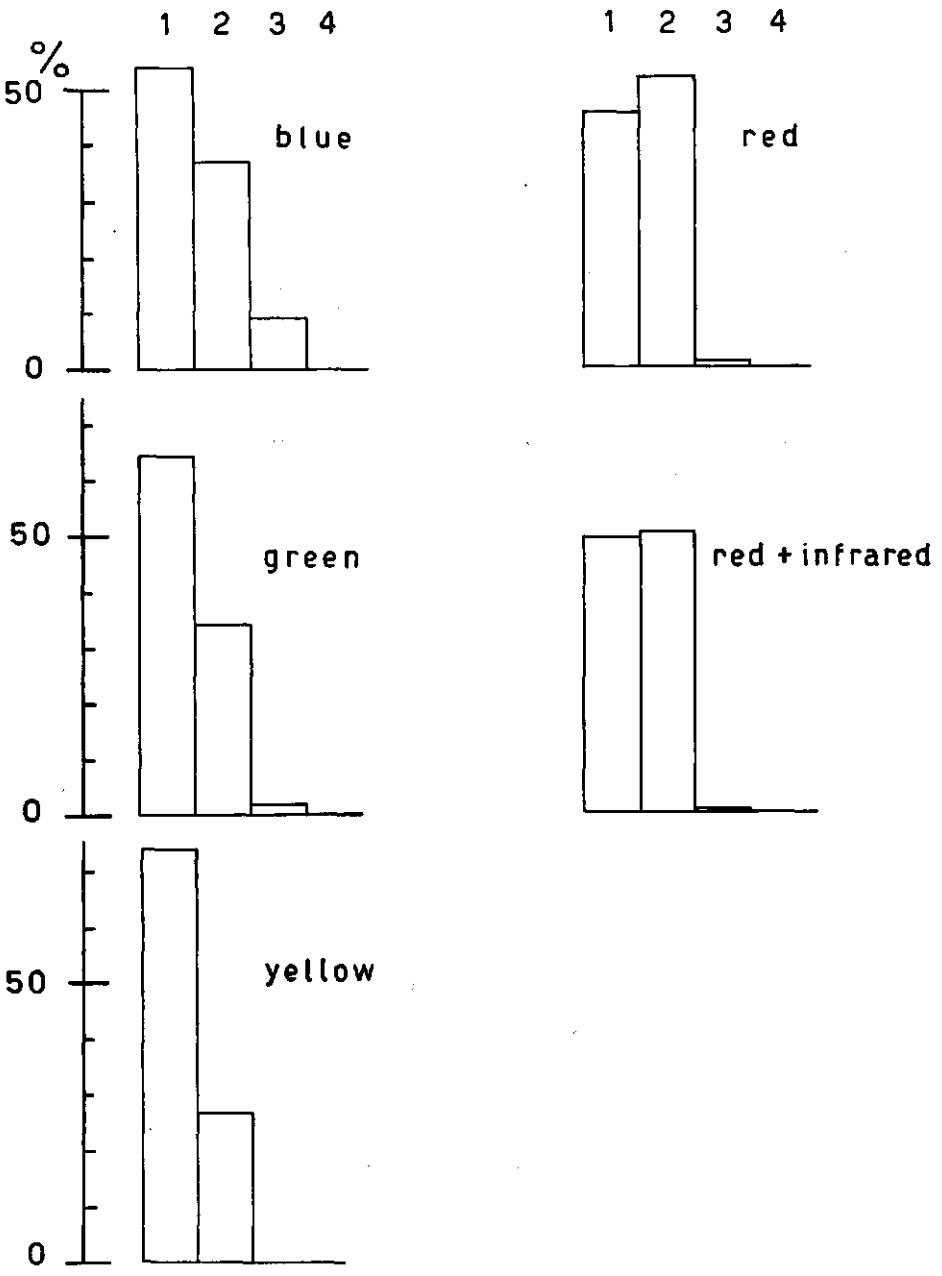

FIG. 9. Frequency distribution of the number of young fronds growing out from one mother frond. Mineral nutrition, high intensity illumination (∞ 10000 ergs/cm²sec., spectral regions).

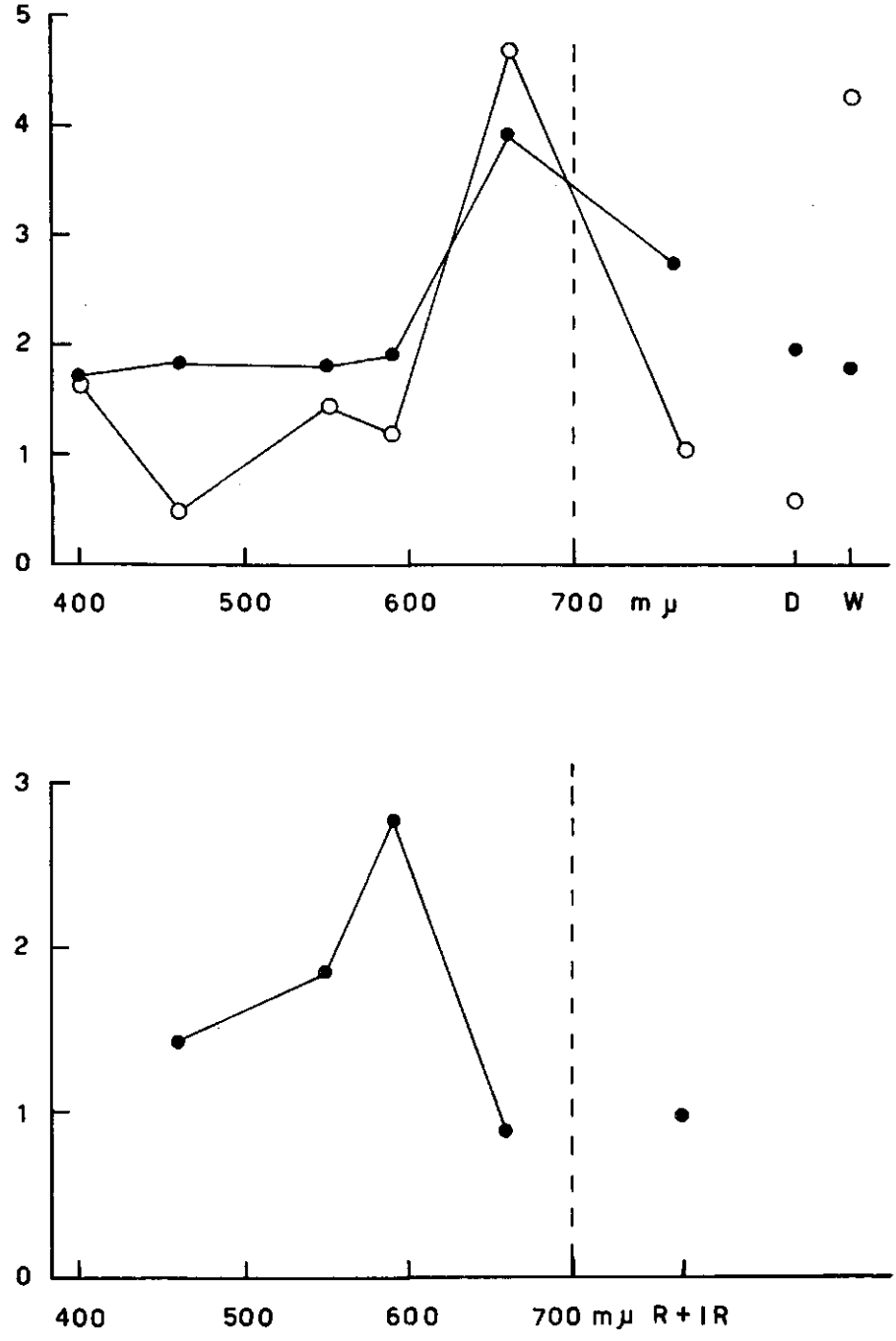

FIG. 10. The quotients of the percentage of the most frequent number of young fronds divided by the next one.
Above: o carbohydrate, continuous coloured light, ∞ 1000 ergs/cm²sec. Number 2/3
• mineral nutrition, supplementary coloured light, ∞ 1000 ergs/cm²sec. Number 1/2
Below: mineral nutrition, coloured light of high intensity (∞ 10000 ergs/cm²sec.) Number 1/2

Frequency distribution in three experiments shows that when photosynthesis is strong (high intensity coloured light or basic white light period) frequency 1 is most often, while under heterotrophic development frequency 2 predominates.

On the other hand, if the quotient of the percentage of the most frequent number over the next one is plotted against wave length (fig. 10), a similarity between the two low light intensity experiments (continuous and supplementary illumination) is obvious. Under these conditions the highest value of the quotient is found in the red, contrary to the results of high light intensity experiments, where in red and red + infrared the value of this quotient is lowest.

DISCUSSION

The results described above are in general agreement with the other observations reported from this laboratory (WASSINK and co-workers 1952–56). In low light intensity, the specific influence of near infrared causing the elongation of some parts of plants was observed many times with different objects. In some experiments similar effects of blue were reported; the influence of this last kind of light, however, seems to be produced by a small admixture of infrared radiation transmitted through the glass fitters. Recently, DE LINT (1957) has observed that when using blue plastic filters completely absorbing the near infrared, no specific effect of blue as compared to other ranges of visible light can be observed. Using blue cabinets with plastic filters we find very small elongation effects in *Lemna* in two cases and no effect in one case.

Although the observation with high light intensity spectral lights are only of a preliminary nature – because not quite equal intensities of illumination were used in different cabinets – the results observed are similar to those reported by WASSINK and STOLWIJK (1952) for *Solanum lycopersicum*. If the *Solanum* plants were growing exclusively in different spectral regions without supplementary white light, conspicuous stem elongation was observed in yellow and red light.

Different morphotic reactions in the same coloured light (for instance red) when applying low or high intensity may be connected with different kinds of action of these two light conditions on the auxin controlled processes, as was discussed by STOLWIJK (1954). According to STOLWIJK's hypothesis, coloured light of high intensity may regulate the auxin level, whereas such light at low intensity, used supplementary to white light illumination, might affect the activity of auxins or the sensitivity to auxins. From this point of view the comparison of two types of low light intensity experiments is interesting. The same qualitative influence on the shape of fronds in both cases suggests that the sensitivity to auxin is the same in both cases, independent of the way the monochromatic light was used; exclusively or as a supplementary illumination, and is thus connected with the low level of intensity of the various spectral regions.

The differences between different spectral regions of light were more expressed when continuous low light intensity illumination was used. This results demonstrated that the use of sugar-fed plants, and coloured light illumination of very low intensity may be usefull in photomorphotic research.

SUMMARY

Photomorphogenetic studies of the type carried out earlier in this laboratory,

using large surface illumination in narrow spectral regions, were applied to sterile cultures of *Lemna trisulca* L.

Three types of conditions were mainly used:

1) low light intensity spectral light applied to cultures containing sugar,
2) low light intensity spectral light applied as a daylength extension to cultures receiving a basic high intensity light period in white light ("daylight" fluorescent tubes),
3) high light intensity spectral light applied exclusively, without white light or sugar addition.

The morphogenetic characteristics studied were: the length/breadth relation of the leafy frond parts, and the relative length of the "petioles", expressed as two "shape coefficients", viz. b/a, being length/breadth of leafy parts, and c/b: length of "petiole" over length of "leaf". The main results appear in figures 4, 6 and 8.

Condition 1 (see above) yields conspicuous elongating effects in near infrared, a slight elongating effect is observed in pure blue.

Condition 2 yields much the same result, however less conspicuous differences, and no or hardly any effect in blue.

Condition 3 yields elongation in red light and in red + infrared as was observed previously in some other plants (e.g. tomato).

Interesting differences between the various spectral regions were also expressed in the number of daughter fronds per mother frond (see figs. 5, 7 and 9). Also the quotient of the percentage of the most frequent number of young fronds over the next in frequency had an apparent relation to the spectral region, irrespective of the actual value of the numbers (e.g. 1/2 or 2/3, see fig. 10).

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