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THE EFFECT OF LIGHT ON THE GROWTH OF SPORELINGS OF THE INTERTIDAL RED ALGA *PLUMARIA ELEGANS* (BONNEM.) SCHM.

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(With Plate I and Text-figs. 1–12)

Reports concerning the effects of various environmental factors on the distribution of marine red algae have included several dealing with the influence of light. Gail (1922), Hubbenet & Voblikova (1928), Lubimenko & Tichovskaia (1929), Montfort (1929, 1930, 1933), Tschudy (1934) and Levring (1947) have all studied the effects of light on the photosynthesis of red algae; and Biebl (1952) has shown that thalli of algae from the mid-littoral zone are more resistant than those of sublittoral species to intense illumination. Fewer studies, however, have been made using sporelings of red algae as test material. Thus, Chemin (1931) observed that sporelings of *Nemalion multifidum* appeared tolerant of bright light; Katada (1949) showed that *Gelidium amansii* sporelings grew at a faster rate in green than in blue-green light; Jones (1959) demonstrated that week-old sporelings of *Gracilaria verrucosa* bleached and ceased development after 4–5 h exposure to a high light intensity (56,000 lux) from a tungsten-filament lamp; and a recent investigation of the light-sensitivity of sporelings of several species of intertidal red algae (Boney, unpublished observations) has given results similar to those of Biebl (1952).

One aspect of this general problem which has received detailed study is the function of the phycobilins (accessory pigments responsible for the colour of red algae) in photosynthesis. Engelmann (1883) first suggested that the red colour of these plants represented an adaptation to the green light which prevails under the sea, and later (Engelmann, 1884) he observed that the red pigments, as well as chlorophyll, contributed to photosynthesis. Haxo & Blinks (1950) confirmed and extended this theory of 'complementary chromatic adaptation' by demonstrating, with several species of red alga, that photosynthesis in these plants is sensitized primarily by the phycobilins, not by chlorophyll.

In the present work, which continues earlier studies on factors influencing the ecological distribution of marine red algae (Boney & Corner, 1959), an

attempt has been made to evaluate the role of the phycobilins in sporelings of *Plumaria elegans*. Very few measurements were made of photosynthesis, cell numbers being considered a more reliable estimate of changes taking place under different experimental conditions. Thus, measurements of photosynthesis with red algae are usually made over such a short period (20–30 min) that the immediate history of the plants becomes of paramount importance. Measurements of cell production, on the other hand, can easily be extended over several days during which the plants have more time in which to adapt themselves to the conditions of the experiment. A second reason for concentrating on growth and not photosynthesis was that the *Plumaria* sporelings provided only a very small bio-mass and were never free from bacteria and diatoms. Hence, measurements of photosynthesis with this material were likely to be of doubtful significance.

It seemed at the outset that phycobilins might fulfil at least two functions in *Plumaria* sporelings. The first was that of protecting other light-absorbing substances from light of unfavourable wavelengths; and the second (and generally accepted function in adult red algae) was that of absorbing light energy which was then used either directly, or indirectly (via chlorophyll *a*), in photosynthesis. However, although this second function is a likely explanation of the role of the pigments in red algae which are permanently submerged, it is difficult to see how it can apply in the case of *Plumaria* which, as it occurs in the mid-littoral zone, is exposed to long periods of unfiltered daylight and therefore receives a higher proportion of red light. It therefore seemed more profitable to examine the first of these possibilities and the results obtained are consistent with the view that the red pigment in *Plumaria* (phycoerythrin) can protect sporelings of the plant from the growth-inhibitory effects of green light of certain wavelengths in the region 500–540 mμ.

A preliminary report of this work has already appeared (Boney & Corner, 1960). The present paper provides a more detailed account.

METHODS

Plant material and spore settlement

Plumaria is particularly suitable for studies on sporeling behaviour because of its protracted fruiting period and copious spore output (Boney, 1960*a*). Collections of plant material and settlement of spores were carried out as described earlier (Boney, Corner & Sparrow, 1959; Boney & Corner, 1959).

Measurement of growth

Measurement of growth was facilitated by the well-defined sequence of early development in *Plumaria* sporelings. This development is typical of the Ceramiales, and is distinguished from that of other Florideophycidae by the early formation of a rhizoid and an erect-growing filament of cells. With *Plumaria*, under the conditions of experiment, this 'erect' filament grows in a horizontal plane over the first 2–3 weeks, so that the total cells produced per filament can be readily counted under low magnification. Before the erect filament develops the sporelings appear as one-, two- or

three-celled spherical or ovoid structures (Pl. I, A, *a*); but after 7 days many have grown to the four- or five-celled stage (Pl. I, A, *b*). Growth stages observed in the second week of development are shown in Pl. I, A(*c*, *d*).

The rate of cell production is shown on a logarithmic scale in Text-fig. 1, from which it will be seen that growth is more rapid during the second week; for this reason measurements of cell production were always made during this period. At the end of an experiment, the total number of cells produced by a crop of sporelings was counted, each sample crop consisting of 200–300 sporelings. Two or three replicates were used. The cell production per 100 sporelings was then calculated, and from this total was deducted the number of cells produced per 100 sporelings during the period before the start of the experiment. The final value was then calculated as the number of cells produced by 100 sporelings per day during the period of experiment (Boney, 1960*b*), the accuracy of duplicate determinations being approximately 3%.

This type of measurement covers but one aspect of the growth of a multicellular organism, that of increase in cell number, without exact reference to increase in cell size. However, during the experiments the cells were repeatedly examined under a microscope and the size of the cells was always normal. In addition, for the purposes of the present work, it was considered that cell production would offer a valid measure of any inhibitory or stimulatory effects on the overall growth process.

Culture medium

Throughout the experiments the sporelings were grown in a mixture of one part Erd-Schreiber medium (Føyn, 1934) and three parts sea water. Diluted medium was always used because although the undiluted supports vigorous sporeling growth (see Text-fig. 1) it also encourages an excessive development of contaminating diatoms.

SCREENING EXPERIMENTS

Optimum light intensity

As the *Plumaria* sporelings appeared to contain only small amounts of red pigment (located at the surface of the chromatophores) it seemed likely that any protective effect the pigment exerted would involve only small quantities of light energy, and that therefore the sporelings must be sensitive to quite small changes in the composition of the incident light. Consideration of these possibilities led to the first part of this investigation in which measurements were made of cell production by sporelings screened from the light source by diluted solutions of phycoerythrin extracted from the adult plant. Before such experiments could be made, however, it was necessary to determine the optimum light intensity for sporeling growth under the conditions prevailing in the present work, i.e. illumination from a fluorescent tube. This was done as follows.

The culture vessels were placed in a constant temperature room (16° C) at various distances from a vertically situated Mazda 'Daylight' fluorescent tube. Illuminations were measured in lux with a Benjamin photometer, corrections being made for the differences between the spectral composition of the light used for experiment and that for which the instrument was calibrated.

The absolute energy values were found using the equation

$$\text{flux (lumens/mm}^2) = \frac{K_{max}}{10^7} \int_{380\,m\mu}^{720\,m\mu} E'_\lambda V_\lambda d\lambda,$$

where K_{max} is the mechanical equivalent of light (682 lm/W for $\lambda = 555$ mμ), V_λ is the relative luminous efficiency of light at wavelength λ (Walsh, 1953) and E'_λ is the energy flux of the source at the wavelength λ in ergs/sec/mm²/mμ.

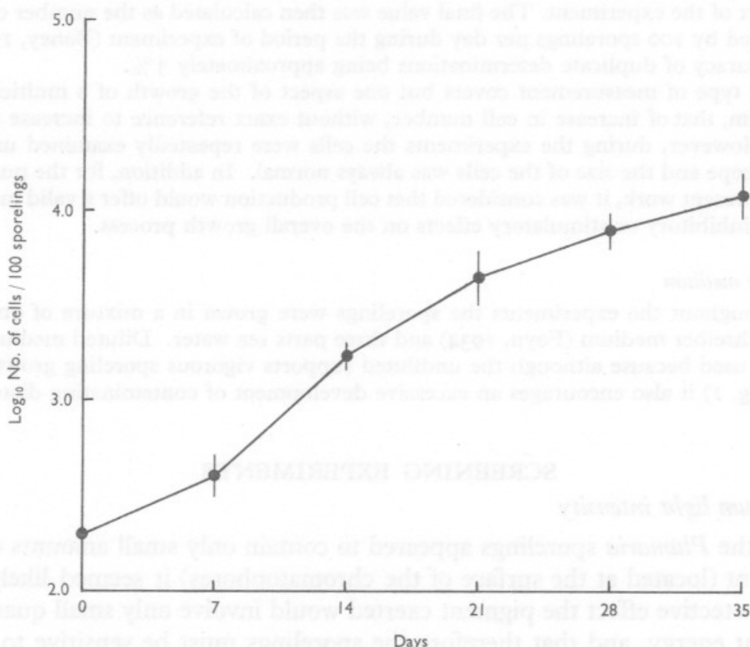

Fig. 1. Cell production by *Plumaria* sporelings during first 5 weeks of growth in Erd–Schreiber medium. Vertical lines represent difference in duplicate values.

As the relative spectral distribution of energy of the source was known (the E_λ of Text-fig. 2*a*), the absolute values of E'_λ could be found. The total energy of the source between two wavelengths λ_a and λ_b is then

$$\int_{\lambda_a}^{\lambda_b} E'_\lambda d\lambda.$$

Growth of the sporelings under continuous illumination of different light energies is shown in Text-fig. 3, from which it will be seen that the maximum rate of cell production was obtained at roughly 10 ergs/sec/mm² in the wave-band 380–720 mμ decreasing only slightly when this value was reduced

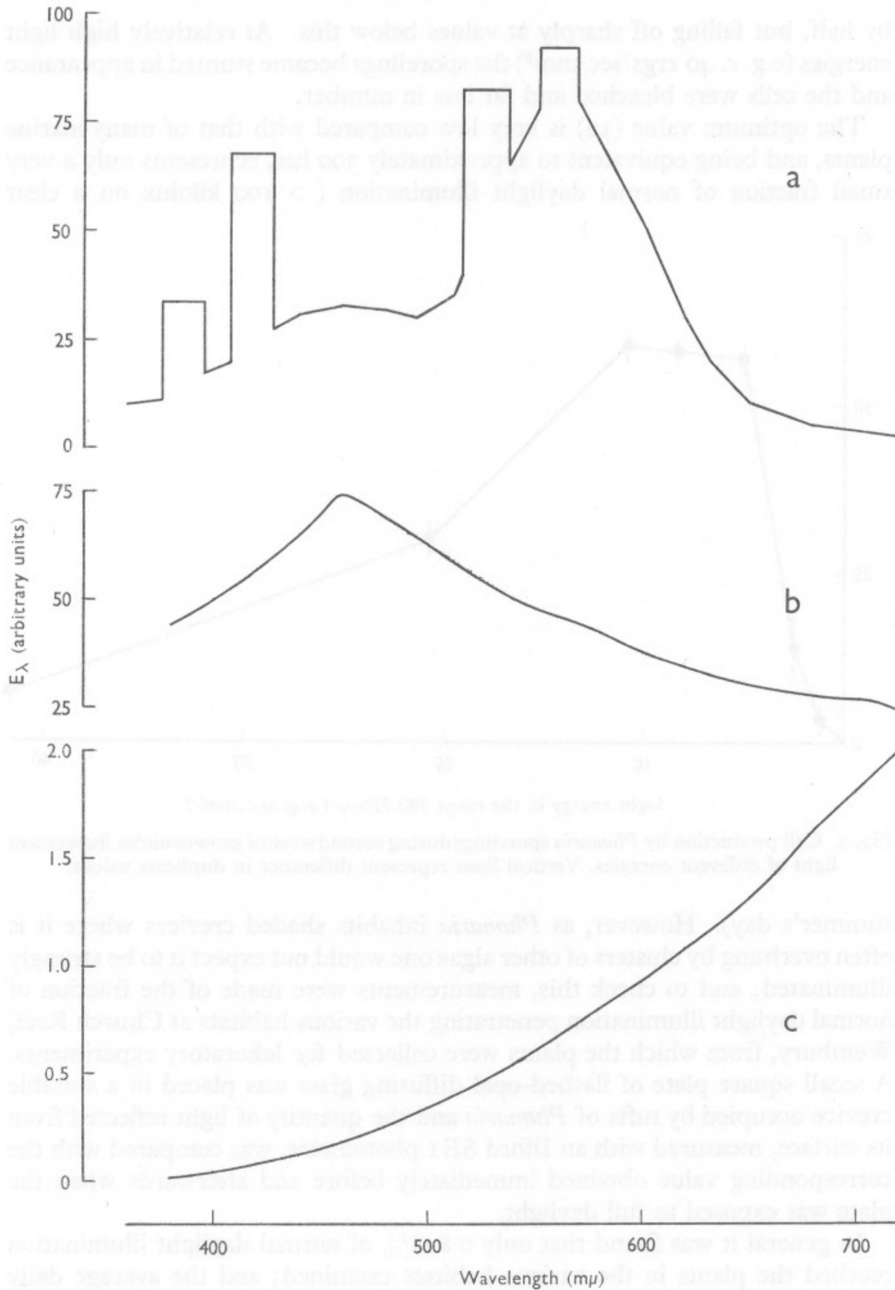

Fig. 2. Spectral energy distribution curves of the three light sources. (*a*) Mazda Daylight fluorescent tube (data supplied by manufacturers); (*b*) north window daylight (data from Le Grand, 1952); (*c*) tungsten-filament lamp (data from Skogland, 1929, for 500 W lamp with a colour temperature of 2500° K). The blocks of energy in (a) represent energy which is eally confined to much narrower wavebands.

by half, but falling off sharply at values below this. At relatively high light energies (e.g. *c.* 40 ergs/sec/mm²) the sporelings became stunted in appearance and the cells were bleached and far less in number.

The optimum value (10) is very low compared with that of many marine plants, and being equivalent to approximately 300 lux, represents only a very small fraction of normal daylight illumination (> 100 kilolux on a clear summer's day). However, as *Plumaria* inhabits shaded crevices where it is often overhung by clusters of other algae one would not expect it to be strongly illuminated; and to check this, measurements were made of the fraction of normal daylight illumination penetrating the various habitats at Church Reef, Wembury, from which the plants were collected for laboratory experiments. A small square plate of flashed-opal diffusing glass was placed in a suitable crevice occupied by tufts of *Plumaria* and the quantity of light reflected from its surface, measured with an Ilford SE 1 photometer, was compared with the corresponding value obtained immediately before and afterwards when the plate was exposed to full daylight.

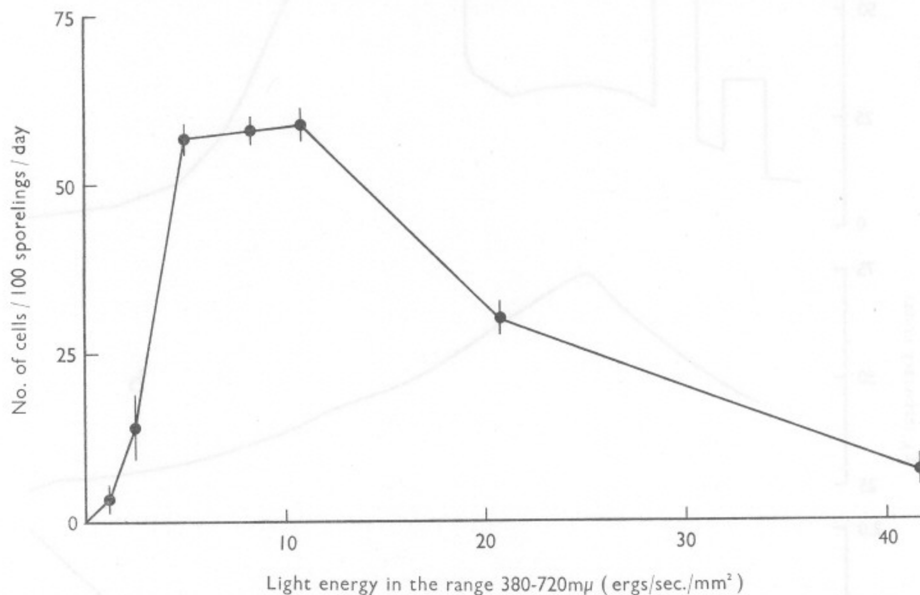

Fig. 3. Cell production by *Plumaria* sporelings during second week of growth under fluorescent light of different energies. Vertical lines represent difference in duplicate values.

In general it was found that only 0·8–8 % of normal daylight illumination reached the plants in the various habitats examined; and the average daily values in lux calculated for the month of October, when regeneration is active, were close to the optimum value (*c.* 300 lux) found in the laboratory experiments (see Table 1). The calculated May values were somewhat higher

(200–2000 lux) but as *Plumaria* sporelings are undoubtedly still further protected from light by the adult plant, and as the laboratory experiments were all carried out under continuous illumination, this result is hardly surprising. Moreover, it is interesting to note that when the incident light is maximal, between May and September, the production of sporelings is minimal.

Finally, concerning this low optimum light intensity for the growth of *Plumaria* sporelings, it might be mentioned that Sundene (1959) also found a low light intensity (500 lux) most suitable for culturing the related species, *Antithamnion plumula*.

TABLE 1. INTENSITIES OF ILLUMINATION IN VARIOUS HABITATS OCCUPIED BY ADULT *PLUMARIA* AT CHURCH REEF, WEMBURY

Daylight illumination values calculated from data described by Harvey (1955) for average illumination at Plymouth during the years 1930–37: May value, 25 kilolux; October value, 8 kilolux.

	Average daily illumination in lux	
Habitat	May	October
Rock crevice facing north	625	200
Shaded rock-shelf facing south-west	2000	600
Rock crevice facing south-west	800	250
Rock-shelf overhung with thick clusters of *Ascophyllum nodosum* and *Polysiphonia lanosa*	200	60

Experiments with phycoerythrin

The phycoerythrin used in the screening experiments was extracted from fronds of the adult plant by first rinsing them in tap water, then storing them in distilled water at room temperature in the dark for several days. The cherry-red aqueous extract was then filtered through an Oxoid membrane (pore diameter 0·5–1·0 μ) and phycobilins were then precipitated from the filtrate by adding an equal volume of a 50% solution of ammonium sulphate. The precipitate was concentrated by gentle centrifuging, suspended in distilled water, dialysed against glass-distilled water for 24 h, and the clear cherry-red solution so obtained then stored in the dark at 0° C until needed for use in the screening experiments. The absorption curve of the extract prepared in this way is shown in Text-fig. 4, where it is compared with the curve obtained by Haxo & Blinks (1950) for the pigment extracted from *Porphyra nereocystis*.

It will be seen that the pigment from *Plumaria* absorbed greatest in the range 480–570 mμ, with peaks at 495 and 560 mμ, as compared with corresponding values of 490 and 560 mμ obtained with the pigment from *Porphyra*.

Slides to which the week-old sporelings were attached were immersed in the culture medium contained in thin-walled glass vessels (80 ml.) and these in turn were placed centrally in small honey-jars (250 ml.) so that the distance

between the walls of the outer and inner vessel was always 2 cm. Sea water, to which sufficient phycoerythrin had been added to give a faint cherry-red tinge, was then run into the gap between the inner and outer vessel and the complete assembly was then placed at a measured distance from the light source for a further week, after which the total number of cells produced per 100 sporelings was estimated in the usual way. It was found that sporelings thus screened from the fluorescent light source grew more rapidly than unscreened sporelings at light intensities both above and below the optimum value (see Text-fig. 5).

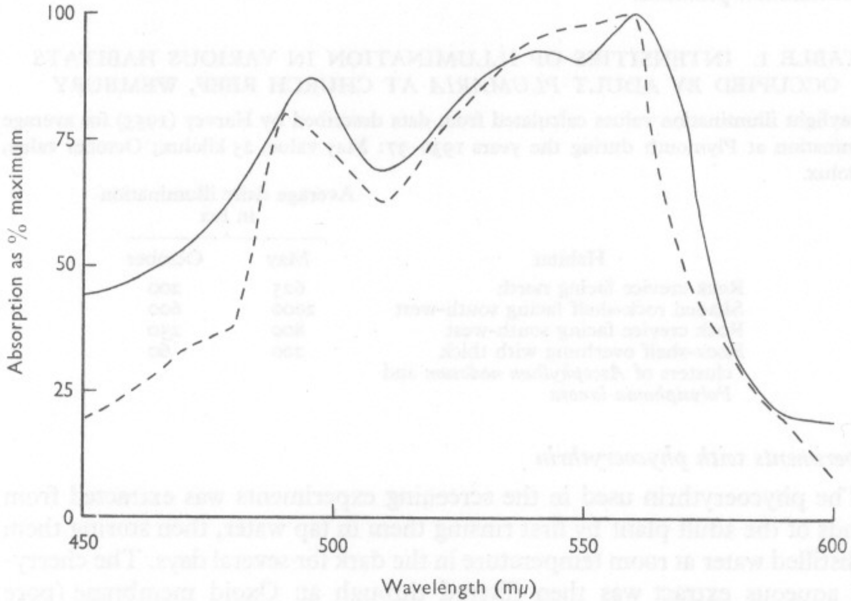

Fig. 4. Absorption curves of Phycoerythrin. (– – –), Pigment from *Porphyra* reported by Haxo & Blinks (1950); (——), pigment from *Plumaria*.

A particularly large stimulation of growth was observed at the lowest light intensity used (*c.* 1 erg/sec/mm^2), the screened sporelings growing at roughly three times the rate of the control sample. This finding therefore excludes the possibility that the screening solution was enhancing growth simply by shading the sporelings, for at suboptimal light intensities shading would obviously inhibit growth.

Experiments with fluorescent dyes

Because of the instability of phycoerythrin in sea water, screening solutions of the pigment had to be prepared freshly each day during the 7-day period of the above experiments. Accordingly, the need arose to find other substances

having a marked absorption in the region 480–570 mμ which would provide an adequate substitute for phycoerythrin. It seemed that dyes structurally related to fluorescein might prove appropriate and tests were therefore made with sea-water solutions of eosin yellow, eosin blue, sodium fluoresceinate and erythrosin.

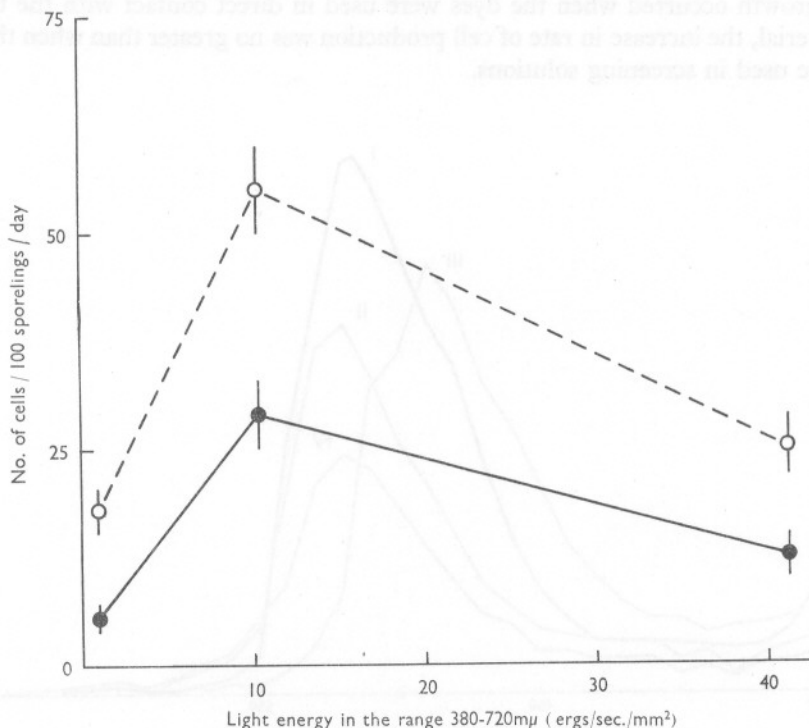

Fig. 5. Cell production by sporelings unscreened (●—●) and screened from the light source with a solution of phycoerythrin (○- - -○) at different light energies. Vertical lines represent differences in duplicate determinations.

The absorption curves of these compounds (each at a concentration of 10 mg/l.) are shown in Text-fig. 6, from which it will be seen that the curve for eosin yellow has a peak at 515 mμ approximately at the centre of the range of greatest light absorption observed with phycoerythrin. Screening solutions of all four dyes were tested for their effects on growth at a concentration of 0·2 mg/l., this being equivalent to an optical density ($\log_{10} I_{inc.}/I_{trans.}$) at 515 mμ identical with that of the phycoerythrin solutions used earlier. The results given in Table 2 show that the rate of cell production was significantly greater when sporelings were protected from the light source by all four dye solutions.

Sellei (1940) observed that very dilute solutions of fluorescein, when added

as a soil-dressing, markedly improved the growth of certain angiosperms. Accordingly, it was thought interesting to see if the dyes used in the present work would stimulate the growth of red algal sporelings when placed in the culture medium itself instead of in the surrounding screening solution. The results of this experiment, included in Table 2, show that although stimulation of growth occurred when the dyes were used in direct contact with the test material, the increase in rate of cell production was no greater than when they were used in screening solutions.

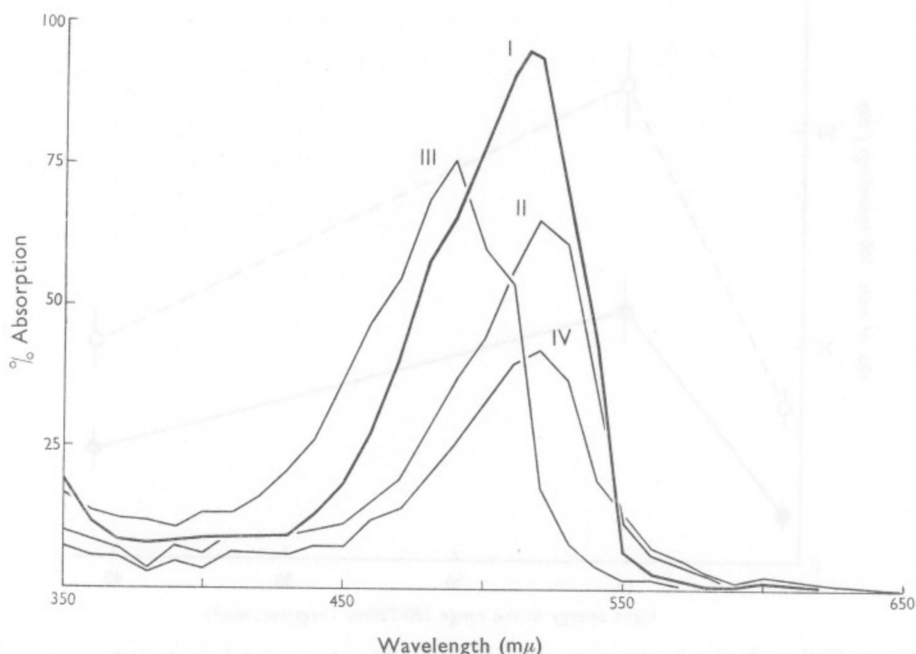

Fig. 6. Absorption curves of aqueous solutions of fluorescent dyes, each at a concentration of 10 mg/l., determined with Unicam spectrophotometer, SP 500. I, Eosin yellow; II, fluorescein; III, eosin blue; IV, erythrosin.

TABLE 2. EFFECT OF FLUORESCENT DYES ON THE GROWTH OF *PLUMARIA* SPORELINGS

Concentration of dye 0·2 mg/l. Other conditions as described in text.

	% increase in cell production compared with that of unscreened sporelings	
	Sporelings screened by dye solution	Sporelings immersed in dye solution
Eosin yellow	59·5	47·2
Fluorescein	41·5	38·6
Eosin blue	41·7	12·4
Erythrosin	13·0	10·5

Effect of different concentrations of eosin yellow

So far, the screening experiments had been carried out using only one concentration of the dyes and it therefore seemed advisable to see if other concentrations caused even greater stimulations of growth. Text-fig. 7*a* shows the results obtained in an experiment, carried out at the optimum light intensity, in which a range of concentrations of eosin yellow was used in the screening solutions. It will be seen that maximal stimulations of growth took place with concentrations of 0·2–0·5 mg dye/l., but that the effect completely disappeared when the concentration was raised to 2·0 mg/l. It seemed remarkable that such large stimulations of growth (> 100%) should be caused by such small quantities of dye in the screening solutions, concentrations which (as will be seen later) removed only minute fractions of the incident light. Accordingly, further experiments were made to see if the same result was obtained when different light intensities were used (as in earlier experiments with phycoerythrin); and also when the fluorescent lamp was replaced by other light sources.

Effect of screening with eosin yellow at different light intensities

The experiment was similar to that made earlier with phycoerythrin in that the growth rates of sporelings screened with dilute solutions of eosin yellow (0·2 mg dye/l.) were compared with those of unscreened sporelings at several light intensities. The results (see Text-fig. 8) showed that, as in the earlier experiment with phycoerythrin, screening with the dye solution caused marked stimulation of growth at light intensities both above and below the optimum value (10 ergs/sec/mm^2), a particularly large effect being observed at the lowest light intensity used.

Experiments with different light sources

All previous experiments had been done using continuous illumination from a fluorescent lamp. Therefore in order to use more natural conditions, experiments were made with April north window illumination. In these experiments the sporelings were not grown under continuous light nor could the light intensity or air temperature be controlled, as it was in earlier studies. Nevertheless, the results obtained (see Text-fig. 7*b*) were similar in that once again screening solutions which contained small concentrations of eosin yellow (0·04–0·4 mg./l.) were found to cause a marked stimulation of growth (> 80% with 0·2 mg dye/l.).

Similar studies were made at an optimum light intensity of 10 ergs/sec/mm^2 using as light source a tungsten-filament lamp (500 W; colour temp. = 2500° K) surrounded by a water-jacket. The results obtained (see Text-fig. 7*c*) were again in accordance with those of experiments with other light sources in that a stimulus of growth took place when the sporelings were

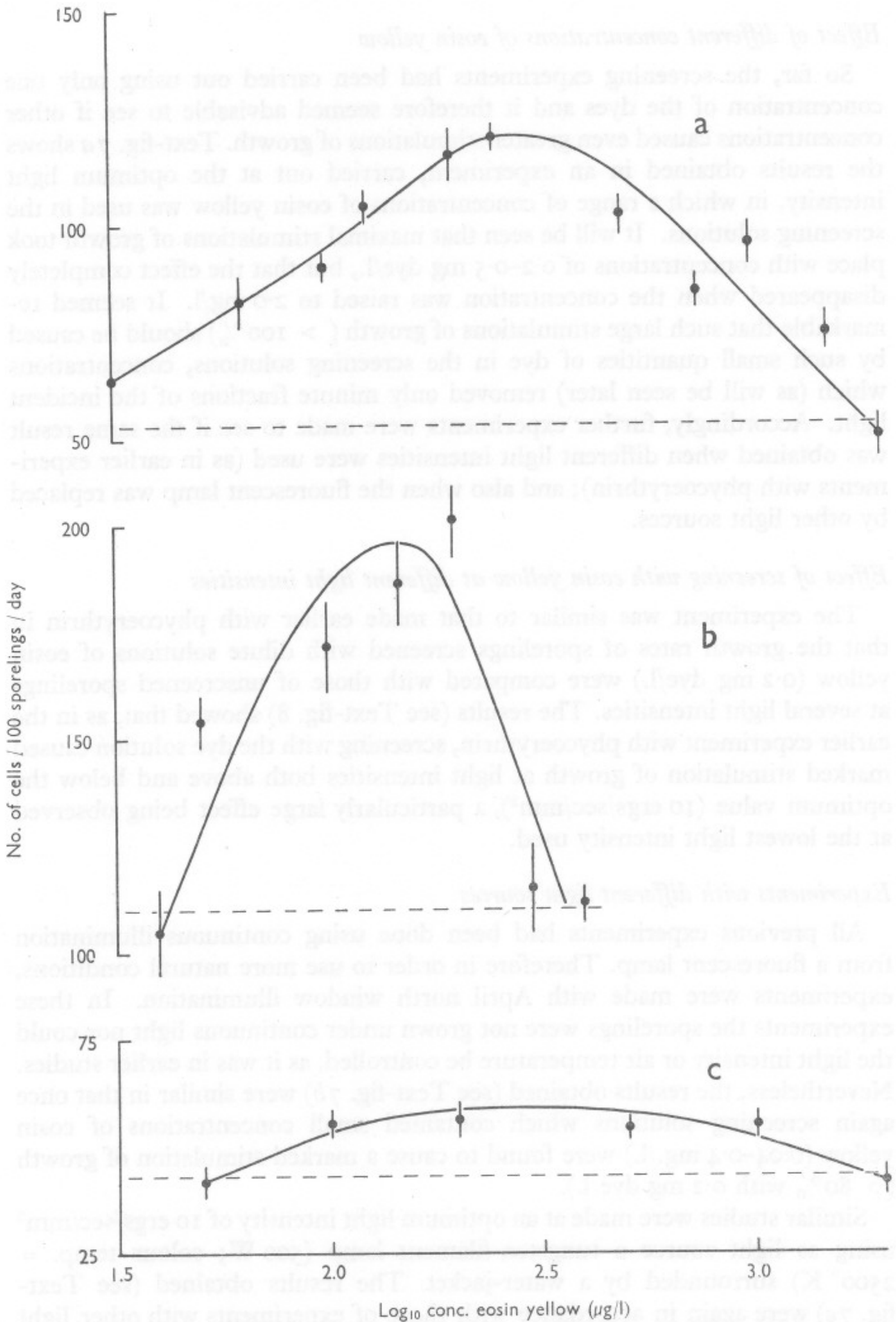

Fig. 7. Cell production by sporelings screened from various light sources by different concentrations of eosin yellow. (*a*) Fluorescent strip; (*b*) April north window illumination; (*c*) tungsten-filament lamp. Vertical lines represent standard deviations based on four determinations. Pecked lines represent cell production by unscreened sporelings used as controls.

screened by dilute solutions of eosin yellow (0·1–2·0 mg/l.). However, in these experiments the effect of the dye was much less marked in that the greatest stimulus of growth observed was little more than 30% (with 0·2 mg dye/l.).

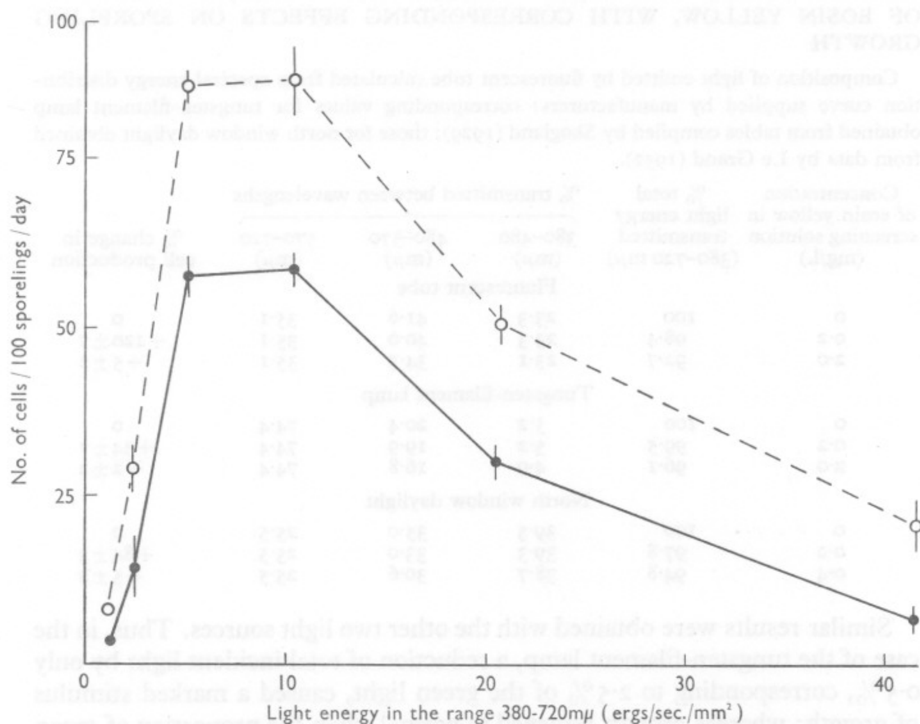

Fig. 8. Growth of unscreened sporelings (●—●) and those screened from fluorescent light source (○– – –○) at different light energies by a 2 cm thick solution containing 0·2 mg eosin yellow/l. Vertical lines represent differences in duplicate determinations.

From the spectral energy distribution curves of the three light sources (see Text-fig. 2) and the absorption curves (see Text-fig. 9) of eosin yellow at different concentrations (determined with a Beckman spectrophotometer, model DK-2) it was possible to calculate the proportion of light in different wave-bands of the visible spectrum removed by various screening solutions. These data, together with corresponding growth measurements, are shown in Table 3. It will be seen from this that in experiments with a fluorescent tube as light source, removal of only a very small quantity (1·6%) of the total incident light caused a marked stimulus of growth; furthermore, this light was removed exclusively from the green region of the spectrum, the proportion of light energy between 480 and 570 mμ being reduced from 41·6 to 40·0 (i.e. by 3·8%) without any change taking place in the proportions of blue or

red light. However, when the proportion of green light was reduced by a much greater amount (from 41·6 to 34·5, i.e. by 17%) growth of the screened material was practically normal.

TABLE 3. AMOUNTS OF LIGHT TRANSMITTED BY DILUTE SOLUTIONS OF EOSIN YELLOW, WITH CORRESPONDING EFFECTS ON SPORELING GROWTH

Composition of light emitted by fluorescent tube calculated from spectral energy distribution curve supplied by manufacturers: corresponding values for tungsten-filament lamp obtained from tables compiled by Skogland (1929): those for north window daylight obtained from data by Le Grand (1952).

Concentration of eosin yellow in screening solution (mg/l.)	% total light energy transmitted (380–720 mμ)	% transmitted between wavelengths			% change in cell production
		380–480 (mμ)	480–570 (mμ)	570–720 (mμ)	
		Fluorescent tube			
0	100	23·3	41·6	35·1	0
0·2	98·4	23·3	40·0	35·1	+120±7
2·0	92·7	23·1	34·5	35·1	+5±2
		Tungsten-filament lamp			
0	100	5·2	20·4	74·4	0
0·2	99·5	5·2	19·9	74·4	+34±7
2·0	96·1	4·9	16·8	74·4	−2±2
		North window daylight			
0	100	39·5	35·0	25·5	0
0·2	97·8	39·3	33·0	25·5	+84±3
0·4	94·8	38·7	30·6	25·5	+5±2

Similar results were obtained with the other two light sources. Thus, in the case of the tungsten-filament lamp, a reduction of total incident light by only 0·5%, corresponding to 2·5% of the green light, caused a marked stimulus of growth; whereas growth returned to normal when the proportion of green light removed was increased to 17%. Again, using north window daylight, a reduction of the total incident light energy by 2·2%, corresponding to 5·6% of the green light, led to a greatly increased cell production which, however, returned to normal when the green light was reduced by 23%.

Ultra-violet light as a possible factor influencing growth rate

As yet the possibility had not been excluded that the large stimulations of growth observed in experiments using a fluorescent lamp might be a result of the dye solutions removing ultra-violet (UV) light inhibitory to growth. To some extent this seemed unlikely because these stimulations of growth also occurred in studies with daylight and a tungsten-filament lamp as light sources. However, more direct evidence was considered necessary and this was obtained in the following experiments.

Qualitative studies were made using a method similar to that described by Denton (1955). The slit of a Hilger UV spectrograph was replaced by one

composed of two razor blades attached to a flat surface. This slit was illuminated by light reflected from a wide white screen placed at an angle of 45° to the axis of the spectrograph. Pl. I, B, *a* shows a spectrum made using a 5 sec exposure with the fluorescent tube used in the screening experiments. It will be seen that this source contained extremely little light in the near UV; furthermore, no light could be detected in the far UV, even in experiments in which much longer exposures were used.

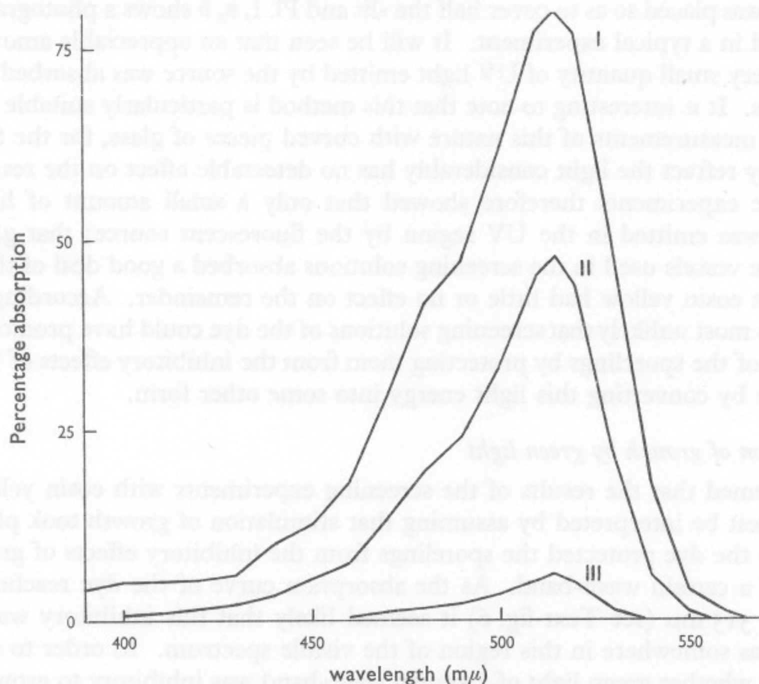

Fig. 9. Absorption curves for aqueous solutions of eosin yellow at different concentrations, determined with a Beckman spectrophotometer (DK-2): I, 5·0 mg/l.; II, 1·0 mg/l.; III, 0·2 mg/l.

Next, a small cuvette of 2 mm width made from two cover-slips was placed close against the slit and filled with a sea-water solution of eosin yellow (2 mg/l.) until the bottom half of the slit was covered. With this arrangement, exposures gave side by side the transmission of the test solution and that of an equal thickness of air. A photograph of this is shown in Pl. I B, *c*. By using exposures of various durations any required part of the spectrum could be made to give an intermediate density on the photographic plate suitable for critical comparison of the light transmitted by the two halves of the slit. In Pl. I, B, *c* the region between 365 and 406 mμ, and the lines at 334 and 313 mμ permit such a critical comparison from which it will be seen that the test solution absorbed negligible amounts of UV light. In this experiment, a

Wood's glass was placed between the light source and the cuvette to exclude the visible part of the spectrum: and if there had been any marked fluorescence of the eosin solution a dark band would have appeared in this region. However, no such band could be observed, even when very long exposures were used.

Using a similar method, the transmission of samples of glass from the vessels used in the screening experiments was examined. In this case, a piece of glass was placed so as to cover half the slit and Pl. I, B, *b* shows a photograph obtained in a typical experiment. It will be seen that an appreciable amount of the very small quantity of UV light emitted by the source was absorbed by the glass. It is interesting to note that this method is particularly suitable for making measurements of this nature with curved pieces of glass, for the fact that they refract the light considerably has no detectable effect on the result.

These experiments therefore showed that only a small amount of light energy was emitted in the UV region by the fluorescent source; that glass from the vessels used in the screening solutions absorbed a good deal of this; and that eosin yellow had little or no effect on the remainder. Accordingly, it seems most unlikely that screening solutions of the dye could have promoted growth of the sporelings by protecting them from the inhibitory effects of UV light, or by converting this light energy into some other form.

Inhibition of growth by green light

It seemed that the results of the screening experiments with eosin yellow could best be interpreted by assuming that stimulation of growth took place because the dye protected the sporelings from the inhibitory effects of green light of a certain wave-band. As the absorption curve of the dye reached a peak at 515 mμ (see Text-fig. 6) it seemed likely that this inhibitory wave-band was somewhere in this region of the visible spectrum. In order to test directly whether green light of a known wave-band was inhibitory to growth, experiments were carried out in which amounts of green light, comparable in intensity with that removed by the screening solution, were added to the light present in the fluorescent source. To obtain light of appropriate magnitude the following method was used.

Two white diffusing screens were arranged in the position normally occupied by the sporelings so that they could be illuminated respectively by the fluorescent source and by a 36 W tungsten-filament lamp. The screens were then observed side by side through a small artificial pupil, P (see Text-fig. 10). A green filter of known transmission was placed in front of the eye of the observer, an Ilford 604 being chosen because its fractional *transmission* roughly corresponds to the fractional *absorption* of the screening solution of eosin yellow which gave the maximum stimulus of growth. The distance of the fluorescent source from the screens was kept constant at a value equal to that used later in the actual experiment. The position of the tungsten lamp

was then adjusted until a visual match was obtained of the light intensities falling on the two screens, the transmission of the Ilford 604 filter being narrow enough to ensure that no appreciable difference in hue interfered with the match. The tungsten lamp was now fixed in this position, the filter (F 1) placed in front of it and the white screen replaced by a glass vessel containing a sample of the sporelings, conditions being such that these now received green light from the tungsten lamp which approximately replaced that removed by the screening solution of eosin yellow. To obtain a more exact

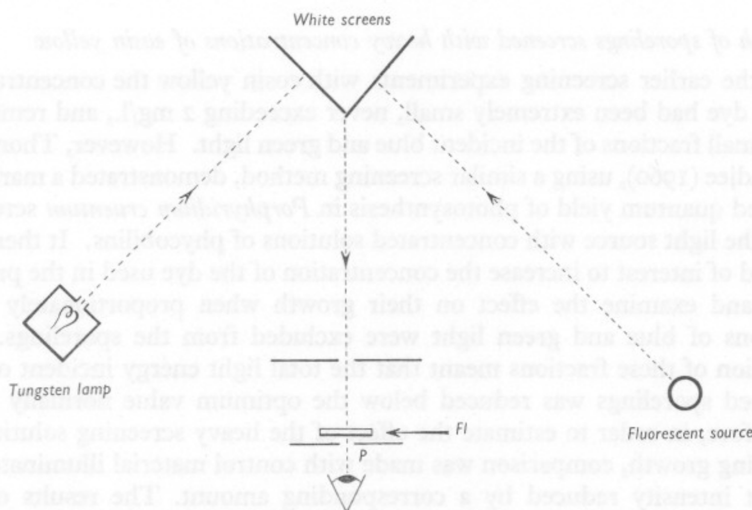

Fig. 10. Optical arrangement for adding measured amounts of green light to the fluorescent source.

replacement, and to vary the quantity of added green light, the tungsten lamp was moved by distances calculated from the known transmissions of the filter and screening solution; and the inverse square law. It may be noted that as the screening solution responsible for maximum stimulation of growth absorbed only 3·8 % of the green light passing through it (see Table 3) this did not appreciably diminish the intensity of the added green light supplied by the tungsten lamp.

TABLE 4. EFFECT OF GREEN LIGHT ON GROWTH OF *PLUMARIA* SPORELINGS

Screening solution contains 0·2 mg eosin yellow/l. Other conditions as described in text.

Condition of sporelings	Nature of light source	Growth (cells/100 sporelings/day)
Unscreened	Normal	25
Screened	Normal	47
Unscreened	Added green light	14
Screened	Added green light	18

Four samples of sporelings were used in the experiment. The first two received light from both the sources, one sample being screened (0·2 mg eosin yellow/l.; 2 cm thickness) and the other not; the second pair of samples, again one screened and one unscreened, received light from the fluorescent strip alone. After 10 days the cell production of the various samples was measured in the usual way and the results are shown in Table 4. It is clear that the additional green light greatly inhibited sporeling growth; also that its inhibitory effect was partially reduced by the screening solution of eosin yellow.

Growth of sporelings screened with heavy concentrations of eosin yellow

In the earlier screening experiments with eosin yellow the concentrations of the dye had been extremely small, never exceeding 2 mg/l., and removing only small fractions of the incident blue and green light. However, Thomas & Govindjee (1960), using a similar screening method, demonstrated a markedly reduced quantum yield of photosynthesis in *Porphyridium cruentum* screened from the light source with concentrated solutions of phycobilins. It therefore seemed of interest to increase the concentration of the dye used in the present work and examine the effect on their growth when proportionately large fractions of blue and green light were excluded from the sporelings. The exclusion of these fractions meant that the total light energy incident on the screened sporelings was reduced below the optimum value normally used. Therefore, in order to estimate the effect of the heavy screening solution on sporeling growth, comparison was made with control material illuminated by a light intensity reduced by a corresponding amount. The results of the experiment are shown in Table 5, from which it will be seen that, with all three light sources, these conditions caused no significant change in cell production by the sporelings. It should be noted that the very high concentration of dye used (100 mg/l.) had no effect on the quantity of yellow and red light transmitted, even though the quantity of blue light was drastically

TABLE 5. GROWTH OF *PLUMARIA* SPORELINGS SCREENED FROM DIFFERENT LIGHT SOURCES BY A HEAVY CONCENTRATION OF EOSIN YELLOW

Experimental details as given in text. Spectral composition of light sources as described in Table 3. Concentration of eosin yellow = 100 mg./l.

% light energy absorbed at different wavelengths in visible range				
% total (380–720 mμ)	% violet/blue (380–480 mμ)	% green (480–570 mμ)	% yellow/red (570–720 mμ)	% change in cell production
		Fluorescent tube		
50·5	90·0	71·0	0	2±4
		Tungsten-filament lamp		
18·7	83·9	70·9	0	4±4
		North window daylight		
57·5	78·0	74·6	0	3±6

reduced (e.g. by 90% in the case of the fluorescent tube), as was that of green (e.g. by nearly 75% in the case of north window daylight). The finding that exclusion of large amounts of blue and green light from the sporelings caused no significant change in their cell production indicated that yellow and red light were adequate for sustaining sporeling growth. To examine this aspect further, experiments were carried out to see if the sporelings would grow under different intensities of pure yellow light supplied by a sodium lamp.

Growth of sporelings under yellow light from a sodium lamp

Samples of the sporelings were placed at different distances from the sodium light and the 'lux' values for these various positions were measured with a Benjamin selenium photocell photometer. To find what these values mean in absolute units the following argument was used.

If a constant reading of the photometer is given when it receives a flux of E'_{Na} ergs/sec/mm^2 from the sodium lamp as well as from the fluorescent tube of intensity E'_λ ergs/sec/mm^2/mμ at the wavelength λ, then

$$E'_{Na} S_{Na} = \int_{380\ m\mu}^{720\ m\mu} E'_\lambda S_\lambda d\lambda.$$

Where S_λ is the known spectral sensitivity of the photometer (S_{Na} being the value for sodium light).

Now we can find the spectral energy distribution E'_λ in the way described on p. 68, and hence E_{Na} can be calculated. In this way it was found that when the photometer gave a nominal reading of 300 lux the energy of the sodium light was 7 ergs/sec/mm^2. For other nominal lux values the energy flux can be calculated because the same constant of proportionality, 1 erg $\equiv \frac{300}{7}$ lux, will hold for all intensities.

The results of the experiment are shown in Text-fig. 11, from which it will be seen that the sporelings grew quite well under the sodium light, cell production being roughly the same as that found in earlier experiments using equivalent amounts of light energy provided by the fluorescent tube. Once again it was found that when high intensities were used the cells became bleached and their growth was aberrant. However, unlike the results obtained with the fluorescent light source, high intensities of illumination from the sodium lamp, although they caused bleaching, did not cause a decrease in the number of cells actually produced.

PHOTOSYNTHESIS STUDIES

One explanation of the increased growth observed with sporelings screened from the light source by dilute solutions of either eosin yellow or phycoerythrin was that these plants had a much higher rate of photosynthesis. To examine this possibility measurements were made of the oxygen produced by

screened and unscreened *Plumaria* at 16° C, under continuous illumination from a fluorescent tube and at a known light intensity. For reasons given earlier, sporelings could not be used in these experiments, which were therefore made with tufts of the adult plant.

Methods

Immediately after the plant had been brought into the laboratory, fronds of suitable size (see later) were rinsed in filtered sea water then pulled slowly through Agar gel and stroked gently with a camel-hair brush to remove epiphytic diatoms and bacteria (Provasoli, private communication). The cleaned material was again rinsed in filtered sea water to remove any traces of the Agar gel and then transferred to Winkler bottles (70 ml. capacity) containing

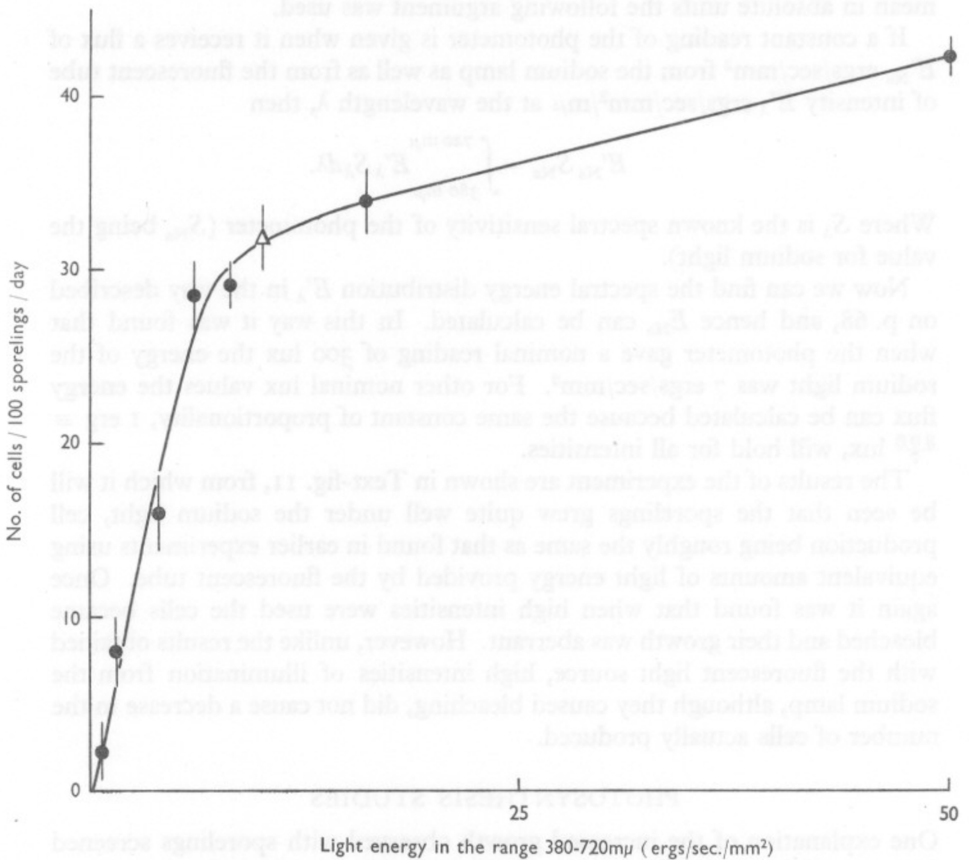

Fig. 11. Growth of sporelings under different light energies of yellow light from a sodium lamp (●—●). Δ gives reference value for optimum light energy from fluorescent source. Vertical lines represent differences in duplicate values.

sea water from which suspended material had been removed by filtration through an Oxoid membrane (pore diameter 0·5–1·0 μ). Stoppered bottles containing the plant tissue, and others with sea water alone (used as controls), were then placed under a series of light intensities determined by distance from the light source. Additional samples, together with sea-water controls, were kept in total darkness. After 24 h the bottles were unstoppered, the plant removed, and the oxygen present in the sea water in each bottle estimated by the Winkler method.

It was found that the oxygen content of the sea water used in the controls usually fell by about 20% but that in the presence of plant tissue it greatly increased, sometimes by as much as 300%. During preliminary experiments using sea water of about average oxygen content (6 c.c./l.) there was evidence of supersaturation in that some of the oxygen produced by photosynthesis collected as bubbles over the surface of the plant and did not dissolve in the sea water. To avoid this, the oxygen content of the sea water was reduced to 3–4 c.c./l. before the experiment was started, and the amounts of plant material used were relatively small (*c.* 20 mg dry wt.). A further reason for using only small amounts of plant material was that if the quantity was increased beyond 30 mg dry wt., oxygen production was markedly reduced, presumably because the sample was too large to receive uniform illumination.

Results

It was found that the compensation point (i.e. no evolution or absorption of O_2 or CO_2) of adult *Plumaria* at 16° C was 50 lux ($\equiv$ 1·6 ergs/sec/mm^2 in the range 380–720 mμ); that light saturation was reached at a light energy of 75 ergs/sec/mm^2, as compared with values of 30–50 reported by Haxo & Blinks (1950) for the shade-loving species *Delesseria decipiens*; and that the oxygen production at this light energy was 166–176 μl. O_2/mg dry wt. of plant/day (see Text-fig. 12). Assuming a respiratory quotient of unity, and knowing the dry wt. of *Plumaria* to account for 16–20% of the fresh wt., the assimilation of CO_2 may be calculated as a mean value of 2·6 mg CO_2/g. fresh wt./h: and it is worth noting that this value compares reasonably well with that of 3·90 reported by Bidwell (1958) for the marine red alga, *Polysiphonia lanosa*.

Further experiments were carried out to compare the amount of oxygen produced by unscreened plants with that from plants screened from the light source by very dilute solutions of eosin yellow which had given rise to a greatly increased growth rate in the earlier experiments with sporelings. In the present study, carried out at 16° C, the weights of plant tissue were all in the range 15–25 mg dry wt., and each determination of O_2 production was made with four duplicate samples of the plant placed under continuous illumination from the fluorescent strip for 24 h at a light energy of 75 ergs/sec/mm^2 (in the range 380–720 mμ). The results of these experiments are shown in Table 6,

from which it will be seen that screening with the dye solutions caused no significant increase in the total quantity of oxygen produced.

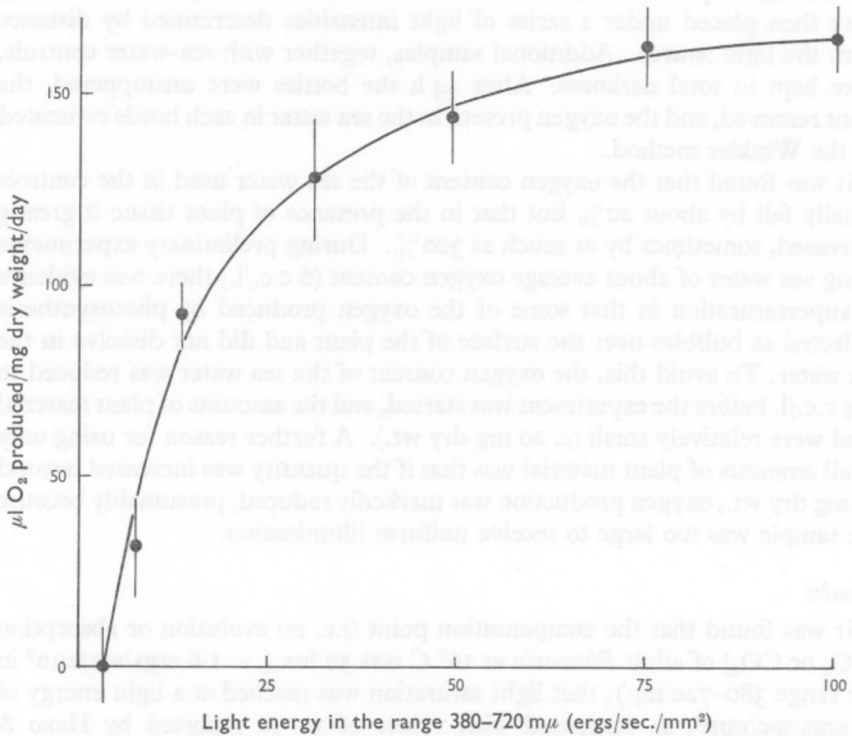

Fig. 12. O_2-Production by adult *Plumaria* under different light energies from the fluorescent source. Vertical lines represent standard deviations based on four determinations.

TABLE 6. OXYGEN PRODUCTION BY ADULT *PLUMARIA*

Experimental details as given in text.

Concentration of eosin yellow in screening solution (mg/l.)	Total oxygen produced (μl./mg dry wt. of plant/day)
0	176 ± 5
0·02	172 ± 3
0·05	182 ± 6
0·10	188 ± 8
0·20	172 ± 6

DISCUSSION

It is essential to the theory of complementary chromatic adaptation in its original form that blue and green light, absorbed by accessory pigments such as phycoerythrin, is vitally important for photosynthesis in the red algae,

which cannot use chlorophyll alone for this purpose. For this reason one would expect such plants, when screened from the light with concentrated solutions of phycobilins (or dyes such as eosin yellow), to be less efficient at photosynthesizing, and attention was drawn earlier to demonstration of this by Thomas & Govindjee (1960), using *Porphyridium cruentum.* However, although these authors found *photosynthesis* to be less efficient in these conditions, present studies with *Plumaria* sporelings have shown that *growth* is unaffected, even when the incident green light energy is reduced by about 75%. In addition, whereas Thomas & Govindjee observed no changes in photosynthesis by *Porphyridium* screened with very dilute solutions of phycobilins, these conditions were found to cause a marked increase in sporeling growth in experiments with *Plumaria*, a finding substantiated by the further observation that when small amounts of green light were added to the source sporeling growth was greatly inhibited.

A possible explanation of these differences is that the very short periods used in measurements of red algal photosynthesis do not allow the plants sufficient time to become adapted to the experimental conditions, whereas in growth experiments this adaptation can and does take place. Thus, Yocum (1951), using discs of thalli of *Porphyra perforata*, found that growth was at first fast in green light, but slow in red. However, after several days, growth increased in red light; and when these light-adapted thalli were tested for photosynthesis the activity of the chlorophyll *a* was greatly enhanced, the action spectrum now showing a distinct 'peak' at wavelengths where this pigment has maximum absorption (685 mμ). Evidence of light-adaptation in *Plumaria* was obtained in experiments in which sporelings of the plant were found to show normal growth under illumination from a sodium lamp at a region of the spectrum (580 mμ) which is only poorly absorbed by either phycoerythrin or chlorophyll *a*. However, as in most red algae, the concentration of the chlorophyll in *Plumaria* is far higher than that of phycoerythrin and therefore, under these conditions, chlorophyll was probably more active in photosynthesis.

In general, the present findings, and those of Yocum, are consistent with the view that a red alga such as *Plumaria*, which inhabits the mid-littoral zone and is therefore accustomed to normal daylight illumination, has become adapted to these conditions by making use of chlorophyll for the purposes of photosynthesis. Interest then attaches to the role—if any—of the phycoerythrin in these plants.

Concerning this question, present findings indicate that the function of this pigment is to protect the plant from the inhibitory effects of green light in the wave-band 500–540 mμ. However, it is interesting to note that only a very small fraction of this green light needs to be removed from the source to cause a marked increase in growth (just as only a correspondingly small fractions needs to be added to the source to inhibit it); and that if this

fraction of green light removed is increased the growth-stimulating effect disappears. It therefore seems that the effect can only be sustained by a critical balance of wavelengths in the incident light, the crucial factor being the amount of light energy emitted in the region 500–540 mμ as a proportion of that emitted at other wavelengths. This is further borne out by the fact that the effect is observed over a wide range of light energies, including some below and some above the optimum value for sporeling growth. Obviously, as these total light energies are varied so too is the amount in the wave-band 500–540 mμ. However, this quantity, as a fraction of the total, which is the crucial factor, remains constant and so the effect is still found. Further evidence of the importance of this balance of wave-lengths is provided by the observation that the stimulation of growth caused by removing very small amounts of green light from the source varies considerably with the composition of the incident light. Thus, in experiments with a fluorescent tube the effect was found to be roughly three times as great as that observed in similar studies using a tungsten lamp; which again emphasizes that the effect must depend on a critical balance between light in the region 500–540 mμ, and that emitted at other wavelengths.

Why the relative amounts of green light of a particular wave-band present in the incident light should have such a powerful influence on sporeling growth is not yet understood. Nor is it easy to see why photosynthesis remained unaffected, no measurable change in oxygen production being observed when the adult plant was kept under experimental conditions that caused a great stimulation of sporeling growth. Possibly the comparatively short period of the photosynthesis experiment (24 h) may have meant that the plants had insufficient time in which to adapt themselves to the various conditions; and in any case, photosynthesis by the sporelings themselves would obviously provide a better means of comparison. It is hoped that these will eventually be obtained in better quantity, and free from contaminating diatoms and bacteria, so that they can be used for long-term photosynthesis experiments in future studies.

One explanation of the remarkable stimulation of sporeling growth observed in the present screening experiments is that compounds responsible for controlling this growth may be less effective under conditions where the incident light in the region of 500–540 mμ is reduced as a fraction of the total. This could be either because the production of these compounds is inhibited or because they are particularly sensitive to the spectral composition of the incident light. Concerning this possibility it is worth noting that fluorescent dyes, especially eosin and fluorescein, have been found to promote root growth in various land plants when applied in certain concentrations (Morretes & Ferri, 1954; Zsolt, 1947; Szabolics, 1949), an effect ascribed to changes caused by the photosensitivity of auxins to the fluorescent substances. These experiments were carried out in a way totally different from that described

in the present work: nevertheless, an investigation of the hormonal control of *Plumaria* growth, and especially its relation to the spectral composition of the incident light, might be of interest, and a future study of this nature is planned.

It seems probable that the role of phycoerythrin in marine red algae is closely related to their ecological distribution. Thus, plants completely submerged and thereby cut off from most red light use their accessory pigment for photosynthesis (energizing chlorophyll *a* indirectly); but plants adapted to long periods of daylight rely to a much larger extent on chlorophyll *a* itself for photosynthesis and utilize their accessory pigment mainly as protection against inhibitory green light. If this be true, one might expect species which are even more exposed than *Plumaria* to strong daylight to be better adapted to using chlorophyll *a* for photosynthesis; and that consequently the protective effects of the accessory pigment in these species might be even more marked. On the other hand, plants which are much less exposed than *Plumaria* to daylight might still use their accessory pigment for the purposes of photosynthesis (together with chlorophyll *a*) so that its protective function in these species is of little importance. Because of these considerations it was thought necessary to extend the present investigation to include similar studies with other species, and a report of these findings is the subject of a second paper.

We are deeply indebted to Dr E. J. Denton for much useful advice and discussion, and for his considerable help in the preparation of the text; to Dr H. W. Harvey, F.R.S. for his encouraging interest; and to Mr F. J. Warren and Mrs Ann Peace for their invaluable technical assistance.

SUMMARY

Sporelings of the marine red alga *Plumaria elegans*, grown under continuous illumination from a fluorescent tube, show a maximum rate of cell production at a light energy flux of 10 ergs/sec/mm^2 in the range 380–720 mμ. This amount of light energy is low, but compares closely with that illuminating *Plumaria* in its natural habitat at Wembury during the period of the year when the plant is producing spores.

Growth of the sporelings is greatly increased when they are screened from the light source by weak solutions of phycoerythrin extracted from the adult plant; and this effect is observed over a wide range of light energies, including some below and some above the optimum value for growth.

The same result is obtained when phycoerythrin in the screening solutions is replaced by an equivalent concentration of the dye eosin yellow (0·2 mg/l.) absorbing 3·8 % of incident green light in the wave-band 480–570 mμ. However, as the concentration of dye is increased this stimulation of growth

diminishes until, when the quantity of green light absorbed exceeds 17 %, the effect is no longer observed and cell production is normal.

Similar effects are found in screening experiments with sporelings illuminated by April north window daylight; and, to a lesser extent, in experiments with a tungsten-filament lamp as light source.

Cell production is normal in sporelings screened from all three light sources by very heavy concentrations of eosin yellow (100 mg/l.) which remove approximately 75 % of the incident green light; and sporeling growth is also well maintained by pure yellow light from a sodium lamp.

Very weak screening solutions of eosin yellow which cause greatly increased cell production by the sporelings have no detectable effect on O_2 production by the adult plant, which remains unchanged at 171–181 $\mu l\ O_2$/mg dry wt./day at a light saturation value of 75 ergs/sec/mm^2 in the range 380–720 mμ.

The screening solutions do not stimulate growth of the sporelings by protecting them from ultra-violet light. On the other hand, evidence that they act by reducing the fraction of incident light in the wave-band 480–570 mμ has been confirmed by the finding that supplementing the fluorescent source with green light in the wave-band 500–540 mμ greatly retards sporeling growth.

The results suggest that although mid-littoral algae such as *Plumaria* may have no need of phycoerythrin as an accessory pigment in photosynthesis, it could still have an important function as a means of protecting the plants against excess green light in the wave-band 500–540 mμ, early stages of plant growth being dependent on a critical balance between energy in this wave-band and that present at others in the incident light. This view is discussed with especial reference to the theory of complementary chromatic adaptation, and future studies are indicated.

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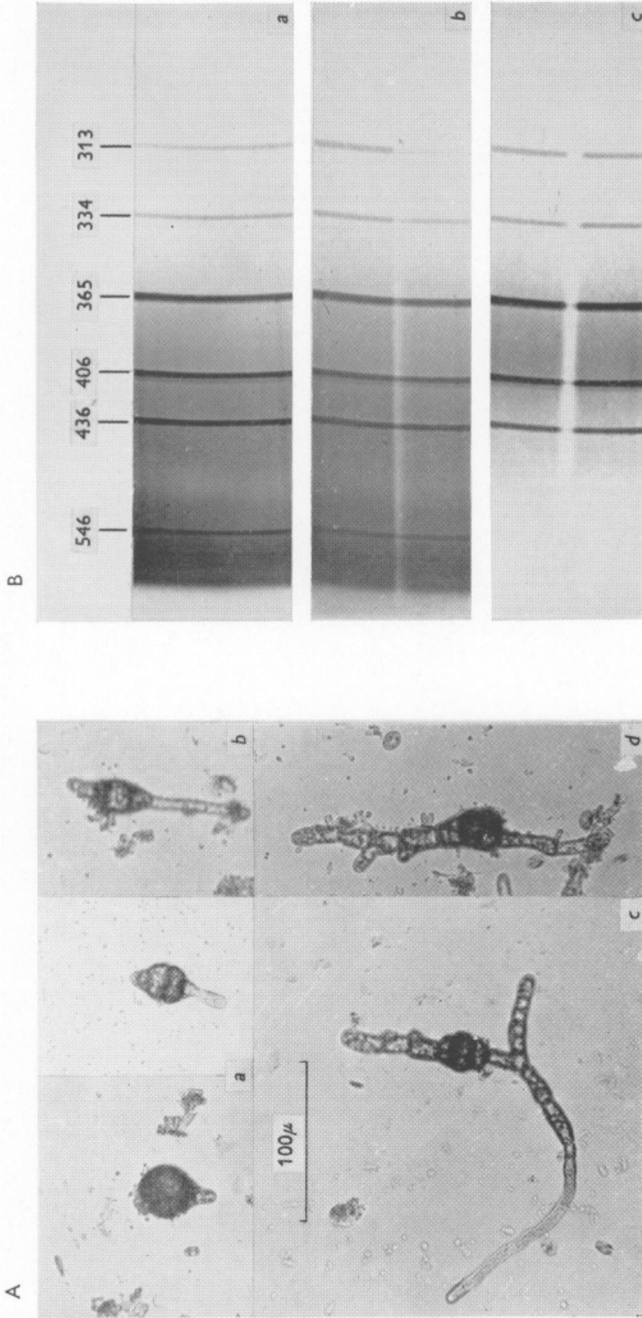
EXPLANATION OF PLATE I

A. Development stages of *Plumaria* sporelings. For explanation see text.

B. (*a*) Spectrum of Mazda 'Daylight' fluorescent lamp (5 sec exposure); (*b*) top half of slit uncovered; bottom half covered with glass from vessels used in screening experiments; (*c*) red and visible light excluded from source by OX7 filter. Top half of slit uncovered; bottom half screened with solution (1 mm thick) of eosin yellow (10 mg/l.).



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