Planta 63, 83—98 (1964)

From Tyco Laboratories, Waltham, Mass., and Harvard University, Cambridge, Mass.

INTERACTIONS BETWEEN DAYLENGTH AND LIGHT INTENSITY IN THE GROWTH AND CHLOROPHYLL CONTENT OF *ACETABULARIA CRENULATA*

By

JOHN TERBORGH and KENNETH V. THIMANN*

With 9 Figures in the Text

(Received June 22, 1964)

Introduction

Of the environmental variables regulating the development of higher plants one of the most important is the daylength. Research with algae, however, has given the impression that their performance is remarkably unaffected by the length of the daily light period, the few observed effects being, for the most part, explainable in other ways. True photoperiodism is typically characterized by: (a) induction, or continuation of the effect after transference to non-inducing photoperiods, and (b) sensitivity to short breaks in the light or dark periods.

Several reports of effects exerted on algae by the lengths of light and dark periods occur in the literature. LEAGUE and GREULACH (1955) found that the number of gametangium pairs counted in randomly selected microscope fields of *Vaucheria* cultures was considerably higher in those given 18-hour photoperiods than in those given 8-hour photoperiods. Similarly, MÜLLER (1962) found that the ratio of plurilocular to unilocular sporangia produced by the brown alga, *Ectocarpus*, could be increased by increasing the daylength at 16° C. However, both authors were able to raise the performance of their short-day cultures to that of the respective long-day cultures, in the first instance by adding glucose and meat peptone to the medium, and in the second by increasing the light intensity. In neither case was it possible to alter the short-day behavior by introducing light-breaks in the middle of the dark period. The effects of daylength on gamete germination in *Ulothrix flacca* and on gamete production in *Ulva lactuca* and *U. thuretti* reported by HYGEN

* Supported in part by a grant, No. GM 10599 from the National Institutes of Health to Profs. K. V. THIMANN and J. G. TORREY, and by a predoctoral fellowship from the same Institutes to JOHN TERBORGH. We wish to thank Dr. KURT BETH of the Stazione Zoologica, Naples, for many stimulating discussions with one of us (K.V.T.).

and FØYN, respectively (vide LEVERING, 1960) may also be attributable to differences in light quantity between treatments, though they do merit further investigation.

What appeared to be an absolute requirement for an alternating light-dark regime was described for the filamentous green alga, *Hydrodictyon*, by PIRSON *et al.* (1954) and PIRSON (1957). They found rhythms in growth, photosynthesis, and respiration which were trainable to a variety of light-dark regimes. Respiration was suppressed in the light and went through a maximum a few hours after the beginning of a dark phase, a finding which was used to account for the failure of growth in continuous light. By this interpretation the requirement of *Hydrodictyon* for a periodic regime would be a consequence of an interaction between its endogenous rhythms, and not a manifestation of true photoperiodism in the sense in which it operates in higher plants.

GOLUEKE (1962) studied the effects of a range of photoperiods varying from 10 to 24 hours per day on the efficiency of conversion of absorbed light energy to dry matter in *Chlorella*. Although short photoperiods resulted in somewhat higher efficiencies, they were less than 20% above those found in continuous light. GOLUEKE observed no qualitative effects of daylength, either on the photosynthesis or the growth characteristics of *Chlorella*.

In studying the interactions of light and temperature on the growth and morphogenesis (gametangium formation) of several species of *Acetabularia*, BETH (1955a) measured the growth in photoperiods of from 1 to 24 hours per day. The rate of stalk elongation was strongly dependent upon the duration of the illumination received up to 12 hours per day. In an attempt to determine the optimal photoperiod for growth he calculated the *efficiency* of growth as the elongation produced by 120 light hours under various regimes. His results, however, did not reveal any consistent relationships. Out of 10 experiments the maximum efficiency was found at a photoperiod of one hour in 2, at 12 hours in 2 and at intermediate levels in the other 6. Understandably, no attempt was made to draw conclusions from these results, which will be considered further in the discussion.

Although none of the studies mentioned above suggests that true photoperiodic effects occur among the algae, this possibility has been reopened by three recent developments.

1. In studies with the red alga, *Porphyra tenera*, IWASAKE (1961) and IWASAKE and MATSUDAIRA (1963) found that its life cycle could be regulated by daylength. The development of this alga consists of two phases having different growth forms. One, the filamentous *Conchocelis*, is found during the summer months in the vicinity of Japan and, cultured under long days, produces only the *Conchocelis* colonies. The other

phase, a leaf-like thallus, occurs during the winter months and in culture grows well in short days (8—11 hours). Given long days, however, the thallus shrivels and eventually disintegrates, releasing "carpospores" which germinate to form *Conchocelis* colonies. These then may produce two types of sporangia, one in continuous light or long days, another in short days. Spores from the former germinate to produce additional *Conchocelis* colonies, while those from short-day sporangia develop into the thallus form. These responses pertain to wide ranges of temperature and intensity and appear truly photoperiod-specific. Unfortunately, the authors' results are largely qualitative and do not include the above classical tests for photoperiodism.

2. The sensitivity of other red algae to photoperiod is implied by the recent finding of FRIES (1963) that *Goniotrichum* and *Acrochaetium* formed spores in a 12-hour regime but did not when given 18, 23, or 24 hours of illumination per day.

3. The discoveries of HAUPT (1959) and HAUPT and THIELE (1961) that chloroplast orientation in *Mougeotia* and *Mesotaenium*, respectively, is a red, far-red reversible reaction indicates the occurrence in algae of phytochrome, the reversible pigment operative in photoperiodism of higher plants, and opens the question of its physiological role in the thallophytes.

Among the green algae, *Acetabularia* has played a prominent role in studies of growth and morphogenesis, particularly with reference to RNA and protein synthesis (cf. VANDERHAEGHE, 1963). For this reason and because of its large size and stepwise development, this alga appeared ideally suited for an investigation of photoperiodic effects. Moreover, BETH's suggestive but inconclusive data on growth efficiency posed a need for a systematic study of its light physiology.

The present paper deals with the interactions of light intensity and daylength in promoting the elongation of *Acetabularia crenulata* cells in the phase of development prior to gametangium formation. It brings to light a definite dependence of growth on photoperiod and considers the relationships between the efficiency of growth and the daily irradiance as varied by intensity and photoperiod. A later paper will deal with the action of light on the morphogenesis of this alga.

Materials and Methods

The experiments described here utilized unialgal, but not axenic, cultures of *Acetabularia crenulata*. This is one of the species used in the well-known grafting experiments of HÄMMERLING (1953) and others. Stocks were grown essentially as by HÄMMERLING (1944) and BETH (1953) in Erdschreiber medium in cotton-stoppered Erlenmeyer flasks at 23° and given 8 hours of white light daily at 300 to 500 ft—c. Continuous growth was maintained in these cultures by periodic transfers to fresh medium.

In order to minimize variation in performance all the cells for each experiment were selected from a single stock culture. Suitable cells were isolated on the basis of their size, appearance, and ease of measurement. Variation in initial length was always confined within a 2.0 mm range. The number of cells in a growth run varied with the experiment from 20 to 32.

Normally, growth was measured as the increase in stalk length, but in a few cases total cell volumes were estimated from measurements of length and mean diameter by assuming a cylindrical shape. Since the growth rate follows an exponential timecurve, the only realistic measure of growth is a logarithmic one. Accordingly, the time required for a cell to double its length or volume, or the fraction of a doubling per unit of time, has been used throughout. Rates of doubling in length were calculated from the elongation in 6 to 10 day intervals and represent the mean values for two or more such intervals.

At the cell densities employed in these experiments (never more than 0.8 grams fresh weight per liter of medium) depletion of nutrients does not result in a decreased growth rate for 14 days or more. Accordingly, samples were transferred to fresh Erdschreiber every 6—10 days, at the time of each growth measurement. Bubbling air through light-saturated cultures failed to augment the growth, as did the addition of $NaHCO_3$. This is in agreement with the finding of ALLEN and ARNON with *Anabaena* (1955) that CO_2 limited the growth of unshaken cottonstoppered cultures only after tissue densities exceeded 6 grams per liter, or more than 7 times the maximum attained in our experiments.

Experimental conditions were provided in two types of light rooms with controlled temperature and humidity. One, maintained at 25° C, contained a bank of continuously operating daylight fluorescent tubes which gave intensities of 300 ft—c down to 12 ft—c depending on the distance from the source. It was calculated that 1 ft—c-hour of this light was approximately equivalent to 144000 ergs/cm². Desired photoperiods were imposed manually by placing the flasks in light-tight boxes for the specified periods each day. Two other rooms operated at 23° C and were programmed for daily light periods of 8 and 16 hours. The light sources in these were combined batteries of tungsten reflector-flood and mercury vapor lamps (General Electric RC 400) from which we were able to obtain intensities from 62 to 900 ft—c. Water temperatures in the flasks were maintained at 23° during the light period with the aid of heat filters where necessary.

Light intensities were measured with a special photocell having a hemispherical cap designed for gathering diffuse incident light (WASSINK and VAN DER SCHEER, 1951). Readings were taken with a Weston microammeter and converted to foot-candles by calibration with a Weston III exposure meter.

Chlorophyll determinations were carried out on samples of cells whose growth had been followed for a month or more under the specified regimens of light intensity and photoperiod. After harvesting, small groups of 5—8 cells were blotted on filter paper and weighed to 0.1 mg on a Roller-Smith torsion balance. These were then extracted in the dark at 4° C with 2 ml aliquots of methanol. Two one-day extractions sufficed to remove all the red-absorbing material. The absorption spectra of the combined extracts were measured in a Perkin-Elmer model 350 spectrophotometer. The ratio of the extinction at 661 mμ to the fresh weight of tissue extracted gave a relative measure of the total chlorophyll concentration.

Experimental Results

1. Cell volume as a function of cell length. *Acetabularia crenulata* grows and differentiates normally in continuous light or in a wide variety of light-dark regimes (BETH, 1953, 1955a and b). Prior to gametangium (cap) formation the cell consists of a thin cylindrical stalk having at its basal extremity a small (simple or branched) rhizoid or holdfast. Growth in volume results from the expansion of both the length and girth of the stalk. Increase in length occurs at a small apical growth zone while radial enlargement takes place along the entire axis of the cell. A doubling in length is accompanied by a constant 7 to 8-fold increase in volume over the cell length range of 5 to 22 mm (Fig. 1). This relation holds for plants growing under both long-day and short-day conditions (circles and crosses respectively in Fig. 1). Thus the fraction of a doubling in length occurring per day is a proportional measure of overall growth at any cell length and is a valid parameter for comparing samples grown under different light regimes.

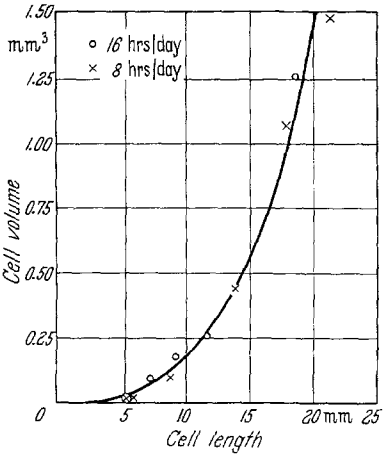

Fig. 1. Cell volume as a function of cell length during growth under 8- and 16-hour photoperiods. Daylight fluorescent illumination, 300 ft—c, 25° C

2. The rate of growth as a function of the intensity of illumination. The relation between growth rate and light intensity at 25° was measured in continuous fluorescent light at intensities between 12 and 300 ft—c (Fig. 2). A strongly light-dependent relationship holds below 55 ft—c, while between 55 and 300 ft—c the growth rate increases only another 28%, indicating that the threshold for growth saturation lies near 70 ft—c. Extrapolation of the steep linear portion of the curve to the abscissa gives an estimate of 8 ft—c as the compensation point for growth.

The results of a similar series of measurements carried out at 23° with the combined tungsten-mercury light sources and employing photoperiods of 8 and 16 hours are shown in Fig. 3. Under the 16-hour regime growth was saturated at 62 ft—c, the lowest intensity available, and was independent of intensity above this value up to 900 ft—c. Considering the somewhat different spectral composition of the light sources and a temperature difference of 2°, these results closely parallel those in Fig. 2 from the continuous light regime. The lower growth

rates in the present case are probably attributable to the difference in temperature.

Performance in 8-hour days differed in two respects from that in 16-hour days. The rate of elongation was limited by light intensity up to 225 ft—c, and above that value it remained constant at only two thirds the rate in 16-hour days. The slower growth in the 8-hour regime cannot be accounted for on the basis of light quantity, since at 900 ft—c

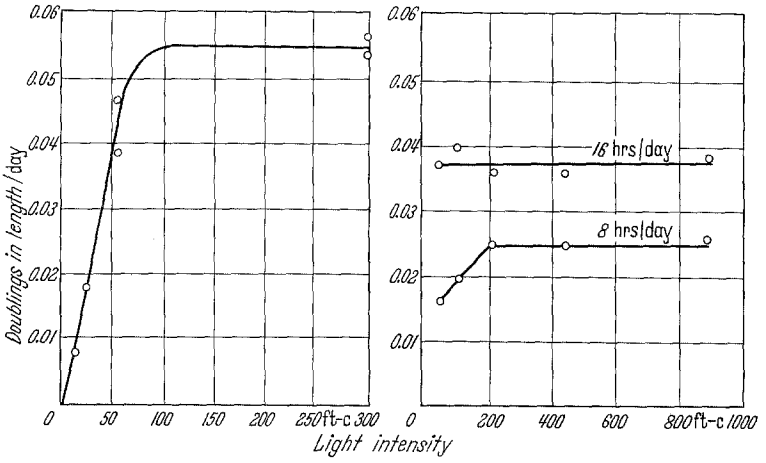

Fig. 2 Fig. 3

Fig. 2 and 3. The rate of doubling in length of *A. crenulata* cells as a function of the incident light intensity. Fig. 2: continuous daylight fluorescent illumination, 25° C. Fig. 3: 8- and 16-hour photoperiods; tungsten and mercury vapor lamps, 23—24° C

the elongation rate was only 65% of that in the 16-hour regime at 62 ft—c where only one eighth as much light was received per day. These results show that under low intensities and short photoperiods growth is limited both by the *intensity and duration* of the light received. When large daily light dosages are given in 8-hour periods, however, *only the duration* of the light period is limiting.

3. The growth rate as a function of photoperiod at different intensities. A second type of saturation curve for growth may be obtained by varying the photoperiod at constant intensity. At 300 ft—c which, as shown above, was well over the intensity required to saturate the growth rate in photoperiods of 8 hours or more, the duration of the daily light period was rate-limiting below about 12 hours and above this level had no effect (Fig. 4). Under these conditions the minimum photoperiod which supports growth is a little less than one hour per day. When intensities were employed that are growth-limiting when given continuously (55 and 25 ft—c in Fig. 4), growth was strongly dependent on photoperiod up to 24 hours per day. Again, with shorter photoperiods growth

is limited both by the intensity and duration of the illumination, as it was in the 8-hour regime below 225 ft—c. Extrapolation of these curves to the abscissa gives estimates of 12—15 hours for the minimum daily light periods required to support growth at the respective intensities.

Thus the rate of elongation of *A. crenulata* can be limited either by the intensity or the duration of daily illumination periods, or by both or neither depending on how these variables are manipulated (see Fig. 5). Intensity alone is limiting only with continuous light of less than approx-

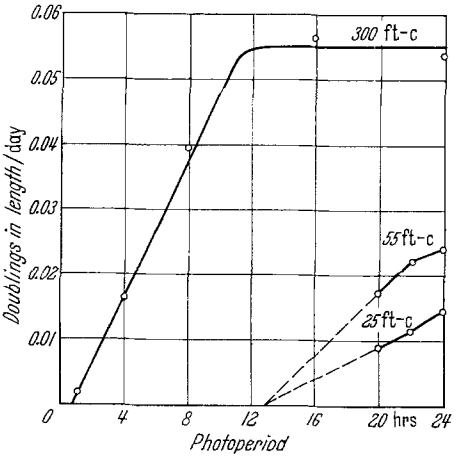

Fig. 4

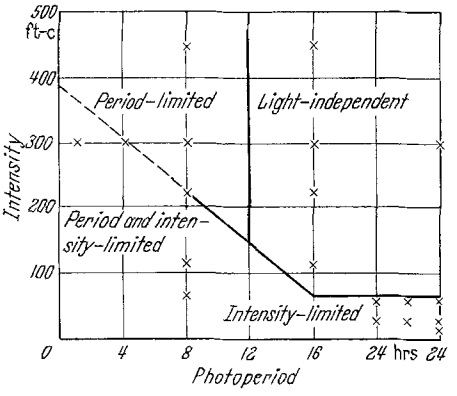

Fig. 5

Fig. 4. The rate of doubling in length of *A. crenulata* cells at intensities of 25, 55 and 300 ft—c as a function of photoperiod. Daylight fluorescent illumination, 25° C

Fig. 5. Limiting and non-limiting light regimes for the autotrophic growth of *A. crenulata*. Points correspond to the regimes represented in Fig. 2—4

imately 70 ft—c. Duration of the photoperiod alone can limit the growth only when photoperiods of less than about 12 hours are coupled with intensities of 150—300 ft—c or more. Both duration and intensity of illumination are limiting at intensities below 55 ft—c in periods of less than 24 hours, or at intensities up probably to 300—400 ft—c in very short periods. When moderate to high intensities are given for periods longer than 12 hours neither factor is limiting. Data relating the rate of growth to the total irradiance received per day in different photoperiods will be presented in the next section.

4. The efficiency of growth as a function of the daily irradiance. The data presented in Fig. 2, 3, and 4 can be recalculated to give the growth rate per unit of light energy (*i.e.* the fraction of a doubling in length produced by 1,000 foot-candle-hours of irradiance) as a function of the total daily irradiance. The resulting curves represent the *efficiency of incident light* in producing cell enlargement.

In continuous light the efficiency of elongation reached a well-defined maximum at irradiances corresponding to intensities of 30 to 70 ft—c (Fig. 6). Under these conditions the growth saturating intensity was approximately 70 ft—c. Thus the highest growth efficiencies were found in the upper part of the limiting intensity range. Below 25 ft—c the efficiency fell off with great steepness to zero at the compensating point for growth, and above 70 ft—c it decreased by a factor of about 2 with each doubling of the intensity.

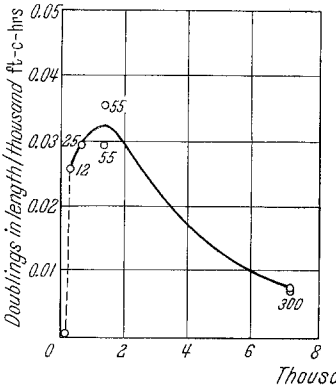

Fig. 6

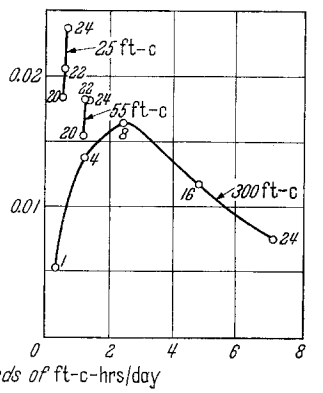

Fig. 7

Fig. 6 and 7. The efficiency of growth of *A. crenulata* cells (expressed as the fraction of a doubling in length produced by 10^3 ft—c—hrs of illumination) as a function of the daily quantity of illumination. Fig. 6: continuous daylight fluorescent illumination, 25° C. Numbers beside points indicate the light intensity (ft—c). Fig. 7: variable daylength, same illumination as Fig. 6; numbers beside points indicate the photoperiod. Curves represent experiments at three intensities, 25° C

Similar curves are plotted in Fig. 8 for cultures given 8- and 16-hour daily light periods. Since the lowest intensity used in these experiments was 62 ft—c, only the descending portions of the curves are seen. Comparisons between the two regimes at equal irradiances show that the elongation efficiency was 43 to 49% higher in 16-hour light periods. If, on the other hand, equal intensity is the criterion for comparisons, then the efficiency was 36 to 41% higher in the 8-hour light periods at all intensities above 225 ft—c. At the lowest intensities used, however, the short-day regime appeared slightly less efficient than the long-day regime in promoting elongation.

The last statement is illustrated more convincingly by the experiments of Fig. 7 in which a range of daily irradiances was obtained by varying the photoperiod at different constant intensities. At 300 ft—c the most effective photoperiods were about 8 hours, at which elongation was nearly three times as efficient as in continuous light. But if the light intensities were those which would have been limiting if given

continuously, then the reverse was the case, maximum efficiency using 55 or 25 ft-c being obtained in continuous light. The introduction of

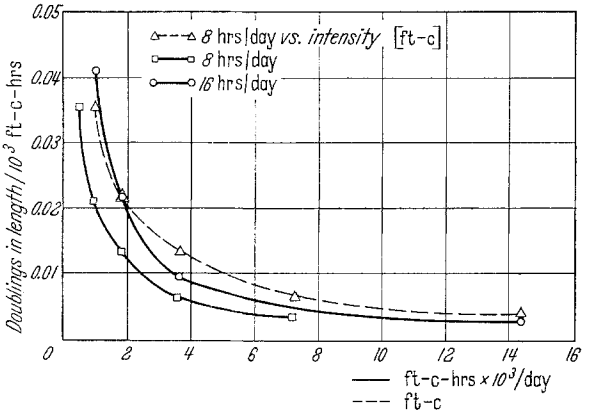

Fig. 8. The efficiency of growth of *A. crenulata* cells (expressed as the fraction of a doubling in length produced by 10^3 ft—c—hrs. of illumination) as a function of the daily quantity and of the intensity of illumination. Squares, growth in 8-hour periods; circles, growth in 16-hour periods; dashed line, growth in 8-hour periods plotted against light intensity (ft—c). Tungsten and mercury vapor lamps, 23° C

dark periods of only 2 or 4 hours resulted in appreciably lower values, particularly at 25 ft—c. It follows that the most efficient photoperiod for promoting the elongation of *A. crenulata* depends on the light intensity. Low (growth-limiting) intensities are most effective when given continuously, while higher intensities are most efficiently employed for shorter photoperiods. The limit of this trend appears to fall at about 8 hours at intensities of 300 ft—c or above, since the relative advantage of this photoperiod was not further increased by intensities up to 900 ft—c. On the other hand, a given *quantity* of illumination is always most efficient for growth when given as continuous light.

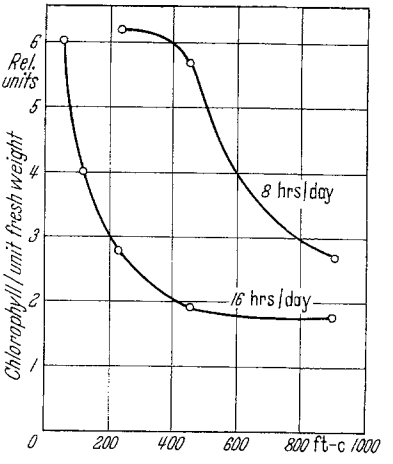

Fig. 9. The chlorophyll content of *A. crenulata* cells grown for 1 month or more under the indicated regimes, plotted as function of the light intensity. Tungsten and mercury vapor illumination, 23° C

5. Chlorophyll content as a function of the daily irradiance. During these experiments it became apparent that cultures growing in short photoperiods were more densely green than those grown in longer days.

The relative chlorophyll contents of groups of cells grown for a minimum of one month at various intensities in 8- and 16-hour photoperiods are shown in Fig. 9. Within each of the regimes the chlorophyll content varied with intensity over nearly a 3-fold range. The actual intensity range within which this "adaptability" was manifested, however, depended on the photoperiod, the half maximal chlorophyll contents being at 550 ft—c in the 8-hour regime and at 100 ft—c in the 16-hour regime. In addition to this intensity-dependent adaptation, the chlorophyll content of cells grown in 8-hour photoperiods was considerably greater than in those given 16-hour photoperiods over the whole intensity range studied. It can be seen in Fig. 9 that at no intensity (above 225 ft—c) does halving the intensity in a 16-hour day produce as great an increase as halving the daylength. Thus the effect of photoperiod on *A. crenulata* is to regulate not only its growth rate but also its pigmentation.

Discussion

The rate of elongation of *Acetabularia crenulata* cells under a wide range of light intensities and photoperiods can be limited by light in three distinct ways.

1. In the simplest case, with continuous illumination at intensities below 60—70 ft—c, intensity was limiting, presumably because of a low photosynthetic productivity.

2. When low to moderate intensities were given in a regime having a dark period, growth could be accelerated *either* by lengthening the light period *or* by raising the intensity. Such was the case in 8-hour photoperiods at light intensities less than 225 ft—c. Again, the basis of this type of limitation seems to be a deficient daily productivity.

3. Growth limitation of a third kind was found when moderate to strong illumination (≥ 225 ft—c) was given for 8 hours a day. Here only the *period* of illumination was limiting, the daily irradiance usually being more than adequate to sustain a higher growth rate if given in longer photoperiods. Thus short-day cultures grew more slowly than those given long days, even though they received up to *8 times as much light*. Plants limited in this manner characteristically possess higher chlorophyll contents than those given long days at the same intensity of daily irradiance.

The efficiency of growth per unit of incident light has been calculated as the fraction of a doubling in length produced by 1,000 foot-candle-hours of irradiance. This is a complex function, the value of which should depend on the relative contributions of three physiological parameters; 1. absorptivity, 2. the quantum yield of photosynthesis, and 3. the efficiency with which gross photosynthate is converted into growth, hereafter referred to as the "conversion efficiency". Optimal

growth efficiency is attained when all three functions reach a combined maximum.

In continuous light, maximum growth efficiencies were found at 30 to 70 ft—c. The relationship between efficiency and photoperiod, on the other hand, was less simple and depended on the intensity. Below approximately 70 ft—c continuous illumination was optimal, while at higher intensities the optimum photoperiod was shorter, reaching an apparent limit of roughly 8 hours above 225 ft—c. At 300 ft—c the efficiency of growth was nearly 3 times greater in 8-hour photoperiods than in continuous light. In part this difference is accounted for by the higher chlorophyll contents of cells grown in the shortday regime; however, growth efficiency might also depend on the availability of certain substances produced by light-independent processes. A dark period could allow such substances to accumulate and thus provide for a higher rate of photosynthesis than could occur in continuous light. Another possibility is that the efficiency of photosynthesis may be regulated by an endogenous rhythm. Such a rhythm has been described for *Acetabularia major* by SWEENEY and HAXO (1961), but its characteristics in different regimes were not investigated. In the case of *Hydrodictyon,* rhythms regulate both growth and photosynthesis (PIRSON, 1957) and must result in a cyclic fluctuation of growth efficiency throughout the day.

Supra-saturating irradiances always resulted in sub-optimal growth efficiencies. For example, growth in the 16-hour regime was only 7% as efficient at 900 ft—c as at 62 ft—c. This disparity probably reflects reduced values for all three of the parameters which influence the efficiency. For one, the chlorophyll content of cells grown at 900 ft—c is roughly 1/3 that of cells grown at the lower intensity. Secondly, the quantum yield of photosynthesis is known to decrease with intensity above the saturating level (RABINOWITCH, 1951). And thirdly, low conversion efficiencies were indicated by the observation that extracellular materials accumulated in cultures grown under high intensities. The release of organic substances has been noticed in diverse groups of algae given excess illumination (O'COLLA, 1962), and it might be a mechanism for disposing of surplus metabolites under conditions where net productivity outruns the demands of growth.

Working with several species of *Acetabularia,* BETH (1955a) was unable to show a regular relationship between the amount of growth produced by 120 hours of illumination and the length of the photoperiod. The discrepancies between his results and ours, however, can probably be accounted for by differences in methodology. For one, a systematic error is inherent in BETH's practice of comparing the net elongation of samples whose mean lengths were different over the period of measure-

ment. Since the absolute rate of elongation is strongly dependent on cell size, valid comparisons of growth between samples can be made only on the basis of the time needed for the size to double, *i.e.* the doubling rate (TERBORGH, 1963). Moreover, judging from BETH'S growth curves, he must have had to extrapolate to obtain a figure for the net growth in 120 light-hours from cultures given only 1 light-hour per day. It has been found in the present experiments that cells placed in severely light-limiting conditions grow 2—3 times more rapidly in the first week than they do a month later. For this reason, an extrapolation of initial growth rates under such conditions is likely to result in a large overestimate of the steady-state rate. This may perhaps explain why, in two instances, BETH found optimal efficiencies with a 1-hour photoperiod.

The chlorophyll content of *A. crenulata* cells has been found to show a dual dependence on the light regime. In short or long days the amount of chlorophyll per unit fresh weight of material varied inversely (over a 3-fold range) with intensity. Similar intensity "adaptations" have long been known for higher plants (*e.g.* GUTHRIE, 1929) and algae (*e.g.* MYERS, 1946; MYERS and KRATZ, 1955). Secondly, within the intensity range of 225 to 900 ft—c, *A. crenulata* plants given 8-hour photoperiods possessed 50—300% more chlorophyll than did those given 16-hour photoperiods. Effects of daylength on the chlorophyll content of algae do not appear to have been observed previously, although there are several such reports for higher plants. The problem has received most attention from SIRONVAL (1957) and CHAILAKYAN and BAVRINA (1957) who in most cases found that chlorophyll levels in daylength-sensitive plants were higher in the regime that promoted flowering. In view of exceptions such as the short-day plant, *Cannabis sativa*, which has more chlorophyll in long days (SIRONVAL, 1957), no generalizations have been proposed. Circadian rhythms also play a role in the chlorophyll metabolism of some plants. The chlorophyll content of *Perilla* has been found to rise and fall in a diurnal cycle and that of *Hyoscyamus* was shown to be sensitive to the timing of lightbreaks in an extended dark period (BÜNNING, 1959).

The effect of daylength on the chlorophyll content of *A. crenulata* can not be due to differences in the quantity of light received in the long- and short-day regimes. Moreover, it is of greater magnitude than the fluctuations observed in the diurnal rhythms of higher plants. Whether the effect can be modified by inserting supplementary doses of light into the regime remains to be determined.

Finally, the question remains as to whether the limitation of growth by the duration of the daily light period is intrinsically a photoperiodic phenomenon or is the consequence of a period-limited photosynthetic

productivity. The first of these possibilities was tested in a variety of experiments in which the dark period was interrupted with white, red, or far-red light or preceded by red or far-red light. *None* of these treatments significantly enhanced the growth of period-limited cultures. The second was tested by measuring the Q_{10} of elongation in experiments in which the temperature differential between samples was imposed only during an 8-hour daily light period. The resulting Q_{10}'s were as high as 2.1, and not appreciably lower than those found in long days; this suggests the interpretation that growth proceeds predominantly in the light. Thus, the amount of growth in an 8-hour period might be limited by the amount of photosynthate accumulated within that period at a maximum, temperature-limited rate of photosynthesis. The rate-limiting process in growth under these conditions would, of course, be non-photochemical. On the other hand, the same conclusion would apply if growth were regulated, either directly or via photosynthesis, by a light-trained diurnal rhythm.

Summary

The growth performance and chlorophyll content of *Acetabularia crenulata* have been examined under a variety of conditions of light intensity and photoperiod. This paper considers only the phase of cell enlargement which precedes gametangium formation or morphogenesis. The cells are filamentous at this stage and increase in volume through simultaneous elongation at an apical growth zone and radial expansion of the entire axis. As the cells elongate from 5 to more than 20 mm the ratio of length to volume remains constant and is the same for cells grown under 8- and 16-hour photoperiods. Thus rates of growth in different light regimes could be compared merely by measuring the rate of doubling in length.

The results show that:

1. Growth at 23 or 25° can be limited by light in 3 ways: 1. by low intensities (<70 ft—c) under continuous illumination, 2. by both the intensity and duration of the daily light period at low to moderate intensities, and 3. at intensities above 225 ft—c only by the *duration* of the daily light period. Given photoperiods of 12 hours or more and moderate to high intensities of illumination, growth was entirely independent of the daily irradiance.

2. The efficiency of growth per unit of irradiance was maximal at photoperiods or intensities just below those which sufficed to saturate the growth rate. At intensities below 60—70 ft—c optimal efficiency was attained in continuous light, but with higher intensities the most efficient photoperiod decreased to an apparent limit of roughly 8 hours

above 225 ft—c. The variation of growth efficiency with photoperiod is discussed.

3. The chlorophyll content of *A. crenulata* cells depended on both the intensity and the photoperiod under which the cells were grown. At any intensity above 225 ft—c in 16-hour photoperiods, however, the effect of halving the photoperiod was much larger than that of halving the intensity. The efficiency of growth under various conditions is considered in relation to the corresponding chlorophyll contents of the cells.

4. The growth rate of *A. crenulata*, while thus sensitive to photoperiod, does not appear to be subject to photoperiodic control in the same sense as applies to higher plants.

Zusammenfassung

An *Acetabularia crenulata* wurden der Ablauf des Wachstums und der Chlorophyllgehalt bei verschiedenen Lichtintensitäten und Lichtperioden untersucht. In der vorliegenden Arbeit wird nur die Phase der Zellvergrößerung berücksichtigt, die der Gametangienbildung, d.h. der Morphogenese, vorangeht. Die in diesem Entwicklungsstadium fadenförmigen Zellen vergrößern ihr Volumen durch Verlängerung an einer apikalen Wachstumszone und eine gleichzeitige Ausweitung über ihre gesamte Länge. Während die Zellen sich von 5 mm bis auf mehr als 20 mm verlängern, bleibt das Verhältnis von Länge und Volumen konstant und hat bei Photoperioden von 8 Std und von 16 Std den gleichen Wert. Demnach konnten die Wachstumsraten unter verschiedenen Lichtbedingungen durch einfache Messung der für eine Verdopplung der Länge benötigten Zeit verglichen werden.

Die Versuche führten zu folgenden Ergebnissen:

1. Das Wachstum bei 23° oder 25° C kann durch Licht auf dreierlei Art begrenzt werden: durch Dauerbelichtung mit niederen Intensitäten (unter 754 lx); durch die Intensität und die Dauer der täglichen Lichtperiode bei niedrigen und mittleren Lichtstärken; schließlich bei Intensitäten über 2422 lx lediglich durch die *Dauer* der täglichen Lichtperiode.

2. Die Wachstumsleistung pro Bestrahlungseinheit erwies sich als maximal bei Photoperioden oder Intensitäten gerade eben unter denen, die zur Sättigung der Wachstumsrate ausreichten. Bei Intensitäten unter 646—754 lx wurde die optimale Wirkung im Dauerlicht erreicht; dagegen erniedrigte sich bei höheren Intensitäten (über 2422 lx die wirksamste Länge der Photoperiode offensichtlich auf einen Grenzwert von etwa 8 Std. Die Abhängigkeit der Wachstumsleistung von der Photoperiode wird diskutiert.

3. Der Chlorophyllgehalt von *Acetabularia crenulata* hängt sowohl von der Lichtintensität wie auch von der Photoperiode ab, unter der die Alge kultiviert wurde. Bei allen Intensitäten über 2422 lx in 16 Std-Perioden war jedoch der Effekt einer Halbierung der Periodenlänge wesentlich größer als die Wirkung einer Erniedrigung der Lichtintensität auf die Hälfte. Die Wachstumsleistung unter verschiedenen Bedingungen wird in Beziehung zum entsprechenden Chlorophyllgehalt der Zellen betrachtet.

4. Obwohl sich demnach die Wachstumsrate von *Acetabularia crenulata* gegenüber der Lichtperiode empfindlich zeigt, scheint sie nicht im gleichen Sinne einer photoperiodischen Kontrolle unterworfen zu sein wie die höheren Pflanzen.

Literatur

ALLEN, M. B., and D. I. ARNON: Studies on nitrogen-fixing blue-green algae. I. Growth and nitrogen-fixation by *Anabaena cylindrica* LEMM. Plant Physiol. **30**, 366—372 (1955).

BETH, K.: Experimentelle Untersuchungen über die Wirkung des Lichtes auf die Formbildung von kernhaltigen und kernlosen *Acetabularia*-Zellen. Z. Naturforsch. 8b, 334—342 (1953).

— Beziehungen zwischen Wachstum und Formbildung in Abhängigkeit von Licht und Temperatur bei *Acetabularia*. Z. Naturforsch. **10**b, 267—276 (1955a).

— Unterschiedliche Beeinflussung von Wachstum und Teilung durch Veränderung von Licht und Temperatur. Z. Naturforsch. **10**b, 276—281 (1955b).

BÜNNING, E.: Diurnal changes in pigment content and in the photoperiodic efficiency of red and far-red. In: Photoperiodism (R. B. WITHROW, Ed.), p. 537—540. Washington 1959.

CHAILAKHYAN, M. KH., and T. V. BAVRINA: Effect of day length on pigment content of plant leaves. Plant Physiol. USSR (transl. Fiziol. Rast.) **4**, 301—309 (1957).

FRIES, L.: On the cultivation of axenic red algae. Physiol. Plantarum (Kbh.) **16**, 697—708 (1963).

GOLUEKE, C. G.: Over-all light energy conversion efficiency of a high temperature strain of *Chlorella pyrenoidosa*. Physiol. Plantarum (Kbh.) **15**, 1—9 (1962).

GUTHRIE, J. D.: Effect of environmental conditions on the chloroplast pigments. Cont. Boyce Thompson Inst. **2**, 220—250 (1929).

HÄMMERLING, J.: Zur Lebensweise, Fortpflanzung und Entwicklung verschiedener *Dasycladaceen*. Arch. Protistenk. **97**, 7—56 (1944).

— Nucleo-cytoplasmic relationships in the development of *Acetabularia*. Int. Rev. Cytol. **2**, 475—498 (1953).

HAUPT, W.: Die Chloroplastendrehung bei *Mougeotia*. I. Mitteilung. Über den quantitativen und qualitativen Lichtbedarf der Schwachlichtbewegung. Planta (Berl.) **53**, 484—501 (1959).

—, u. R. THIELE: Chloroplastenbewegung bei *Mesotaenium*. Planta (Berl.) **56**, 388 bis 401 (1961).

IWASAKI, H.: The life-cycle of *Porphyra tenera* in vitro. Biol. Bull. **121**, 173—187 (1961).

—, and C. MATSUDAIRA: Observation on the ecology and reproduction of free-living Conchocelis of *Porphyra tenera*. Biol. Bull. **124**, 268—276 (1963).

LEAGUE, E. A., and V. A. GREULACH: Effects of daylength and temperature on the reproduction of *Vaucheria sessilis*. Bot. Gaz. **117**, 45—51 (1955).

LEVERING, T.: Submarines Licht und die Algenvegetation. Bot. Marina **1**, 67—73 (1960).

MÜLLER, D.: Über Jahres- und Lunarperiodische Erscheinungen bei einigen Braunalgen. Bot. Marina **4**, 140—155 (1962).

MYERS, J.: Culture conditions and the development of the photosynthetic mechanism. III. Influence of light intensity on cellular characteristics of *Chlorella*. J. gen. Physiol. **29**, 419—427 (1946).

—, and W. A. KRATZ: Relations between pigment content and photosynthetic characteristics in a blue-green alga. J. gen. Physiol. **39**, 11—22 (1955).

O'COLLA, P. S.: Mucilages. In: Physiology and Biochemistry of algae, p. 337—356 (R. A. LEWIN, Ed.). New York: Academic Press 1962.

PIRSON, A.: Induced periodicity of photosynthesis and respiration in *Hydrodictyon*. In: Research in photosynthesis, p. 490—499. New York: Interscience Publ. 1957.

— W. J. SCHÖN u. H. DÖRING: Wachstums- und Stoffwechselperiodik bei *Hydrodictyon*. Z. Naturforsch. **9**b, 349—353 (1954).

RABINOWITCH, E.: Photosynthesis and related processes, vol. II, part 1, p. 964—1082. New York: Interscience Publ. 1951.

SIRONVAL, C.: The chlorophyll metabolism, a photoperiodical phenomenon. II. Internat. Conf. Photobiol., Torino 1957. p. 387—395.

SWEENEY, B. M., and F. T. HAXO: Persistence of a photosynthetic rhythm in enucleated *Acetabularia*. Science **134**, 1361—1363 (1961).

TERBORGH, J.: Studies on the growth and morphogenesis of *Acetabularia crenulata*. Thesis, Harvard University 1963.

WASSINK, E. C., and C. VAN DER SCHEER: A spherical radiation meter. Meded. Landbouwhogeschool Wageningen **51**, 175—183 (1951).

Prof. KENNETH V. THIMANN, The Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge 38, Massachusetts, USA