saure und Chlorogensaure. Chemiker-Zeitung 56: 991. 1932.
9. OPARIN, A. Das grune Atmungspigment und seine Bedeutung bei der Oxydation der Eiweisskorper in den keimenden Samen des *Helianthus annuus*. Biochem. Zeit. 124: 90–96. 1921.
10. ROBERTS, D. W. A. Physiological and biochemical studies in plant metabolism. IV. Effect of illumination and darkness on the physiological heterogeneity in the first leaf of wheat. Can. Jour. Bot. 29: 451–466. 1951.
11. ROBERTS, E. A. H. and WOOD, D. J. The polyphenols and amino acids of the tobacco leaf. Arch. Biochem. 33: 299–303. 1951.
12. SHIROYA, M., SHIROYA, T. and HATTORI, S. Studies on the browning and blackening of plant tissues. IV. Chlorogenic acid in the leaves of *Nicotiana tabacum*. Physiol. Plantarum 8: 594–605. 1955.

# ALGAL GROWTH IN CROSSED GRADIENTS OF LIGHT INTENSITY AND TEMPERATURE[1,2]

PER HALLDAL[3] AND C. S. FRENCH

DEPARTMENT OF PLANT BIOLOGY, CARNEGIE INSTITUTION OF WASHINGTON, STANFORD, CALIFORNIA

Two of the major factors that determine the growth responses of plants are the light intensity and the temperature. The effect of either of these variables on the growth rate is strongly influenced by the level of the other factor. A complete study of just these two variables, even for a single species, is a large-scale job.

The apparatus described here provides a visual picture of algal growth as a function of two factors varying along rectangular coordinates.

The principle is to use a thin layer of agar with algae growing on its surface. The agar layer is provided with a temperature gradient from left to right and a light intensity gradient from front to back. There is, at any given intensity, a range of temperature, and at any given temperature a range of intensities. Growth response is observed visually or photographed at intervals, thus adding the dimension of time.

Visual interpretation of algal growth response to the crossed gradients of light intensity and temperature are subjective and only roughly quantitative. Precision of the response estimate is improved if response is measured, for example, in terms of pigment formation (1, 2).

This paper gives details of construction and operation of the device which has been used to determine growth patterns of several unicellular algae (3, 4).

## APPARATUS

The base of the crossed gradient culture chamber is a $12 \times 16$ inch aluminum plate $\frac{3}{4}$ inches thick. The $11\frac{3}{8} \times 12$ inch center part of the plate, the actual growth chamber, is bounded by metal edges $\frac{1}{8}$ inch high and is painted with chemically inert white Tygon. A Lucite spacer resting on the ridge surrounding the growth chamber supports the cover, a shallow Lucite box, through which warm water is circulated to prevent condensation of water. Figure 1 shows these parts in cross section.

The aluminum base plate extends 2 inches on either side of the growth chamber. On each side transverse borings carry water from the back of the plate to the front and again through the plate to a rear exit port. The left edge of the plate is kept uniformly cold and the right edge at a high temperature by continuous circulation of water from two constant temperature baths. The flow of heat through the aluminum produces a temperature gradient across the plate, which is mounted on cork for thermal insulation. The cork lined frame is supported on leveling screws.

The light comes from three 300-watt projector spot lamps run from a voltage regulator. Just below the lamps is a Lucite tank containing a 4.5-inch-deep heat filter of distilled water supplied from a reservoir with an automatic level device.

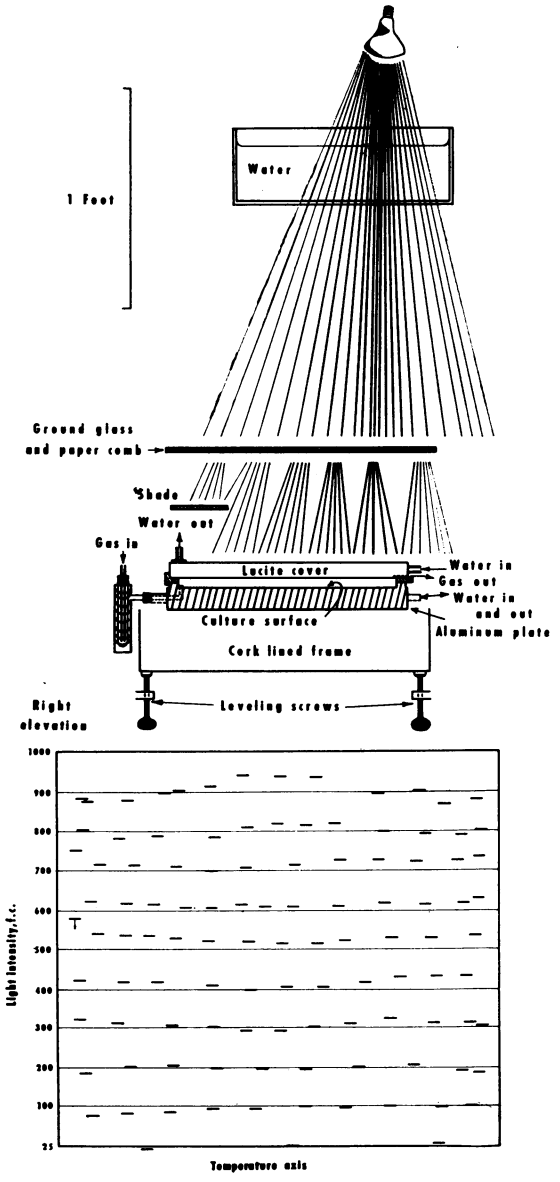
The intensity gradient on the culture surface is provided by directing the projector spot lamps toward the rear of a ground-glass plate, 4 inches above the agar surface. There is also a comb-shaped piece of translucent paper cut to appropriate shape by successive trials. The comb is on top of the ground-glass and is protected by a clear-glass plate. In addition to these arrangements for producing the intensity gradient, there is an opaque shade spaced between the ground-glass diffusing surface and the growth chamber. The arrangement of these parts is shown in figures 1 and 2.

Five percent $CO_2$ in air enters the growth chamber through 6 ports spaced along its front edge. Before entering these ports the gas stream, regulated by separate needle valves, bubbles through water in individual test tubes. To prevent the agar from drying out, the water is electrically heated to 80° and 90° C respectively in the tubes which saturate the gas entering the two high temperature ports.

[1] Received February 3, 1958.

[2] Dedicated to Professor Richard Harder, University of Göttingen, on the occasion of his 70th birthday, March 21, 1958.

[3] Present address: Botanical Laboratory, Lund, Sweden.



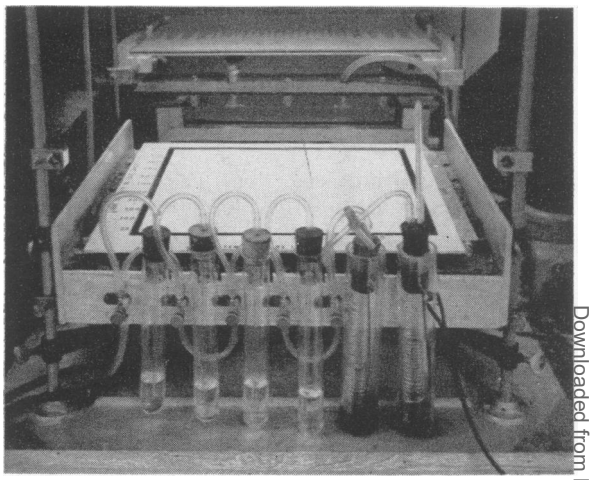



FIG. 1 (*top left*). The crossed gradient culture apparatus. (Cross section from right-hand side.)

FIG. 2 (*top right*). Front view of crossed gradient culture apparatus as arranged for photographing the culture. The ground glass screen with its paper comb, the shade below it, and the Lucite box cover have been pushed back on racks and the printed cardboard scale put in place. The culture surface is within the black lines.

FIG. 3 (*bottom left*). Actual intensity contours as measured over the surface of the culture plate (dashes), compared with the nominal intensity (full lines).

## OPERATING PROCEDURE

The temperature is checked by a thermocouple. The measuring junction may be taped to the plate, or inserted in the agar layer.

To check the light intensity gradient the aluminum plate is removed and the Lucite cover is held in its normal place with appropriate supports. A photographic exposure meter is mounted on a frame which holds its sensitive surface in the plane normally occupied by the agar surface. This frame rests on the base of the apparatus over a sheet of paper and provision is made in the frame for marking the paper directly under the center of the photosensitive surface. To adjust the intensity gradient 10 lines are ruled across the paper representing even intervals from the front to the back of the culture surface. These lines are labeled from 0 to 1000 ft-c. Trial adjustments of the lamp position and of the shape of the paper comb are made and the desired intensity at various places is compared with the actual reading of the exposure meter. Separate variac controls of the individual lamp intensities are made and the lamp positions adjusted. After a reasonable match of the desired and the actual intensities is achieved the final intensity contours are plotted by moving the photocell to those positions which give exact readings of each 100 ft-c on the scale and marking the paper accordingly. These marks at 100 ft-c intervals give a contour plot of the actual intensity. An illustration of such a plot is given in figure 3.

Before pouring the agar, the plate is leveled and then heated electrically to above the solidifying temperature of agar. The agar (200 ml), containing appropriate nutrient salts, is poured on to give a layer about 2 mm deep. After the agar has cooled the plate is inoculated.

Spraying and painting proved to be impractical inoculation procedures. The most successful way is to pour on a layer of liquid culture medium containing the suspended algae and then allow the plate to dry overnight with the cover off. This gives a uniform inoculum and the excess water either evaporates or is taken up in the agar.

In some cases it is better to illuminate the plate at room temperature with weak uniform light to start growth. After the inoculum has started grow-

ing uniformly on the plate, the temperature and intensity gradients are set up.

Photographs are taken under identical conditions once or twice a day. The camera is permanently mounted and four photoflood lamps are fixed to the side walls of the surrounding chamber to give reproducible and uniform illumination. These lamps are used only for photography. To take pictures it is necessary to slide the water tank, the Lucite water box cover, and the ground-glass plate out of the way on tracks with rollers. A white cardboard frame printed with the experimental data is put around the plate before taking pictures.

To make quantitative measurements of the pigment formation from some of the algae, small pieces of the agar were removed and placed directly in the spectrophotometer with opal glass plates. The results of this work with blue-green algae are described elsewhere (1, 2).

The most serious difficulties in operation were drying out of the agar and achieving a uniform inoculation. Contamination of the algal cultures caused very little trouble because of the largely inorganic media, the abundant inoculum, and the short duration of the runs.

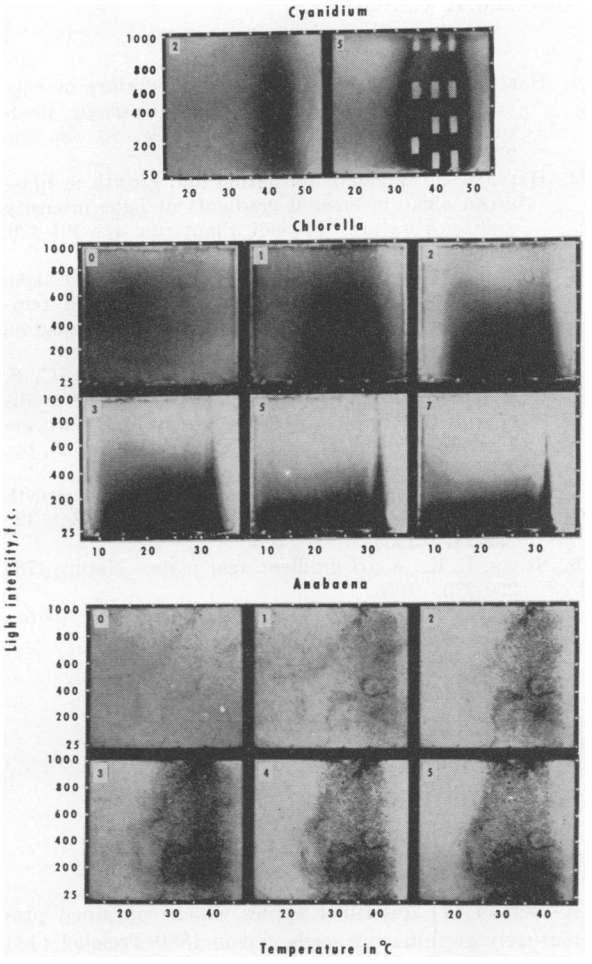



FIG. 4. The growth of algae on the crossed gradient plate. The days elapsed since inoculation are given on each photograph.

TABLE I

LIGHT INTENSITIES AND TEMPERATURES FOR BEST GROWTH OF ALGAE AS OBSERVED ON THE CROSSED GRADIENT PLATE

| SPECIES | AGE HRS | APPROX LOWER LIMIT | OPTIMUM | APPROX UPPER LIMIT |
|---|---|---|---|---|
| *Chlorella pyrenoidosa* | 24 | 22<br>50 | *29*<br>*250* | 33° C<br>400 ft-c |
| Anabaena sp. | 48 | 28<br>150 | *36*<br>*400* | 43° C<br>1000 ft-c |
| *Anacystis nidulans* | 12 | 36 | *40*<br>Equal growth at all intensities from 25 to 1000 ft-c | 43° C |
| *Cyanidium caldarium* | 48 | 38<br>100 | *43*<br>*350* | 47° C<br>700 ft-c |
| *Haematococcus lacustris* | | 12<br>50 | Growth over entire range of temperature and intensity | 33° C<br>1000 ft-c |

The following media solidified with 1.5 % agar were used: (A) Chlorella and Haematococcus: $KNO_3$, 1.25; $KH_2PO_4$, 1.25; $MgSO_4 \cdot 7\,H_2O$, 1.0 g/L with 1 ml Hutner's trace element mixture. The inoculum of Haematococcus was grown at 5° C. (B) Anabaena and Anacystis: Myers and Kratz 1955 D medium. (C) Cyanidium: $NH_4Cl$, 0.535; $MgSO_4 \cdot 7\,H_2O$; 0.246; $CaCl_2$, 0.055; $KH_2PO_4$, 0.272 g/L; $H_2SO_4$ 0.01 M with a trace of Fe and 1 ml Hutner's trace element mixture.

## RESULTS

The pattern of algal growth on the gradient plate changes greatly in the course of a few days. The later patterns are much sharper and more characteristic than those seen at first. These striking final patterns have been described for most of the species so far cultivated. Only recently, however, has it become clear that the patterns of the older cultures are distorted by secondary effects and hence should not be taken to indicate the optimum growth conditions for the algae.

The complication of the secondary effects are best shown by the experiment with *Cyanidium caldarium* (fig 4). After two days this alga had produced a green zone within the range shown in table I. After five days most of this region was no longer green, but yellow, while the adjacent region, where the growth was poor at first, had become deep green. It appears that the early luxuriant growth had exhausted the nutrient supply in the optimum region so further growth there was impossible. The pattern produced later was therefore artificially distorted by

the nutrient deficiency. The apparent narrow temperature range of maximum growth found in the old cultures of Chlorella and Anacystis also seem to be caused by such secondary effects.

We have therefore prepared table I from the earliest clear pattern that developed. It should represent the optimum conditions for cultivating these algae.

In figure 4 the development of a *Chlorella pyrenoidosa* and of an Anabaena culture are also shown. Some difficulties in preparing a uniform inoculum of the Anabaena are evident in the figure but do not distort the conclusions appreciably.

With Haematococcus some growth was evident over the temperature range of 12 to 33° C and light intensities from 50 to 1000 ft-c with greater growth toward the high temperature side. At the higher intensities the color was red and shaded to brownish green at intensities below about 200 ft-c. The optima were broad and not clearly shown in this experiment.

## Discussion

Crossed gradient growth studies should be useful in selecting appropriate algal strains for the temperature and intensity prevailing in large-scale algal farms. Studies of the behavior of mixed cultures on this crossed gradient plate would appear to be of value in showing how to adjust the conditions of a large-scale culture to give any desired strain a competitive advantage.

Another promising application of the crossed gradient plate would be as a means of isolating from nature algal strains having specified temperature and intensity tolerances.

The influence of light and temperature on growth of Lemna, a small higher plant, and on the germination of light-sensitive seeds, is being investigated with this device by Mrs. Brian J. Elliott. A number of other applications of the crossed gradient principle, using variables other than temperature and light, appear to be feasible. Several papers have appeared (5, 6) describing pH and chemical gradients in thin layers of agar. These gradients are established by pouring the plate slightly off level with half of the agar and then pouring an equal volume of a different composition with the plate tilted the opposite way. The plate here described would allow such a chemical gradient to be used on one axis with either temperature or light intensity on the other.

## Summary

A device is described for growing algae on an agar surface having a temperature gradient from left to right crossed by a light intensity gradient from front to back. The optimum growth conditions for several algae have been determined with this apparatus.

The culture plate and Lucite parts were made by Mr. L. R. Kruger. The supporting frame, thermostats, and auxiliary components were made and kept in operating condition by Mr. R. W. Hart who also drew the illustrations. Several of the experimental runs were done by Miss Helen S. Huang. The algal cultures were kindly sent by Drs. Jack Myers, M. B. Allen and R. C. Starr.

## Literature Cited

1. Halldal, P. Effect of light and temperature on pigment ratios in blue-green algae. Carnegie Institution of Washington, Year Book No. 56, pp. 272–275. 1957.
2. Halldal, P. Pigment formation and growth in blue-green algae in crossed gradients of light intensity and temperature. Physiol. Plantarum 11: 401–420. 1958.
3. Halldal, P. and French, C. S. The growth of algae in crossed gradients of light intensity and temperature. Carnegie Institution of Washington, Year Book No. 55, pp. 261–265. 1956.
4. Halldal, P., Huang, Helen S. and French, C. S. Growth patterns of various algae in crossed gradients of light intensity and temperature. Carnegie Institution of Washington, Year Book No. 56, pp. 275–278. 1957.
5. Kratz, W. A., and Myers, J. Nutrition and growth of several blue-green algae. Amer. Jour. Bot. 42: 282–287. 1955.
6. Sacks, L. E. A pH gradient agar plate. Nature 178: 269–270. 1956.
7. Weinberg, Eugene D. Double gradient agar plates. Science 125: 196. 1957.



# ABSENCE OF SEED DORMANCY IN A WHITE MUTANT STRAIN OF HELIANTHUS ANNUUS L.[1]

RAYMOND H. WALLACE and HELEN M. HABERMANN[2]

Department of Botany, University of Connecticut, Storrs, Connecticut and Research Institutes (Fels Fund), University of Chicago, Chicago 37, Illinois

The premature germination of seeds which normally require a period of dormancy has been reported in many plant species. As early as 1875 Ascherson (1) described apples which contained prematurely germinating seeds and in 1880 Treichel (13) reported observing germinating seeds within several kinds of fleshy fruits. About three decades ago several viviparous mutants of maize were described in papers by Mangelsdorf (7, 8, 9), Eyster (4, 5, 6) and

[1] Received February 14, 1958.

[2] Present address: Dept. of Biol. Sci., Goucher College, Baltimore, Md.