

THE ASEQUAL REPRORUCTION OF *SCHIZOMERIS* AND *ULOTHRIX* (CHLOROPHYTA) IN RESPONSE TO VARIOUS LIGHT INTENSITIES

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ABSTRACT.—The influence of light intensity on the reproduction of a species of *Schizomeris* and the interaction of light intensity and exogenous dextrose on the reproduction of a species of *Ulothrix* were determined. The behavior of *Ulothrix* zoospores with respect to direction of light source and aeration was noted and discussed.

Experiments involving the influence of growth regulating compounds on the growth and reproduction of algae were begun by the first author and his students in 1964. The study reported herein was performed in order to determine optimal light intensities for use in these experiments.

Bold (1942) and Pringsheim (1949) reported that full sunlight was generally inhibitory to the growth of algae. The latter proposed that algae were adapted to growth under relatively low light intensity in nature. Myers (1953) found 400 foot-candles to be optimal for growth of a strain of *Chlorella pyrenoidosa*. Phillips and Myers (1954), working with the same species, reported that 600 foot-candles was optimal when given as short flashes rather than continuously. Myers (1951) reported a light intensity-temperature interaction. Sorokin and Krauss (1958)

found that light saturation ranged from 250 to 500-foot candles for five kinds of algae grown at 25°C. They found that light saturation for a high temperature strain of *Chlorella pyrenoidosa* changed from 500 to 1400 foot-candles as the temperature was raised from 25°C to 39°C. Optimal light intensity has been found to vary with the duration and frequency of the light periods (Phillips and Myers, 1954), genetic make-up of the species or strains of algae used (Sorokin and Krauss, 1958), carbon dioxide content of the nutrient medium (Spoehr and Milner, 1949, and Tamiya, 1957), and temperature (Myers, 1951, and Sorokin and Krauss, 1958).

MATERIALS AND METHODS

The culture room was placed on a cycle of 16 hours of illumination and 8 hours of darkness. A light intensity range of 0 to 700 foot-candles was chosen, with 40 watt "cool-white" General Electric fluorescent tubes as the source of illumination, as suggested by Starr (1960). Distance from the light source was used to vary the light intensity, which was measured using a General Electric type-213 light meter. All experiments were performed at 72°F.

A modification of Bristol's solution (Table I) was used as the basic culture

TABLE I.—Composition of modified Bristol's Solution. A liter of final solution contains 10 ml each of stocks 1 through 6 and 1 ml each of stocks 7 through 10.

Stock Solution	Quantity per Liter	Compound
1	25 g	NaNO ₃
2	2.5 g	CaCl ₂
3	7.5 g	MgSO ₄ ·7H ₂ O
4	7.5 g	K ₂ HPO ₄
5	17.5 g	KH ₂ PO ₄
6	2.5 g	NaCl
7	50 g	EDTA
	31 g	KOH
8	4.98 g	FeSO ₄ ·7H ₂ O
	1 ml	H ₂ SO ₄ , Conc.
9	11.42 g	H ₃ BO ₃
10	8.82 g	ZnSO ₄ ·7H ₂ O
	1.44 g	MnCl ₂ ·4H ₂ O
	1.57 g	CuSO ₄ ·5H ₂ O
	0.71 g	MoO ₃
	0.49 g	Co(NO ₃) ₂ ·6H ₂ O
	1 ml	H ₂ SO ₄ , Conc.

medium. The growth containers were 16 x 150 mm test tubes containing 15 ml of nutrient medium. The tubes of medium were autoclaved at 250°F for 25 minutes. Each experiment was evaluated after seven days under the conditions specified.

A clonal culture of *Schizomeris* sp., having bacterial contaminants, was obtained from H. L. Monoson, then at Kansas University, and utilized in this study. *Schizomeris* was inoculated by delivering 0.1 ml from a 15 ml matured culture by means of a pipette. It was grown under eight light intensities, varying from 0 to 600 foot-candles. Ten replications of the basic design were used in each trial. There were five trials in this experiment, each trial being performed on a different date using fresh inoculum and medium. Reproduction was determined by counting populations of new filaments within the 16 units (1.05 mm²) of a grid insert placed within the ocular lens system

of a microscope. Each test tube was placed on a tilted microscope stage and was evaluated at eight evenly-spaced locations along the tube's circumference in a plane midway between the liquid surface and the bottom of the tube, using a magnification of 100 X.

An axenic culture of *Ulothrix acuminata* Mattox and Bold from the Culture Collection of Algae at Indiana University was also studied. Inoculations were made by transferring one loop (2.5 mm diameter) full of material from an agitated tube of matured filaments. This alga was grown at eight light intensities ranging from 0 to 700 foot-candles. Six replicates of the basic design were included in each trial. There were four trials using the basic culture medium and three with the basic medium plus 1.25 g/l dextrose.

Glass microscope slides were cut longitudinally and one half-slide was placed in each test tube prior to autoclaving. Reproduction was determined by counting the number of new filaments per 1.05 mm² in ten evenly-spaced locations on the side of the glass slide facing the light. The top half-inch of the slide was discounted since it picked up additional filaments during its withdrawal from the medium.

RESULTS

The optimal light intensity for *Schizomeris* within the specified environmental conditions proved to be between 400 and 500 foot-candles (Table II). Maximum populations were found at 400 foot-candles in trials 3 and 4 and at 500 foot-candles in trials 1, 2 and 5. It was determined that *Schizomeris* did not reproduce in the absence of light. New filaments originated from zoospores and the filament count was taken as an approximation of zoospore release.

All trials using *Ulothrix* yielded maximum filament counts at 500 foot-candles with the exception of trial seven in the series having dextrose, which yielded maximum count at 600 foot-candles (Table III). It

TABLE II.—Number of filaments of *Schizomeris* per field (1.05 mm²). Each number represents a mean of eight fields per test tube times ten replications.

	LIGHT INTENSITY (FOOT-CANDLES)							
	0	50	100	200	300	400	500	600
Trial 1	0	2.3	11.5	20.8	30.9	57.5	96.2	55.2
Trial 2	0	2.1	8.6	10.2	21.4	29.6	38.3	26.1
Trial 3	0	0.8	2.2	6.7	4.1	31.7	18.6	11.9
Trial 4	0	1.8	4.8	29.5	33.6	42.3	20.8	16.7
Trial 5	0	2.3	5.4	5.8	13.2	23.0	40.1	23.2
Mean	0	1.8	6.5	14.6	20.6	36.8	42.5	26.6

TABLE III.—Number of filaments of *Ulothrix* per field (1.05 mm²). Each number is the mean of ten fields per slide (test tube) times six replications. The nutrient media were modified Bristol's solution in trials 1 through 4 and modified Bristol's solution with 1.25 g/l dextrose in trials 5 through 7.

	LIGHT INTENSITY (FOOT-CANDLES)							
	0	100	200	300	400	500	600	700
Trial 1	—	0	4.1	19.6	23.0	27.0	16.8	16.1
Trial 2	—	6.0	18.5	31.1	42.8	94.5	58.6	37.5
Trial 3	—	2.1	8.1	28.8	44.0	75.5	41.5	41.1
Trial 4	2.5	7.3	12.6	14.5	19.5	39.3	24.8	16.3
Mean (1-4)	2.5	5.1	10.8	23.5	32.3	59.2	35.4	27.7
Trial 5	9.5	17.1	28.5	28.8	56.0	72.3	49.6	37.1
Trial 6	9.8	7.1	11.6	21.0	23.5	24.8	24.1	13.0
Trial 7	21.5	13.5	19.5	30.3	33.6	56.3	62.8	32.0
Mean (5-7)	13.6	12.6	19.9	26.7	37.7	51.1	45.5	27.3

was found that *Ulothrix* would reproduce in darkness and that the zoospores would develop into nearly colorless filaments.

Trial three in the inorganic medium series (Table III) was investigated to determine whether phototaxis, which appeared to exist, really occurred. The population counts were consistently higher on the side of the glass slide facing the light

than on the side away from the light source (Table IV). The zoospores moved toward the light source prior to cementing themselves to solid objects in their vicinity. This was the case at all light intensities between 100 and 700 foot-candles. Furthermore, the zoospores exhibited a strong aerobic tendency by swimming to the surface of the medium prior to their development. The lat-

TABLE IV.—Number of filaments of *Ulothrix* per field (1.05 mm²). Each number is the mean of ten fields per slide (test tube) times six replications in trial 3 without dextrose.

Orientation	LIGHT INTENSITY (FOOT-CANDLES)						
	100	200	300	400	500	600	700
Facing light	2.1	8.1	28.8	44.0	75.5	41.5	41.1
Opposite light	0.5	7.8	18.8	29.6	38.1	24.3	22.5

ter was concluded from observations rather than from population counts.

DISCUSSION

The data, indicating maximum reproduction of *Schizomeris* and *Ulothrix* at approximately 500 foot-candles, are generally in agreement with the results of Myers (1953), Phillips and Myers (1954), Sorokin and Krauss (1958), and Starr (1960). The literature indicates that saturation light intensity can be shifted by aeration (Spoehr and Milner, 1949, and Tamiya, 1957), temperature (Myers, 1951, and Sorokin and Krauss, 1958), light duration (Phillips and Myers, 1954) and genetic characteristics (Sorokin and Krauss, 1958). It is likely that light quality, inorganic and organic nutrients, pH, and other factors also contribute to determining light saturation.

It has been impossible, thus far, to separate filament size increase from zoospore formation in *Ulothrix*, as both have been stimulated by the same factors. However, the filaments produced under 600-700 foot-candles exhibited shortness, few cells, and an anomalous sort of branch or accessory holdfast production. The dark grown cells were very elongate, with the plastids considerably shorter than the cells' lengths.

The fact that low light intensities are inhibitory to reproduction is of considerable interest as is the reversibility of this inhibition with dextrose. It is not clear, however, whether exogenous dextrose or photosynthate are necessary (1) as respirable materials, (2) as assimilable materials, or (3) as stimuli to the processes of zoosporogenesis and zoospore release. Kulfinski and Soice (1965) found that dextrose was inhibitory to *Ulothrix*, although stimulatory to *Lepatosira obovata*.

Kulfinski and Dash (1966) reported dextrose to be stimulatory to growth of *Tribonema aequale*. It is of interest, therefore, that dextrose is stimulatory to *Ulothrix* at certain light intensities and inhibitory at others. Diffusion of gases between the nutrient medium and the atmosphere may be the limiting factor in the utilization of dextrose at 500 foot-candles and perhaps this inhibition can be reversed by aeration of the medium.

Evidence was found that the zoospores of *Ulothrix* are positively phototactic throughout the range of light intensities employed. The presence of greater numbers of filaments at the surface of the medium indicated that the zoospores are strongly aerotactic.

SUMMARY

1. Light saturation with respect to zoospore formation and release was found to exist in the vicinity of 500 foot-candles for both *Schizomeris* and *Ulothrix* in modified Bristol's solution.
2. *Ulothrix* reproduced in total darkness. Reproduction in the dark was increased by addition of dextrose.
3. Dextrose was inhibitory to reproduction at saturation light intensity but stimulatory above and below this intensity.
4. The zoospores of *Ulothrix* moved toward the light source, at the light intensities employed, and toward the surface of the liquid nutrient medium, as determined by population distribution after zoospore release.

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