~~PHOTOROPISMS WITH MONOCHROMATIC LIGHT IN FUCUS AND VOLVOX.~~

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ANNIE MAY HURD.

Submitted in partial fulfillment of the requirements

for the degree of Doctor of Philosophy.

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ORIENTATIONS AND PHOTOTROPISMS IN FUCUS AND VOLVOX WITH MONOCHROMATIC LIGHT OF EQUAL INTENSITIES.

Mrs. ANNIE MAY HURD-KARRER

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I Introduction

The power of light stimuli to produce orientations and tropisms is a phenomenon which has been widely demonstrated in both the plant and animal kingdoms. Not only can~~s~~ unilateral illumination direct movements and growth but in some species of plants, namely Equisetum, Fucus, ~~and~~ Puccinia, and related forms, it can establish the direction of the first cleavage plane of the germinating spore. Since in such cases the cell on the shaded side of the spore becomes the rhizoidal cell, the polarity of the plant is determined by light irrespective of gravity.

In any attempt to discover the mechanics of such tropistic reactions, the first problem is to find what wave lengths of light are responsible, and to what extent they are a function of the quality of the stimulus apart from its quantity or intensity. This, then, is the purpose of the present investigation with respect to the phototropisms shown by Fucus sporelings and by Volvox colon[ies].

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Another source of inaccuracy has been the neglect or oversight of the great variation in the intensity or quantity of radiant energy transmitted by the color screens. Biological experimenters have for the most part failed to take into consideration the fact that the quantity as well as the quality of the light stimulus varies with the different colors and that the former variable must be eliminated before results can be attributed to differences in wave length alone. Quite recently (1914) such work has been done and published with the statement that the results reported are only qualitative because there is no way of comparing lights of different colors as to their amounts of radiant energy. There have of course been several methods devised by means of which the relative intensity of monochromatic lights can be measured.

The first exact work of this nature was done by Kniep and Minder (1909). They used a blue and a red color screen and a green solution, with sunlight as the source of light. The wave lengths to which each was transparent were known; and the energy behind each was determined by means of a thermopile and a d'Arsonval galvanometer. The interference of the long heat rays was prevented by inserting a water layer in a parallel sided container between the thermopile and the source of light.

Day (1911) obtained light of known wave length by means of a spectrum from Nernt Glowers formed by a carbon bisulfide prism and cut down by a diaphragm with narrow vertical slits which could be adjusted so as to permit any desired region of the spectrum to be used. In this adjustment a spectroscope was used to determine the exact range of wave-lengths passing through the slit in each of the four illuminations used, --- red, yellow, green, and blue. He measured the intensity of each with a Boys radiomicrometer, and balanced them by varying the number of glowers employed in the lamps. Thus there was one glower for the red light, two for the yellow, and three for the green and blue.

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Gross(1913) also used these same methods in determining the reactions of Arthropods to monochromatic light.

An instrument has been devised by MacDougal and Spoehr ( ) which measures the total radiant energy of any light in terms of its dissociation effect on a photosensitive substance. This is measured by a galvanometer. The advantages in the use of this "photoelectric cell" are said to be its extreme sensitiveness to the wave lengths of the blue end of the spectrum, and the fact that its action in light is "more nearly that of the organism than that of any other light measuring instruments available."

There have been therefore three different methods worked out for biological experiments for the quantitaive analysis of light stimuli; viz., those of Kniep and Minder, Day, and of

MacDougal and Spoehr. The interesting apparatus of Patten(1914) and of Loeb and Northrup whereby a quantitave measurement of the reaction of organisms subjected to two beams of light of different intensity, is obtained. The measurement is in terms of the angular deflections from an initial path of locomotion. The same methods might be applied to work with colored lights. A quantitative measurment of the greater effectiveness of one spectral region over another of equal intensity might be measured by the angular deviation of the path of a motile organism from a line perpendicular to a line connecting the two sources.

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All of these methods with the exception of those of Kniep and inder involve special apparatus often not easily available. It was necessary for the purposes of this investigation to find a simpler method of accomplishing the same results. This report therefore is concerned with two problems; first, a means of making the intensity of the monochromatic light obtained with a set of light filters equal; and second, a comparative study of the tropisms obtained with different wave lengths when the intensity variable is eliminated. The particular phenomena chosen for this study are the power of monochromatic lights to (1) establish the polarity of Fucus sporelings and the origin and consequent direction of the rhizoids; (2) to produce the negative phototropism of the rhizoid; (3) to direct the movements of Volvox.

II. Apparatus and Methods for Exposure to Monochromatic Light of Equal Intensity.

As biological science becomes more exact with the tend- to reduce the expression of natural phenomena to mathematical formulae it is obviously essential to define stimuli of all sorts quantitatively. Indefinite or incomplete records of light stimuli can no longer be attributed to the lack of means of measuring them because access to a spectroscope and thermopile make it possible to analyze any light qualitatively and quantitatively.

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There are two methods for obtaining monochromatic light for biological experiments ---the projection of a spectrum upon the organisms, or the use of filtered light passed through a color screen. The former is theoretically the better for exact work but technical difficulties such as the limited dispersion and low intensity make it impractical for many investigations. Light filters of glass are the most convenient means of securing approximately monochromatic light when unilateral illumination is desired. Ordinary color screens transmit too wide a range of wave lengths for exact work and at present there are very few whose light is of sufficient homogeneity. The best is the Wratten filter screen, made in London, which consists of a dyed gelatine film between two glass plates. MacDougal and Spoehr (1917) have described some colored glass screens designed by them for biological work, but the range of wave lengths to which each of them is transparent is considerably greater than for the Wratten filter.

In the experiments to be described, seven Wratten light filters were used, each of which was fitted as a window in the end of a dark box. Each transmitted only a narrow range of wave lengths but all together they embraced the whole of the visible spectrum. The wave lengths to which each screen was transparent were determined by testing the light transmitted by each with a direct vision spectroscope with a wave length scale attached. Thus the quality of the light stimulus acting in each box is accurately known.

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The source of light best adapted for use with filters for which all the wave lengths of the visible spectrum are required, is the electric arc. The advantages of its use are, that since it emits all the desired wave-lengths all the filters can be used in each exposure, insuring identical conditions of temperature, constancy of illumination, etc. The disadvantages are several. In the first place, the intensity is constantly changing as the carbons burn and the arc gets longer. Worse, the lessening of the intensity may not be the same for all the wave lengths. In the second place, fluctuations in the current cause large variations in the intensity. In the third place, unless an automatically adjusted arc is available, it is necessary to adjust the carbons by hand every five to fifteen minutes, and when an eight hour illumination is desired, this entails considerable inconvenience.

The dark boxes were 10x13 cm. and 8 cm. high, in one end of each of which a hole was cut so that one of the light filters, 6 x 6 cm. might be fitted into it. The boxes were made light tight with tightly fitting covers, and were painted black inside to guard against reflections within the box. The dishes used for the cultures were made of microscope slides cemented together with zinc cement so as to make shallow oblong dishes 7.5x2.5 cm. and 1 cm. deep. It was deemed necessary to use such flat sided dishes in order to prevent possible complications from reflected and refracted light in the

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The spectroscopic analysis of the light passing the screens determines definitely the quality of light entering each box. It is at once evident that the quantity or intensity of light behind filters placed at equal distances from the source, varies, both because the intensity of light transmitted by the different screens is different, and because the different colors are not radiated by the arc with equal intensity. This being the case, differences in results obtained behind the screens could not be attributed to differences in the quality of the light stimulus alone.

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The method worked out for eliminating the intensity variable in the use of the filters employed for these investigations, consisted in finding the distance from the light source at which each box with its colored window should be placed in order that the intensity, as indicated by the deflection of the galvanometer when the thermopile was exposed to the light behind the filter, was equal in each case.

It seems necessary, on account of the questions which have been raised during the course of this work, to state here that the thermopile is equally sensitive to the energy of the red and of the violet end of the spectrum, and is, therefore, an accurate measure

As already pointed out much work has been done and results interpreted as if only one factor, wave length, were concerned. That differences in the amount of radiant energy might be partly or wholly responsible has either been overlooked or regarded as an insurmountable obstacle to exact tropistic work. The writer has been able to find only three accounts of apparatus planned to measure the relative intensities of colored lights. Recently MacDougal and Spoehr have published a description of a photo- electric cell designed to measure relative intensities by their dissociation effect on a light sensitive substance.

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One of the simple means of comparing the radiant energy of colored lights is by the use of the thermopile and sensitive galvanometer. The thermopile is very sensitive to the energy of any ether vibrations whether they be the longer infra-red or so-called heat rays, or the shorter, actinic rays of the spectrum. The method worked out for eliminating the intensity variable in the use of the filters employed for these investigations consisted in finding the distance from the light source at which each box with its colored window should be placed in order that the intensity, as indicated by the deflection of the galvanometer when the thermopile was exposed to the light behind the filter, was equal in each case.

It seems necessary, on account of the questions which have been raised during the course of this work, to state here that the thermopile is equally sensitive to the energy of the red and the violet ends of the spectrum, and is, therefore, an accurate measure of the total amount of light acting behind each color screen. ~~This is true because~~ The difference between heat and light is only a matter of wave length. ~~It is true that~~ The thermopile measures light in terms of the electric current produced by a difference in temperature of the exposed and unexposed junctions; but it does so by virtue of the fact that the energy of whatever vibrations fall upon it, be they long and therefore heating in their physiological effect, or short and therefore only perceived as light, is converted into heat energy upon being absorbed by the exposed junction of the thermopile. In other words, the light of the blue end of the spectrum produces an electromotive force much less than that of the infra- red but no less measurable.

The instruments used in the energy calibration of these screens were a Hilger ~~an ordinary lecture~~ table thermopile with junctions of bismuth and silver ~~antimony,~~ and a moderately sensitive galvanometer (d'Arsonval). ~~With an~~ An electric arc similar to the one later used in the experiments themselves was the source of light. The thermopile with the open end screened by one of the light filters, was exposed to the light until the galvanometer indicator reached a maximum deflection which took, ordinarily, about thirty seconds. The number of divisions through which the spot of light reflected from the galvanometer mirror was displaced on the scale was noted. This was repeated six times and the average deflection recorded. The other filters were then used in turn to screen the thermopile, and thermopile and screen moved to such a distance from the arc that the displacement of the galvanometer indicator were approximately equal in each case. This distance was also found ~~measured~~ for the ~~unscreened~~ thermopile when screened by a piece of clear glass, which represented the removal of the control from the source. For the experiments the quantity of light used can be varied for the whole set of screens by multiplying or dividing these distances by the same number and the intensity in all the boxes will remain equal. The actual amount of light in candlepowers can be

measured by means of a photometer. Then from the law of inverse squares, viz., that the intensity of light per unit surface varies inversely as the square of the distance from the source, the intensity at any distance from the arc can be computed.

The calibration of the set of screens was repeated seven times or until satisfactory checks of the distances were obtained. With some thermopiles of less rapid action than the one used here, it is impossible to get results by waiting for the galvanometer indicator to come to a steady state. In such a case the deflections produced by exposure to the light for equal intervals of time can be compared. A series of measurements for five second exposures agreed very well with those obtained by the other method.

TABLE SHOWING DISTANCES OF WRATTEN LIGHT FILTERS FROM AN ELECTRIC ARC AT WHICH THE INTENSITIES OF LIGHT TRANSMITTED BY ARE EQUAL.

Filter No.	Wave- lengths	Color	Distance from light
70	6600-7000 A.U.	Red	320 cm.
71	6200-6800 A.U.	Red	275 cm.
72	5900-6200 "	Orange	230 cm.
73	5600-5900 "	Yellow	250 cm.
74	5200-5600 "	Green	280 cm.
75	4700-5200 "	Blue	250 cm.
76	4000-4700 "	Violet	250 cm.
Control		White	340 cm.

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III. TROPISTIC REACTIONS TO MONOCHROMATIC LIGHT IN FUCUS SPORELINGS.

(a) The nature of the problem

The power of external factors to determine the polarity of a germinating spore is, without doubt, the power to orient the spindle of the first dividing nucleus, if that polarity is established by the direction of the first cleavage plane. The work on such orientations is very limited and has often resulted in negative results. Drietsch (Oct) established the polarity of sea-urchin ~~working on frog's~~ eggs ~~it has been possible~~ ~~was able to orient~~ the directed the axis of ~~embryo~~ by subjecting the eggs ~~them~~ to pressure. The spindle formed parallel to the flattened sides of the egg. This is consistent with Hertwig's theory that the spindle should form parallel to the longer axis of a dividing cell. It is conceivable that the power of light to orient a ~~the~~ spindle might result from its power to cause more rapid growth along one axis, which might mean ~~with the result~~ that the elongated cell in turn orients the spindle. No such effect of light has been demonstrated with animal eggs; but in certain plants, viz., <u>Equisetum</u>, <u>Fucus</u>, Ascophyllum, <u>Dictyota</u>, <u>Laurencia</u> and <u>Cystoseira</u>, and Puccinia it has been found that one-sided illumination with daylight causes the first cleavage plane to be formed perpendicular to the direction of the incident light and the cell on the darker side of the spore to become the rhizoidal cell. Equal illumination on all sides retards or prevents germination. Gravity and contact cannot establish the polarity of these spores.

All these experiments were conducted with natural light as the source of illumination. The first purpose of the present investigation was to determine the power of monochromatic light to establish the the polarity of germinating spores of <u>Fucus evanescens</u>. It was hoped that the results of such experiments might give a clue to the mechanics of light ~~such~~ orientations. If all wave lengths should produce the same effect it might be the result of a general intensity gradiant; if only the blue light is effective, the problem is one of a chemical effect; if only the red it might be assumed that

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(b) Summary of the Literature

The first work establishing the now well known fact that one-sided illumination will cause the first division plane of a germinating spore to be perpendicular to the direction of the incident light was done by Stahl (1885) on Equisetum. He found that the first wall is formed perpendicular to light rays striking the spores on one side only, and that if all sides are illuminated by rotating spores on a clinostat, the formation of the wall is retarded or prevented. The cell on the shaded side of the spore becomes the rhizoidal cell. In darkness the formation of the first wall follows no rule and the rhizoids extend in every direction. Stahl refers to earlier work on Marsilia and Chara which indicates that gravity is a controlling factor in the orientation of the first division plane.

Rosenvinge (1889) showed that in Fucus spiralis there is no relation between gravity and the first division plane nor did contact with a solid body have any effect. He got the same orientation to light in Ascophyllum and Fucus that Stahl did with Equisetum. but with puzzling exceptions. Where the spores were in groups the cells toward the interior of the group became the rhizoidal cell; and in the lower part of hanging drops the rhizoids appear on the upper side of the spore regardless of the light direction. He concluded then, that not only light but a difference in the concentration of oxygen on the two sides of the spores could determine their polarity. He says that as a result of their respiration the water in the center of the groups of spores is less rich in oxygen and as a consequence the rhizoids are formed on that side. In support of this theory is the fact that although light can determine the polarity of all

the species studied except Fucus serratus; viz., Ascophyllum nodosum, Fucus vesiculosus, F. spiralis, and Pelvetia canaliculata, their sensibility to light differs and the oxygen factor or internal causes produce frequent exceptions in all but Pelvetia. The rhizoids of the latter species are always formed on the darker side of the spore, and this is the one species in which the egg is surrounded by an oogonial wall which might prevent any of the effects of varying oxygen concentration, which can act more potently than light on the spores of the other species. Rosenvinge quotes Kny as finding that neither light, gravity, nor contact can influence the point of origin of the pollen tube from pollen grains, but that in the neighborhood of other grains the tube will be sent out from the side away from them, on which side the supply of oxygen or nutritive elements would be greater.

Farmer and Williams (1896) stated that if Fucus spores are illuminated on all sides they tend to remain spherical instead of producing a rhizoid by the elongation of one of the two cells. Again (1898) they experimented with one-sided illumination with the usual result that most of the rhizoids originated on the shaded side of the spore and the others were turned that way. The fact that some grew out at an angle to the incident light was attributed to "the character of the egg itself".

Winkler (1900) found the same orienting effect of light on the spores of Cystoseira barbata but failed to find any effect of a difference in the oxygen content of the water. He too said that gravity and contact are not factors in the establishment of the polarity of the sporelings. He found that the direction is determined before the first division takes place. Since this polarity is established during the first four hours of illumination and connot be changed afterwards by any change in the direction of the incident light, he concludes that light can orient the spore only during fertilization.

14.

the species studied except *Fucus serratus*; viz., *Ascophyllum nodosum*, *Fucus vesiculosus*, *F. spiralis*, and *Pelvetia canaliculata*, their sensibility to light differs and the oxygen factor or internal causes produce frequent exceptions in all but *Pelvetia*. The rhizoids of the latter species are *always* formed on the darker side of the spore, and this is the one species in which the egg is surrounded by an oogonial wall which might prevent any of the effects of varying oxygen concentration, which can act more potently than light on the spores of the other species. Rosenvinge quotes Kny as finding that neither light, gravity, nor contact can influence the point of origin of the pollen tube from pollen grains, but that in the neighborhood of other grains the tube will be sent out from the side away from them, on which side the supply of oxygen or nutritive elements would be greater.

Farmer and Williams(1897)stated that if *Fucus* spores are illuminated on all sides they tend to remain spherical instead of producing a rhizoid by the elongation of one of the two cells. Again(1898) they experimented with one-sided illumination with the usual result that most of the rhizoids originated on the shaded side of the spore and the others were turned that way. The fact that some grew out at an angle to the incident light was attributed to "the character of the egg itself".

Winkler(1900) found the same orienting effect of light on the spores of *Cystoseira barbata* but failed to find any effect of a difference in the oxygen content of the water. He too said that gravity and contact are not factors in the establishment of the polarity of the sporelings. He found that the direction is determined before the first division takes place. Since this polarity is established during the first four hours of illumination and cannot be changed afterwards by any change in the direction of the incident light, he concludes that light can orient the spore only during

Randolph and Pierce(1905) performed one-sided illumination experiments on Dictyota, Dictyopteris, Laurencia, and Cystoseira, and pointed out the certainty of the action of other factors because rhizoids are formed in the dark and in all-sided illumination. They said that although Winkler(1900) suggested the possibility of stopping germination by changing the direction of light every three hours, it could not be done with Dictyopteris. They emphasized the possibility of influences preceding the illumination affecting the polarity.

The work of Fromme (Rev. 1914) on the urediniospores of Puccinia rhamni is interesting because it refutes the idea that the orientation of the sporelings by light is due to the power of one-sided illumination to cause an aggregation of chloroplasts. He said that in darkness the germ tube grew from any side of the spore, but that in unilateral light the tubes almost always issued from the darker side of the spore.

(C) Results of Phototropism Experiments.

All attempts to solve the problem as to what wave lengths of light are responsible for the orientation of the first cleavage plane of Fucus spores, germinated in unilateral light, have failed so far, and apparently cannot succeed with the apparatus described in the first part of this paper. However it seems worth while to report the methods used and why they have failed. Work will be continued on this investigation at the earliest opportunity, as it is merely a matter of obtaining the right conditions of growth behind the light filters, together with a sufficiently strong illumination.

Doubtful

1. The fruiting plants of Fucus evanescens were collected at Sausilito at low tide of one day and kept over night in damp newspapers. The next morning they were dried slightly by exposing them to the air for about half an hour or less. The fruiting tips were then cut off and submerged in water in the culture dishes. After fifteen minutes many eggs and sperms have settled to the bottom of the dish or can be scraped off into the water. The piece of plant is then removed and the dish is placed in the rack which fits into a dark box behind a filter screen. Each box with its cultures is placed at the proper distance from the light so that each is illuminated with equal intensity. These distances for each filter and the method by which they were obtained are given above. Each distance given there, however, was divided by four or by five as the case might be in order to increase the intensity of the illumination equally for all. The illumination with the electric arc was continued for eight hours in an otherwise darkened room, the carbons being adjusted every five minutes. This time was judged more than sufficient to produce the desired effect since it was found in Cystoseira (Winkler 1900)

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that the polarity of the egg is so firmly established by four hours exposure to daylight that it cannot be changed.

The fact that Fucus evanescens is a monoecious species makes it impossible to tell the exact time of fertilization, but it occurs soon after the eggs escape from the oogonial sac into the water. The sperms at this time can be seen escaping from the antheridia and swimming rapidly around the eggs, then scattering as, presumably, one of them succeeds in entering. The first cross-wall can be seen very plainly twenty- four hours after the cultures are started. The mucilage accompanying the eggs caused them to adhere so firmly to the dish that it is not necessary to use solid media to keep the sporelings from being displaced when the cultures are moved to the microscope stage for examination.

However, none of the eggs germinated behind the color screens, and illuminated for the first eight hours after fertilization, showed any orientation with respect to the direction of the light. They grew ~~normally~~ as if in darkness with the direction of the cleavage plane following no rule and the rhizoids extending in every direction. Evidently the intensity of the light was too low to have any effect, or the time or duration of the stimulation was not right. Attempts to increase the intensity by bringing the cultures closer to the arc, that is, within about seventy-five centimeters, resulted invariably in the death of the spores before any development occured. A cooling device, placed 15 cm. from the naked arc, consisting of a layer of water 6 cm. thick between two glass plates, reduced the temperature from 29 to 23° ~~degrees (Centigrade)~~ ~~but was black~~ but also so reduced the intensity of the light that no orientation was obtained. Attempts to use direct sunlight as the source of light also caused the rapid death of the eggs, presumably on account of the high temperature accompanying it.

Future work on this problem ~~must~~ should therefore involve the use of stronger sources of illumination, and cooling devices to that the polarity of the egg is so firmly established by four hours exposure to daylight that it cannot be changed.

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INteresting results were obtained in an experiment to discover the weakest daylight stimulus which would produce the characteristic orientations of natural illumination. Cultures were placed at intervals of 15 cm. from an east window and exposed 8 hours on a cloudy day. Upon examination a few days later every culture was found to show strikingly the strong orienting effect of unilateral daylight. Even in the one fartherest away, 350 cm. from the window, the eggs were practically all oriented, with the cross wall exactly perpendicular to the direction of the window, & the rhizoids originating from the cell darker side. Every culture showing this orientaion has more or less frequent exceptions to the general rule. Every worker on this problem has reported such exceptions and they have been explained by the assumption of the existence of an inherent polarity which as a rule is overcome by the stronger light stimulus. The fact that in absolute darkness, germination and normal growth are as rapid or more so than in light, also points to an inherent polarity. But the tendancy varies enormously in individual spores. Therefore it is easy to understand that slight variations in the quantity and quality of illumination might easily produce large changes in the sensitiveness of the spore toward light, which means failure of experiments with monochromatic light until the right combination of other external factors in produced.

It was soon discovered in the course of this investigation that although the intensity of the electric arc used was too low to orient the cleavage plane of the egg and too low to cause the cell away from the light to become the rhizoidal cell, it was strong enough to produce a conspicuous negative heliotropism of the rhizoids of these same spores if the illumination was resumed after the rhizoids had developed. ONly seven hour exposures were used to

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Table II

Filter No.	COLOR	WAVE LENGTHS	* DISTANCE FROM LIGHT	HELIOTROPISM CULTURES NO. I	+ I
70	red	6600-7000 A.U.	80 cm.	—	—
71	orange	6200-6800 "	68 "	—	—
72	orange	5800-6200	58 "	—	—
73	yellow	5600-5900	62 "	—	—
74	green	5200-5600	70	—	—
75	blue	4700-5200	62 "	+	+
76	violet	4000-4700	62 "	+	+
	white	4000-7000 +	80 ~~(Foot-note)~~	+	+

* These are the distances of Table I divided by 4.

~~A similar~~ The same experiment was tried with sunlight as the source of illumination. The young plants were exposed behind the filters all day in a south window. The same results were obtained as when the arc was used. Then the experiment was repeated with diffused light. The boxes were placed in an east window for eight hours---10 A.M. to 6 P.M.---on March 28. Again the rhizoids in the blue and violet light showed the ~~tropism~~ response, but in addition a considerable but much smaller portion were affected in the same way behind the green filter. Evidently the conditions of growth in these cultures made the plants more sensitive to light stimuli.

These experiments indicate that the light of wave length 4000-5200 (angstrom units) ~~are~~ is responsible for the phenomenon, but that some rhizoids, more sensitive, will respond to those of 5200-5600. It seems very possible in view of the results on

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72	orange	5900-6200	54 "	–	–
73	yellow	5600-5900	62 "	–	–
74	green	5200-5600	70 "	–	–
75	blue	4700-5200	62 "	+	+
76	violet	4000-4700	62 "	+	+
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Volvox reported in the third part of this paper that with a sufficiently greater intensity, the rays of the red end of the spectrum might cause the same negative phototropism.

Only the growing tips of the rhizoids are sensitive to light. This results in a sharp angular turn if the direction of illumination is changed through 90 or 180 degrees, or if the plant be brought from darkness into unilateral light. As pointed out by Loeb and others such tropisms are probably due to the difference in the speed of the chemical reactions going on in the two sides of the growing tip.

The first protuberance of the germinating spore is not affected by light striking it from the side; and if it is so illuminated during the early stage of elongation of this cell, the first bend occurs at the cross wall separating it from the next rhizoidal cell. In other words, the first rhizoidal elongation continues in the original direction established by the first cleavage plane, and the wall separating it from the next cell of the growing rhizoid appears as the axis of the tropism.

IV. "Group Orientation" in Fucus inflatus.

In every culture of Fucus evanescens whether germinated in darkness or in strong unilateral light a most striking orientation of the first cross-wall with reference to adjacent spores appears. Wherever a group of spores are lying within about 0.2 mm. of each other, the first cleavage plane is perpendicular to the direction of the center of the group. The cell toward the interior invariably becomes the rhizoidal cell. This phenomenon was reported by Rosenvinge ( ) in other species of Fucus and in Ascophyllum. ~~For want of a better term I have called it group orientation.~~

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A study of this phenomenon was made to determine the strength of the stimulus producing this effect, compared to ~~and~~ that of light, in their power to produce the orientation of the plant. It was at once very evident that for most spores the former stimulation predominates when the spores are within a short distance of each other--- .2 mm. or often more--- and beyond this distance, the chemical(?) stimulus becomes too weak and only the light is able to determine the polarity of the plant. Only the comparatively isolated spores therefore ever show the orientation to light with the sources of illumination used here.

The phenomenon is very conspicuous in groups of 2, 3, or 4 eggs as well as in masses of fifty or a hundred. In these large groups it is made evident by the invariable rule that no rhizoid ever extends outwards from a group. When two spores are within the distance through which the stimulus is effective, the first cleavage planes of the two are often parallel and the rhizoids grow towards each other and often meet tip to tip. The groups of five or six often make symmetrical star-like designs when the rhizoids have grown and project beyond the group.

The spores are more rarely affected in this way when the distance between them is over 0.3 mm., but the phenomenon is sometimes observed in spores as much as 0.5 mm. apart. Within a distance of 0.2 mm. there are practically no exceptions.

The relative sensitiveness of a spore towards light and towards this chemical(?) stimulus varies greatly for different spores. When cultures were placed in the window to get as strong a light stimulus as possible in order to determine at what distance from each other the eggs had to be not to show a greater sensitiveness towards the chemical stimulus than towards the light, it was found that this distance followed no rule, the spores showing the greatest individual differences. Of two spores lying within 0.3 mm. of each other one might be entirely oriented by the adjacent spore while the other, apparently like it, would show only the action of the light stimulus.

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In many cases two such spores would seem to show a resultant effect of the two stimuli so that both would be half turned towards each other with both rhizoidal cells showing a tendency to take a direction away from the light at the same resultant angle.

Rosenvinge ascribes this group orientation to a difference in the concentration of oxygen or of nutritive substances on the two sides of the spore. He thinks the rhizoid forms on the side toward the center of a group or towards another egg because the water on that side is less rich in the active substance than on the outer side of the spores as a result of their metabolism. Winkler(1900)working with Cystoseira barbata found that a difference in oxygen concentration has no such effect. Apparently the phenomenon does not occur naturally in this species since his figure given shows nothing but the effect of light. I have never seen a culture of Fucus evanescens with spores germinating so near each other, which showed only light orientation and not the group orientation. Almost invariably when the spores of this species germinate in such close proximity, light appears to have no power to establish the polarity of the plant.

The possibility that the group orientation is due to a polarity established by the position of the egg in the oogonium is suggested by finding many groups of eight lying just as they escaped from the oogonial sac and conspicuously oriented with respect to each other. The fact that groups of ten or of two are as regularly oriented, would refute the suggestion; but in order to prove that the phenomenon is the result of a stimulus acting after the eggs leave the oogonium, a group of them were transferred to a watch crystal and mixed with the point of a needle until their relative positions were entirely changed. But when they germinated the characteristic orientation with respect to each other was found to be without an exception.

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A striking phenomenon which seems to be further evidence of the power of a chemical stimulus to orient the spores was found in three different cultures. Small pieces of a foreign substance, apparently sand, caused the orientation of the spores adjacent to them, with the result that the rhizoids all extended inward towards the substance. This material was present in too small amounts for chemical analysis. A search for some difficultly soluble substance, or an insoluble substance which might be slightly soluble in the weak carbonic acid produced by the growing spores, which, when added to the cultures, might orient the eggs adjacent to it, has failed to bring any positive results. They do show, however, that the phenomenon is not due to a contact stimulus. The substances tried were Scheelite ($Ca_2WO_4O_2$), Magnetite (Fe_3O_4), Barite ($BaSO_4$), Cassereite (SnO_2), Hornblend, Rubellite, beach sand, granite, and silver filings.

The substance or condition originating in the activity of adjacent spores which has so powerful an effect in orientating the first cleavage plane and in determining which cell shall become the rhizoidal cell has no power to cause any chemotropism of the rhizoids after they are started. No rhizoid has been found to have its direction modified by the presence of other spores adjacent to it. In the absence of any light stimulus the rhizoids continue in the direction that they take originally from the spore.

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(d) Summary

To summarize briefly this work on light and chemical stimuli as they affect the polarity of Fucus eggs and the direction of growth of the rhizoids:

(1) Green, blue, and violet light (4000-5600 A.U.) of an intensity great enough to produce negative phototropisms of the growing rhizoids is without effect on the direction of the first cleavage plane of the egg.

(3) The chemical (?) stimulus which orients the direction of the first cleavage plane and determines which cell shall become the rhizoidal cell in group orientations ~~when the eggs are within a certain distance of from another egg~~ has no power to cause a chemotropism of the rhizoids.

(4) The negative phototropism of the rhizoids in monochromatic light is primarily a function of the quality of the light since, with equal intensity of illumination, the light behind the color screens of the red end of the spectrum are without effect.

2. The group orientation reported by Rosenvinge in Ascophyllum nodosum, ~~and~~ Fucus vesiculosus, F. spiralis and F. serratus is also a conspicuous phenomenon in every culture of F. evanescens ~~inflatus~~. In fact, the stimulus acting in such orientation must be stronger in this species than in any of the other species reported, since when spores are within a distance of .2 mm and often more, light stimuli as a rule fail to overcome it.

V. -- Phototropisms of Volvox in Monochromatic Light of Equal Intensity.

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Oltmanns (1892), Holmes (1903) and Mast (1907) have reported in detail the reactions of Volvox to light stimuli, No one, however, has studied its response to monochromatic illumination. The purpose of the present study is a comparison of its phototropisms in different spectral regions for which the intensity is made equal.

The recent article by Mast (1917) sums up in a table the literature on the reactions of plants and animals to colored light so completely and so concisely that there is no necessity for a repetition here. He summarizes briefly results given in the table as follows:

"For seedlings of green plants, plumules and radicles, the region in the spectrum of maximum stimulating effect is in the blue or violet. For the fungi it is somewhat nearer the red. For Bacterium photometricum it is in the infra-red and the orange. For Oscillaria and Paramecium bursaria is it questionable, activity and aggregation being probably determined by chemical changes in the solution associated with the colors. For Chlamydomonas it is in the green; for all other unicellular forms tested it is in the blue, as it is also for the coelenterates and vermes and for a few of the molluscs and arthropods. But for most of the molluscs and arthropods it appears to be in the green or yellow."

That the most effective wave lengths are not the same for even related forms in the lower organisms is further borne out by Masts' (1917) results. He found that the maximum efficiency is near 483 $\mu\mu$ for Euglena, Trachelomonas, Phacus, Gonium, Arenicola and Lumbricus;

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The recent article by Mast (1917) sums up in a table the literature on the reactions of plants and animals to colored light so completely and so concisely that there is no necessity for a repetition here. He summarizes briefly results given in the table as follows:

"For seedlings of green plants, plumules and radicles, the region in the spectrum of maximum stimulating effect is in the blue or violet. For the fungi it is somewhat nearer the red. For Bacterium photometricum it is in the infra-red and the orange. For Oscillaria and Paramecium bursaria it is questionable, activity and aggregation being probably determined by chemical changes in the solution associated with the colors. For Chlamydomonas it is in the green; for all other unicellular forms tested it is in the blue, as it is also for the coelenterates and worms and for a few of the molluscs and arthropods. But for most of the molluscs and arthropods it appears to be in the green or yellow."

That the most effective wave lengths are not the same for even related forms in the lower organisms is further borne out by Mast's (1917) results. He found that the maximum efficiency is near 483 μμ for Euglena, Trachelomonas, Phacus, Gonium, Arenicola and Lumbricus;

near 524 $\mu\mu$ for Pandorina, Eudorina and Spondylomorum;and near 503 $\mu\mu$ for Chlamydomonas and blowfly larvae. The work of Schaeffer( ) on amaebae is interesting as his results indicate that the power of a beam of red light to cause the organisms to move towards it is as great as a beam of white light, and has more attraction than the blue. Most investigators of phototropisms find either that the red is less effective than the blue or that it has no effect. A few find that in equal brightness, the effect is nearly the same for all colors.

The Volvox colonies upon which the following experiments were made were collected by Dr. N. L. Gardner in a small lake in Golden Gate Park on April 2, 1918. They were so abundant that the water was quite green with them.

On April 5 they were exposed to direct sunlight behind the seven Wratten light filters used in the work on Fucus. The same rectangular, flat-sided dishes made of microscope slides cut and cemented together were used. The dishes, filled with a Volvox suspension were placed in the dark boxes in one end of each of which a color screen was fitted. The series of eight boxes were then exposed to direct sunlight. At intervals the covers were raised to determine roughly the percentage of organisms responding, as shown by the completeness of their aggregation. The results of this series are summarized below, the plus sighs indicating an obvious positive heliotropism with the organisms collected against the glass on the side toward the sun, a zero indicating no evidence of any effect of the light.

Table No. 1.

Volvox Reactions to Sunlight behind Wratten Colored Filters.

Filter	Wave Length in Angstrom units.	Color	Exposure 5' I	5' II	10' I	10' II	20' I	20' II
70	6600 - 7000	Red	0	0	+	0	+	0
71	6200 - 6800	Orange	0	0	0	0	+	0
72	5900 - 6200	Orange	0	0	0	0	0	0
73	5600 - 5900	Yellow	+	+	+	+	+	+
74	5200 - 5600	Green	+	+	+	+	+	+
75	4700 - 5200	Blue	+	+	+	+	+	+
76	4000 - 4700	Violet	+	+	+	+	+	+
Control.	4000 - 7000	White	+	+	+	+	+	+

Table No. 2.

Filter	Wave Length	Color	3'	10'	15'	20'	30'
70	6600 - 7000	Red	0	0	0	0	+
71	6200 - 6800	Red	0	0	0	+	+
72	5900 - 6200	Orange	0	0	0	+	+
73	5600 - 5900	Yellow	+	+	+	+	+
74	5200 - 5600	Green	+	+	+	+	+
75	4700 - 5200	Blue	+	+	+	+	+
76	4000 - 4700	Violet	+	+	+	+	+
Control.	4000 - 7000	White	+	+	+	+	+

Table 1 shows that wave lengths from 4000 to 4700 A. are decidedly more powerful in directing the movements of Volvox than are those of the remainder of the visible spectrum, 4700 to 7000 A. U. There is an indication, however, that some individuals respond to the long waves.

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72	5900 - 6200	Orange	0	0	0	0	0	0
73	5600 - 5900	Yellow	+	+	+	+	+	+
74	5200 - 5600	Green	+	+	+	+	+	+
75	4700 - 5200	Blue	+	+	+	+	+	+
76	4000 - 4700	Violet	+	+	+	+	+	+
Control.	4000 - 7000	White	+	+	+	+	+	+

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71	6200 - 6800	Red	0	0	0	+	+
72	5900 - 6200	Orange	0	0	0	+	+
73	5600 - 5900	Yellow	+	+	+	+	+
74	5200 - 5600	Green	+	+	+	+	+
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Control.	4000 - 7000	White	+	+	+	+	+

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Table 2 shows that the stimulating efficiency of the different colors is dependent on the duration of the exposure. At the end of only three minutes, the organisms of the green, blue and violet were all over to the brighter side of the dish. At the end of 15 minutes the red, orange and yellow had still produced no visible effect. But, after 20 minutes, all but the longest waves, 6600 to 7000 had caused a definite aggregation; and after thirty minutes, the effect was seen behind this red screen.

As striking as this relation of the time factor to the power of light stiumli to produce the phototropism was the difference in the response behind the four filters of the green, blue, and violet. After only 3 minutes of exposure the aggregation effect was most marked in the violet, less in the blue and still less in the green. The difference in the appearance of the four dishes was due to the difference in the number of individuals that had responded positively and the completeness of their response, i.e. in 4000 – 470 practically every colony was against the glass on the outer side of the dish; in λ = 470 – 520 $\mu\mu$ there were more scattered about in the water. In 520 - 560 $\mu\mu$ still more and in 560 - 590 $\mu\mu$ there were just barely enough affected to make it evident that some were positively ~~affected~~ phototactic. After 10 minutes this gradiant was still visible. After about 15 minutes, the dishes all looked alike. This again shows the importance of the time factor, since the less sensitive organisms which respond less rapidly are after a longer time affected to the same degree.

In all the succeeding exposures with sunlight this rule held good, that the organisms behind 76 and 75 respond most rapidly and most colors is dependent on the duration of the exposure. At the end of only three minutes, the organisms of the green, blue and violet were all over to the brighter side of the dish. At the end of 15 minutes the red, orange and yellow had still produced no visible effect. But, after 20 minutes, all but the longest waves, 6500 to 7000 had caused a definite aggregation; and after thirty minutes, the effect was seen behind this red screen.

As striking as this relation of the time factor to the power of light stimuli to produce the phototropism was the difference in the response behind the four filters of the green, blue, and violet. After only 3 minutes of exposure the aggregation effect was most marked in the violet, less in the blue and still less in the green. The difference in the appearance of the four dishes was due to the difference in the number of individuals that had responded positively and the completeness of their response, i.e. in 4000 - 470 practically every colony was against the glass on the outer side of the dish: in λ = 490 - 520 μμ there were more scattered about in the water. In 5200 - 5600μμ still more and in 560 - 590μμ there were just barely enough affected to make it evident that some were positively ~~affected~~ phototactic. After 10 minutes this gradient was still visible. After about 15 minutes, the dishes all looked alike. This again shows the importance of the time factor, since the less sensitive organisms which respond less rapidly are after a longer time affected to the same degree.

In all the succeeding exposures with sunlight this rule held good, that the organisms behind 75 and 76 respond most rapidly and most completely, followed by those behind 74 and then 73. It was often quite impossible to detect any difference in the response behind 75 and 76.

It was noticeable in every series exposed that in the white light of the control, complete aggregation of the colonies as the result of their phototropism was not so rapid as in $\lambda = 400 - 470\ \mu\mu$ Repeatedly the observation was made that the control looked more like the culture in $470 - 520\ \mu\mu$. The explanation is probably either that the intensity was so much greater that some individuals were caused to respond negatively, or were neutral; or that the greater reflection from the back of the dish made all parts of the water more nearly alike in intensity of illumination and so tended to equalize the stimulus acting on the different sides of the organism.

When a series of cultures was exposed in the same way with the sun hidden by clouds no response could be obtained behind the red, orange and yellow filters.

These experiments show the greater stimulating efficiency of the blue end of the spectrum since comparison of the order of efficiency of the screens in producing the positive response is not in agreement with the order of the intensity of the light behind them. (see table 1) In fact the stimuli of least energy produce the most rapid response. They also show that with a longer exposure or more intense stimulus the less effective wave lengths may produce the same degree of response. In none of the exposures to sunlight were there any negative reactions or reversals of the response.

On April 6, the experiments were repeated with the electric arc as the source of light, and with the dark boxes at such distances from it that the intensity behind all was the same. The results are completely, followed by those behind 74 and then 73. It was often quite impossible to detect any difference in the response behind 73 and 76.

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Filter	Wave Length	Color	Distance	Exposure	
70	6600 - 7000	red	80 cm.	3'	5'
71	6200 - 6800	red	68	0	+
72	5900 - 6200	orange	58	+	+
73	5600 - 5900	yellow	52	+	+
74	5200 - 5600	green	70	+	+
75	4700 - 5200	blue	62	+	+
76	4000 - 4700	violet	62	+	+
Control.	4000 - 7000	white	85	+	+

The rapid response in the red was surprising since the energy must have been less than in sunlight. It took 20 minutes to get a noticeable reaction in the orange and yellow in sunlight the day befor Here with the arc as a source of light it took but three minutes. It took 30 minutes to get the response in the red that was produced by the arc in 5 minutes. The explanation might be that the colonies were more sensitive to light on April 6. It was unfortunate that this day was cloudy so that the experiment with sunlight could not be repeated. That the ability of the colonies to respond is dependent to a large extent on their own condition as affected by external factors of their invironment was shown strikingly by the fact that organisims kept over night in a small mouth bottle would not respond to any light stimulus, while others of the same collection kept in shallow pans reacted rapidly. Mast ( ) found considerable difficulty in getting consistent responses on account of the great change in the reactions of his organisms with change in their condition due supposedly to the light and temperature conditons.

ism. Also it is probable in view of Patten's work (1916) that the phototropic reactions of all organisms change with their age.

Another explanation which was suggested by the fact that white light was often slower in producing a response than the b lue and violet, is that the greater energy of the red in sunlight might have tended to reverse the positive reaction or neutralize it; or it might have been so reflected within the dish as to act more nearly with the optimum intensity on all sides of the organism. Be that as it may, the fact remains that repeated trials prove conclusively that at the end of a five minute exposure, all wave lengths of the visible spectrum have produced equal effects when the source of light is the electric arc. This is further proof of Mast's (1917) contention that: "There is no evidence indicating that stimulation in any of the species studied is independent of luminous intensity, for if the light in the spectrum on either side of the maximum be made sufficiently intense it becomes more effective than at the maximum."

On April 20, another collection of Volvox was made. It was hoped that a rough approximation of applicability of the Roscoe-Bunsen Law in the reactions of this form to light. This law states that other conditions being equal, the product of the intensity and the duration of the exposure is a constant. Owing, however, to the short time required for response and the variations in the sensitiveness of individuals making it impossible to determine the end points exactly nothing can be concluded as to the truth of this law for Volvox until more accurate means of measuring the beginning and end of the reactions is obtained. To show the difficulty produced by ism. ; Also it is probable in view of ~~Loeb's~~ Patten's work (1916) that the phototropic reactions of all organisms change with their age.

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This effect of changing the distance was repeated with the No. 70 filter, since as it transmits the longest wave lengths the time of the reaction should be longer. However, they were still too short to obviate the large errors due to the indefinite end points. At a distance of 25 cm. there was a noticeable collection on the lighter side of the dish. After 5 minutes nearly all had moved over. At a distance of 50 cm., 100 cm., and 200 cm., the same positive results were obtained with the five minute exposure. Although failing to show the applicability of the Roscoe-Bunsen Law this experiment shows the potency of the long red rays to cause the same prototropisms produced by the blue waves when they act with the right intensity.

Every exposure throughout these experiments was made with a fresh culture so that in no instance was there a chance of the sensitiveness being different for the successive exposures because of the effect of a preceeding illumination.

Holmes, (1903) reports that the positive phototropism in medium light is reversed by strong light. Attempts were made therefore to find the turning point for each screen by lessening its distance from the arc. But the control behind clear glass was the only one in which any negative phototropism could be obtained and the response here varied at different times. In one experiment, the

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An entire carbon arc spectrum was projected on one dish of organisms. By looking down it could be seen immediately that while those organisms finding themselves in the yellow, green, blue and violet light swam towards the light, those of the red moved not towards the front of the dish but in a direction parallel to it, i.e. towards the yellow. The yellow appeared to the eye the brightest part of the dish. The organisms in it were doubtless decidedly phototropic because of the relatively greater intensity, while those of the blue were so in almost equal degree because of the greater effectiveness of these wave lengths even at low intensity. In the red the intensity was not great enough to overcome the ineffectiveness of the long rays, so the organisms responded either to an intensity gradiant which led them thwards the yellow, or to a wave length gradiant which would also lead them toward the yellow. Or the result might have been due to a diffusion of the more efficient rays into the red. The same red rays in the naked arc exposure there was no deflection of the path leading directly towards the source of light.

It is interesting in this connection to note Holt and Lee's (1900) conclusions as to the role of the intensity factor in phototropisms. They state that, "There is no evidence that organisms respond to any other property of light than its intensity. All phototatic response is explained by the intensity of the light, and the direction from which it comes." But my experiments with Volvox show conclusively that for any given intensity the blue and violet wave lengths have the greatest stimulating efficiency, but that by increasing the duration of the exposure the same effect is produced behind the other screens; and by modifying the intensity of the other colors they can be made to produce an equally rapid response. This is in accordance with Mast's (1917) conclusions that phototropic responses are not entirely independent of intensity.