RELATION OF PHOTOTROPISM TO THE WAVE-LENGTH OF LIGHT*

EARL S. JOHNSTON, F. S. BRACKETT, AND W. H. HOOVER
(WITH TWO FIGURES)

Many conflicting theories and statements appear in plant physiological literature regarding phototropism. Such conditions frequently exist in a young science where much of the earlier work is qualitative. In many of these early experiments dealing with the bending of plant stems toward light, the lights used were such that it was impossible to determine accurately whether the color or the intensity was the predominating cause of the bending. The relative influence of the different colors, or wave-lengths, could not be determined because different intensities were used. Both of these light factors must be considered, as a greater value of one may off-set the lesser value of the other. In order to determine which colors are most effective in phototropic bending, it is necessary to use wave-lengths of equal intensities.

One method of evaluating the wave-length effects is described by Hurd.\(^1\) Wratten light filters were used between the light source and the fucus spores and rhizoids with which this work was performed. The intensities were made equal by increasing or decreasing the distance of the electric arc from the measuring instrument. Parr,\(^2\) working with Pilobolus under carefully controlled conditions, found that light in all regions of the visible spectrum brought about responses in this plant. The presentation time decreased gradually from red to violet, with no indication of intermediate maxima or minima. This work appears to be at variance with statements appearing even in recent text books on plant physiology, that the more highly refrangible rays of light are most effective in phototropic movement with the effect diminishing from blue to yellow, and again increasing in the red and infra-red.

Priestley\(^3\) has recently attempted to give a rational explanation to the phenomenon of phototropism. He shows that phototropic curvature in coleoptiles is consistent with the "light-growth" hypothesis, in spite of

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\(^1\) Hurd, Annie May. Some orienting effects of monochromatic lights of equal intensities on fucus spores and rhizoids. Proc. Nat. Acad. Sci. 5: 203–204. 1919.


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many seemingly discrepant experiments. The amount of light required to induce phototropic curvature in normal light-grown shoots is greater, and must be continued longer, than that required to bring about similar curvatures in etiolated shoots. It is thus evident that light affects normal and etiolated shoots quite differently. Briefly, the mechanism of bending in etiolated shoots as discussed by PRIESTLEY, is as follows: The walls of the cells making up the tissue contain fat and protein. These substances prevent the ready passage of sap and water from the vascular system to the meristematic tissue which, under favorable conditions, is capable of rapid growth. BLAAUW points out the similarity between light and its photochemical effect on a photographic plate on the one hand and an etiolated coleoptile on the other. Relatively small quantities of light produce photochemical actions on these shoots. Protein and fatty materials disappear from the cell walls, with the latter substances migrating mainly to the cuticle. The passage way between the meristematic cells and their water and food supply is opened up. In the words of PRIESTLEY, "Increased surface growth now ensues. Growth as a whole may be as active as ever on the more brightly lit side of the etiolated shoot, but it is differently distributed. More cells are added to the surface of the stem and leaf, and less proportionately contributed to the inner layers of the shoot axis. The result is, therefore, in the aggregate, a retardation of growth in length on the illuminated side, and a positive phototropic curvature."

Two classes of explanations for the modification in growth rate have been proposed: First, those assuming the effect to be predominantly due to a local temperature change brought about by unsymmetrical absorption of radiant energy; second, those postulating some type of photochemical action. In this second group would fall both those theories involving a local and direct photochemical change at the point of absorption of light, and those assuming a type of hormone action. The first group, involving a purely thermal hypothesis, would lead to a prediction of phototropic bending for all regions of the spectrum where the radiant energy could be absorbed by the cell materials. Infra-red for all wave-lengths longer than 1.1 \( \mu \) is absorbed by the water, even in relatively small layers. The remainder of the cell materials would absorb light pretty generally through the entire visible spectrum. Where etiolated plants are used, with the absence of chlorophyll, a very selective action would not be expected. In the case of a photochemical hypothesis, however, one might have a highly selective type of absorption, either showing maxima and minima, or beginning weakly at some particular wave-length and increasing towards the shorter wave-lengths or blue end of the spectrum. Such selective absorption in the visible or ultra-violet is characteristic of electronic changes in energy within the molecule.
In the present experiment it was proposed to determine whether or not selective regional phototropic responses (i.e., to different colors) would be found for equal intensities. Second, to determine, in case of selective responses whether the effect of different colors could be off-set by modification in intensities. Such an experiment would crucially decide between the two classes of hypotheses.

The general underlying idea of determining the relative influence of colors in a differential manner by exposing the plant to two different colors from opposite sides was proposed earlier by Johnston. The present experiment was simply an elaboration of this proposed method, wherein particular attention has been given to the physical quantities involved. The wave-length regions have been restricted by filters whose transmission curves were definitely determined. The intensities were measured by means of an especially constructed thermocouple and galvanometer system of high sensitivity, with which accurate determinations could be made over an unusually large range of relative intensities.

The details of the apparatus are as follows: The thermocouple-galvanometer system constitutes essentially a blackened thermometer whose rise in temperature above the surrounding room temperature is proportional to the amount of radiation falling upon it, and is practically independent of the wave-length of the radiation. The rise in temperature was indicated by a galvanometer deflection which was read by the displacement of a small band of light along a metric scale. The system consisted of a single closed electrical circuit of which the d’Arsonval galvanometer coil was a part. Where dissimilar metals are used in a circuit, the unequal heating of the points of contact causes an electromotive force. For small differences in temperature this electromotive force, and the current arising, is proportional to the difference in temperature.

The galvanometer coil and most of the circuit were constructed of copper. The remainder of the circuit was made up of a short length of fine bismuth wire and a short length of bismuth-tin alloy. The juncture of the copper with the bismuth, and the juncture of the copper with the bismuth-tin were each maintained at room temperature. To the point where the bismuth joins the bismuth-tin wire, a blackened receiver was attached. Radiant energy falling upon such a receiver raises its temperature above that of the junctures to the copper, and so causes the current. The alloy of bismuth-tin was made up of 95 per cent. bismuth and 5 per cent. tin. It is interesting to note that this small percentage of tin yields a material of opposite thermoelectric characteristics to pure bismuth.

In the choice of materials for construction of such a thermocouple, not

only the thermoelectric power, but also the resistance and the thermal conductivity should be considered. This combination of metals is the best known at the present time. The wire used was about 25 microns in diameter and between 2 and 3 mm. long. The receiver was a circular piece of platinum foil about 2 mm. in diameter. The thermocouple was then placed in a vacuum of better than $10^{-4}$ mm. pressure thereby serving a double purpose. First, the sensitivity was increased by the removal of convection loss, and second, small disturbances which ordinarily would reach the thermocouple through convection were eliminated. When radiant energy is focused upon such a receiver by means of a lens, it is equivalent to greatly increasing its area, and thus yielding a greater response than an actual increase in area would give, because the thermal losses would be increased at the same time. It is necessary, however, that the same effective area be used throughout the experiment. Because of the very great range of intensity to be observed, it was necessary to use a number of resistances in series and parallel, in order to change the sensitivity by several known factors.

The plant photometer box was 238 cm. in length and 30.5 cm. wide. It was divided into five compartments instead of three as in the earlier experiment. The end, or lamp compartments, were 59 cm. long and 59 cm. high, while the other three were 44 cm. high. The central, or plant compartment (87 cm. in length), was insulated from each lamp compartment by a filter chamber 10 cm. wide. Before the light could reach the plant from either side, it was passed through a water filter immediately surrounding the lamp, through a plate glass window, then through the filter chamber which was cooled by a stream of air, and finally through the desired color filter and a water cell, one window of which was made of heat absorbing glass. This water cell was set at an angle to avoid reflecting light to the plant from the opposite side of the plant chamber.

By the introduction of the cooled filter compartments between the lamp compartments and the plant chamber, heat necessarily arising in the lamp chambers was prevented from reaching the plant compartment. By means of the water cell with the heat absorbing window on the lamp side, only visible radiation was allowed to reach the plant. Water absorbs all the radiation in the infra-red longer than 1.1 $\mu$, while the heat absorbing filter cuts out the near infra-red region between 1.1 $\mu$ and the visible. In the plant chamber the seedling was surrounded by a double-walled glass cylinder, the space between the walls being filled with water and the entire cylinder slowly rotated around the plant axis in order to equalize temperature conditions in the immediate environment of the seedling. The interior walls of all five chambers were painted a dull black. A general view of the photometer box is shown in figure 1.
The coleoptile of the oat was selected for these experiments. The seeds were germinated between glass plates covered with wet filter paper. The plates were so arranged in moisture chambers that the seedlings grew vertically. The seedlings were carefully selected for straightness and transferred to small Erlenmeyer flasks fitted with cork stoppers. Each seedling was supported by means of a little cotton in a small hole of the stopper. The flask was filled with distilled water so that the roots were entirely immersed. By means of a cross hair in a telescope the seedling was adjusted to a vertical position in the plant compartment between the light filters.

In conjunction with the heat absorbing cell, four different light filters (2" x 2") were used in these preliminary experiments. Three were Wratten filters numbers 24 (red), 61 (green), 47 (blue) made by the Eastman Kodak Company, and one a "heat resisting yellow" (yellow shade) made by the Corning Glass Company. The curves presented in figure 2 indicate the transmission of these filters. From these curves it will be observed that the red filter transmits freely all wave-lengths greater than 6000 Å. and absorbs all wave-lengths shorter than 5800 Å., in fact, cutting off practically all
wave-lengths shorter than 5900. The yellow filter transmits freely all wave-lengths longer than 5400 and cuts off all wave-lengths shorter than 5200, effectively removing all wave-lengths shorter than 5350 Å. The green filter shows a maximum transmission at about 5100, practically cutting off all wave-lengths beyond 6000 on the red side, and 4800 on the blue side, with no measurable transmission shorter than 4700. The blue filter transmits effectively a region from 3900 to 5000.

Fig. 2. Curves representing percentages (ordinate) of transmission of the light filters; blue (B), green (G), yellow (Y), and red (R). Wave-lengths are expressed in Ångström units along the abscissa.

The results obtained were as follows. In the first place where the plant was exposed to radiation from only one side with the water cells all removed, and a filter introduced which absorbed all the visible light, no phototropic bending could be observed. In other words, infra-red radiation between the visible and 2.5 μ produced no phototropic bending which could be observed. Second, with the heat absorbing filters together with the red filter and no light from the opposite side, no measurable phototropic bending could be detected. Hence no wave-lengths longer than 6000 Å could be found to produce a measurable phototropic effect. Similarly, with the yellow filter substituted for the red filter, the plants were grown with no opposing light. In this case, a noticeable bending was soon apparent. Hence a phototropic influence is certainly to be found in the region between 5200 and 6000 Å units, and probably in the narrow region between 5350 and 5900 Å. This is the region usually termed yellow. It is impossible from the present experiment to say which portion of this region may be effective. For the green and blue filters the unbalanced phototropic effect was very marked, the bending taking place in a very few minutes. The first differential balance was observed with the yellow light on one side and the green light on the other. A balance was actually secured, however, only when the yellow light was 1,000 times more intense than the green light as determined by the non-selective thermocouple-galvanometer mea-
surements. In the same way a balance was reached between the green and blue when the green light was 30 times more intense than the blue.

In each experiment a single seedling was used. The general procedure was to place the seedling between two different light sources and, after a time interval, observe the coleoptile through the telescope in order to determine any growth curvatures. If, for example, the seedling was exposed between the blue and green filters and a distinct bending toward the blue was shown at the end of half an hour, the lights were so adjusted by position and current controlled by rheostats, that the green intensity was increased and the blue decreased. Another seedling was then placed in the photometer and the experiment repeated. After several trials a balance point was reached where the effect of one light was neutralized by the effect of the other, and the plant continued to grow in a vertical position even after an exposure of several hours. When this balance point was reached the seedling was removed and the especially constructed thermocouple placed in the position of the plant. The relative intensities of the lights were then measured by means of the galvanometer.

It is of interest to note that repetition of the experiment yielded balance points differing by less than 5 per cent. from the previous experiment. Such reproducibility is somewhat unusual in biological measurements. It suggests that by this method one is observing a characteristic of some underlying photochemical reaction. The absence of effect in the red and infra-red together with a very sharp increase in passing from yellow to green, and the subsequent rise in the blue, is typical of an electronic photochemical reaction. The experiment must be regarded, therefore, as crucial evidence against a purely thermal theory and strong support for a photochemical theory.

The results seem of sufficient importance to justify a more elaborate experiment wherein narrower spectral regions are used, that is, more restricted color ranges, thus enabling one to determine the phototropic effect at many points through the spectrum. For this purpose a monochrometer must necessarily be used. In such an experiment it seems desirable also that the phototropic influence of all the wave-length regions be determined in terms of a single comparison band. The intensity of the comparison light could be varied to counterbalance successively the phototropic influence of each band or color, taken preferably all at the same intensity. The experiment should also be carried into the ultra-violet in order to gain the additional information which may throw some light upon the nature of the photochemical reactions involved.

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