

EFFECTS OF RED LIGHT ON GEOTROPISM OF AVENA AND THEIR POSSIBLE RELATIONS TO PHOTOTROPIC PHENOMENA

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ABSTRACT

Red light enhances the geotropic response of excised *Avena* coleoptiles.

With higher dosages this enhancement occurs in the main in the upper centimeter of the organ and is compensated by a decrease in the geotropic response of the base. In both of these aspects the red light induced alterations are dosage dependent in the range of irradiancies investigated (from 2000 to 240 000 erg/cm²; intensity: 1000 erg/cm² sec).

The influence of even a relatively small dosage of red light (2000 erg/cm²) is evident for at least four hours after the illumination.

Possible relations with phototropic phenomena are discussed. From the current hypotheses about the mechanism of the red light-induced alterations in tropic responses, that of red light influencing the pigment system active in phototropic perception is considered to be the least attractive one.

INTRODUCTION

In recent years several authors have reported about red light induced changes in the phototropic behaviour of etiolated corn, oat and barley seedlings. Results obtained by CURRY (1957),¹⁾ BRIGGS (1963), ZIMMERMAN¹⁾ (1962), and ZIMMERMAN and BRIGGS (1963) agree in that preillumination with red light for one or several hours before the phototropic stimulation, induces a shift in the log dosage-response curve in the direction of larger dosages, in so far as first positive and "negative" curvatures of corn and oats are concerned.

Observations in the same field by BLAAUW-JANSEN (1959) and ASOMANING and GALSTON (1961) appear to be in disagreement with the results mentioned above. Some of these disagreements, however, can probably be attributed to the circumstance that the illumination schedules of the latter authors differed markedly from the schedules used by the authors mentioned in the preceding paragraph.

Attempts of BRIGGS (1963) to reproduce the phenomena observed by BLAAUW-JANSEN—viz. an enhancement of the first positive curvature of *Avena* brought about by red light administered immediately before or after the phototropic stimulation—were unsuccessful. The cause of this might be his using white light as a phototropic stimulus (incandescent, 1000 MCS) which contains a very large amount of red light, and the intensity of this light stimulus being much larger than Blaauw-Jansen's, who used blue light of an intensity of 0.5 erg/cm² sec. In similar attempts of ZIMMERMAN and BRIGGS (1963) blue

¹⁾ As the original papers were not available to us, our information on these investigations is second hand.

light was used as a phototropic stimulus, but again, Blaauw-Jansen's observations could not be reproduced. In these investigations, however, an exact repetition of her experimental procedure was also not aimed at.

In the present author's view the light regime used by Asomaning and Galston is not comparable with that of the other authors.

Regarding the mechanism of the red light influence several hypotheses have been suggested. The first hypothesis (BRIGGS, 1963) is that red light may induce a change in the amount or physical state of the phototropic pigment complex. ASOMANING and GALSTON (1961) and COHEN and GOODWIN (1962) have shown that red light increases the amount of yellow pigments. The results obtained by Blaauw-Jansen, however, do not support this first hypothesis.

Another hypothesis suggests phototropic sensitivity being altered indirectly by alterations in the endogenous auxin relationships. To the present author the latter hypothesis appears to be more likely because it may explain not only red light induced phototropic alterations but geotropic alterations as well. Moreover, red light indeed diminishes the amount of diffusible (VAN OVERBEEK, 1936; SCHNEIDER, 1941; BRIGGS, 1963) as well as of solvent extractable (BLAAUW-JANSEN, 1959) auxin from *Avena* coleoptile tips. The work of SCHNEIDER (1941), however, demonstrates that the effect of red light on coleoptile growth is highly complex and cannot be explained by contemplating auxin levels alone.

As a third hypothesis it has been proposed that red light influences the tissue sensitivity to auxin and this also seems to be an attractive possibility, producing an explanation for red light induced changes in both phototropism and geotropism. Moreover, this hypothesis gains strong evidence from BLAAUW-JANSEN's (1959) discovery of the "red light factor". She isolated this substance from red irradiated coleoptiles. It promotes the effectivity of low auxin concentrations, so low as to exhibit only little growth promoting effect by themselves. LIVERMAN and BONNER (1953) tried to explain the influence of red light as eliciting the formation of an auxin receptor. These authors, however, used fairly high auxin concentrations in their investigations. In both instances a material basis to the vague conception of "enhanced tissue sensitivity" can be presented.

The present author (BLAAUW, 1961) did some work on the influence of monochromatic light on the geotropic response of *Avena* seedlings. He noted an enhanced response after red or far red illumination (dosages in the order of 10^4 erg/cm²). With 1-hr unilateral exposition to gravity the most pronounced effects were found when a waiting period of 30 minutes was inserted between the illumination and the beginning of the geotropic exposition.

Blaauw-Jansen concluded from her experiments on the influence of red light on phototropism and in the *Avena* curvature test that the primary leaf might be the site of perception of the red light. In his investigations mentioned above the present author found that seedlings from which the primary leaf had been removed are, with respect to their geotropic response, influenced by red light in the same way

as intact seedlings. Similar observations in the realm of phototropism were reported by ZIMMERMAN and BRIGGS (1963) with excised coleoptiles in agar. As, however, the red light effects appear to be rather complex, the present author, in his experiments to be described in this paper, preferred to rule out all possible inferences of the primary leaf and other organs and therefore worked with excised coleoptiles. In the experimental procedure to be described in the next section a further advantage of this method is that the isolated coleoptiles show a much lower variability than intact seedlings do.

MATERIALS AND METHODS

Husked seeds of *Avena sativa* (cultivar "Victory") were, after having been moistened by shaking them with tap water, put to germinate on moist filter paper in a closed Petri dish at 22° C. During the 19 hours germination period the seeds were exposed to orange light from an incandescent lamp combined with a Schott OG2 filter. Next the seeds were grown for 72 hours in moist vermiculite in absolute darkness at a temperature of 22° C. and a relative humidity of 80 %. Seedlings obtained in this way have first internodes 12–20 and coleoptiles 20–30 mm long.

Working with very dim green light from a Balzer interference filter (λ 560; 0.3 erg/cm² sec) and for the greater part by touch, coleoptiles were excised and after removal of their primary leaves were put upon pins with their narrow sides facing each other. The pins are placed in small cavities cut in perspex strips (Fig. 1), each strip containing 12 pins. The cavities are filled with water as a supply for the coleoptiles; they are so small, that the water does not flow out when the strips are tilted or turned over. After these manipulations the coleoptiles are kept in darkness for at least two hours.

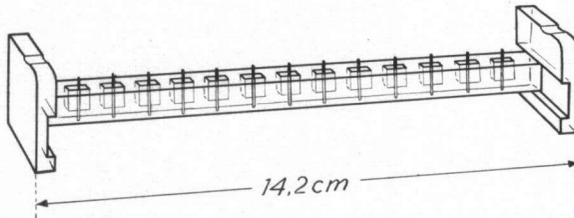


Fig. 1. Perspex strips with pins on which coleoptiles are fixed.

Then the coleoptiles were irradiated from above with red light. The red light was obtained from an incandescent lamp (60 Volt, 50 Amp) with an appropriate system of lenses and filtered through a Zeiss double band interference filter (λ 660). In this way an area of about 20 cm in diameter was illuminated with an intensity of 1000 erg/cm² sec.

In the course of the development of the geotropic reaction at 30 minute intervals shadowgraphs were made on Kodak Royal X Pan sheet film. Light of 560 m μ was used to this end. The sensitivity of

the film being 1250 ASA (= 32 DIN), very little green light suffices to produce a satisfactory blackening.

Curvatures were measured by means of a goniometer. Radii of the curves were estimated with the help of the device represented in Fig. 2. It is a piece of transparent sheet with engravings consisting of a straight line plus part of an arc of a circle, the numbers giving the radii of the arcs in mm. By sliding the sheet over the image of a coleoptile, the arc is found to which the curve of the coleoptile fits.

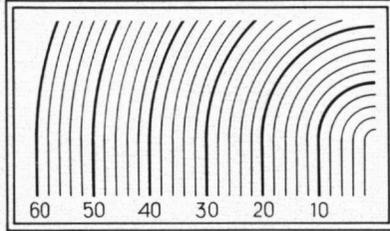


Fig. 2. Device used for estimating the radii of the curves of the coleoptiles.

EXPERIMENTS AND RESULTS

A number of introductory experiments showed, that preillumination of excised *Avena* coleoptiles with relatively small dosages of red light enhances the geotropic curvature and that this effect can be perceived up to several hours after the preillumination. It was also found that the shape of the curve of the coleoptile developed after unilateral exposition to gravity, depends on the dosage of red light administered. In the experiment next to be described in detail, the treatments tried separately in the introductory experiments were combined. The results obtained were in close agreement with the earlier observations.

Sixteen rows of excised coleoptiles were illuminated two by two with a series of 8 red irradiancies amounting from 2000 erg/cm² to 240 000 erg/cm², the intensity being 1000 erg/cm² sec. Four rows were kept in darkness. Thirty minutes after the illumination all coleoptiles were put in a horizontal position and shadowgraphed with 30 minute intervals during a 3½ hours' exposition period. Irradiancies used and geotropic curvatures developed are presented in Table 1 and in Fig. 3. The last two columns of data in Table 1 give the values of the radii of the curves for those cases in which these curves can be described as consisting of one single arc of a circle (see Fig. 4, a and b). In cases in which the curves of the coleoptiles appear to consist of two arcs (as in Fig. 4c), an estimation of the radii of those arcs is rather difficult. It seems that the size of the geotropic curvatures developed by the preirradiated coleoptiles might be a function of the red light dosage, but only to a small extent. The radii of the curves, however, are strongly dosage dependent. In Fig. 3 we can see that the rates of development of the curvatures of the preilluminated coleoptiles remain greater than those of the dark control throughout the whole course of the experiment. As there was a waiting period of half an hour

TABLE 1

row nr	illumination erg/cm ²	number of coleoptiles	curvatures developed after minute (in degrees)							radii of curves after minute (in mm)		
			30	60	90	120	150	180	210	120	210	
A1	2 000	12	2.4	13.9	25.1	34.4	44.7	53.1	59.6	}	21	15
A2	2 000	9	2.1	12.4	22.1	30.2	40.6	49.3	57.2			
A3	4 000	10	2.3	12.5	22.0	29.9	38.5	45.7	53.0			
A4	4 000	9	2.2	13.6	23.0	30.6	39.9	47.2	54.4			
B1	8 000	11	3.4	15.4	28.2	36.3	45.9	53.8	61.2	}	12.5	9.5
B2	8 000	11	2.8	15.3	26.8	35.5	43.6	50.5	58.5			
B3	16 000	11	3.0	16.3	29.3	38.6	47.0	54.8	62.3			
B4	16 000	10	2.9	13.2	16.4	37.0	43.6	51.8	57.6			
C1	30 000	11	1.6	14.2	26.2	37.0	45.0	52.3	57.5	}	11.5	8.5
C2	30 000	12	2.2	13.6	24.1	33.6	40.8	47.2	52.9			
C3	60 000	10	1.8	16.0	29.0	43.4	52.1	61.8	67.8			
C4	60 000	8	1.1	11.9	24.2	34.2	43.0	49.8	54.6			
D1	120 000	10	3.0	14.9	26.2	37.0	45.6	53.8	60.8	}	33	24
D2	120 000	8	2.5	15.4	27.4	38.5	48.9	56.6	62.4			
D3	240 000	11	3.5	17.4	30.5	42.8	53.9	64.2	70.7			
D4	240 000	8	1.5	16.4	29.5	40.4	50.3	56.9	62.0			
A5	dark	8	0.9	7.2	13.9	20.5	25.8	30.2	35.6	}	33	24
B5	dark	10	2.6	8.8	16.0	21.0	25.7	30.1	35.0			
C5	dark	10	1.6	8.5	16.1	20.7	26.1	31.0	35.0			
D5	dark	11	1.6	8.3	15.6	21.1	26.1	29.8	35.6			

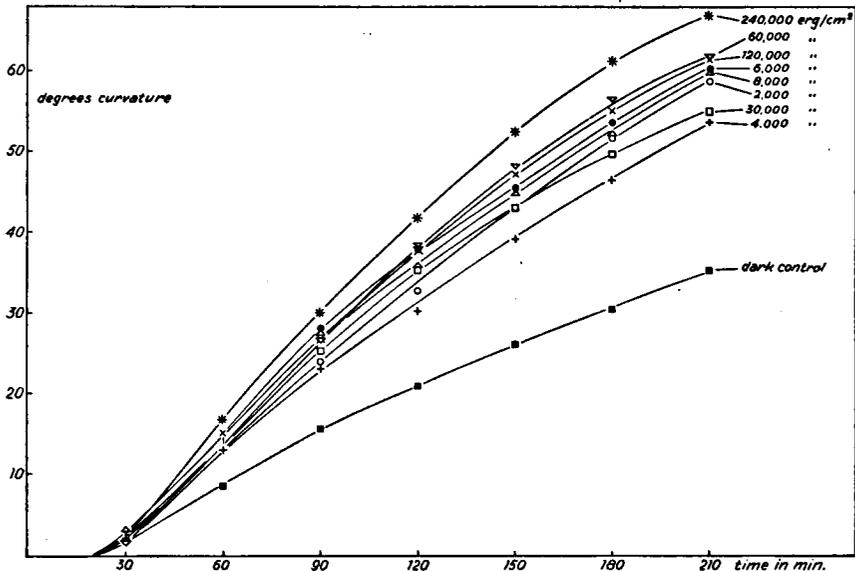


Fig. 3. Response curves found after short lasting preilluminations with red light, administered half an hour before the beginning of the geotropic exposition.

between the illumination and the beginning of the unilateral exposition to gravity, it thus becomes evident that the influence of the preillu-

mination on the rate of development of the curvature is to a full extent perceptible after 4 hours. The same statement holds with respect to the shape of the curves.

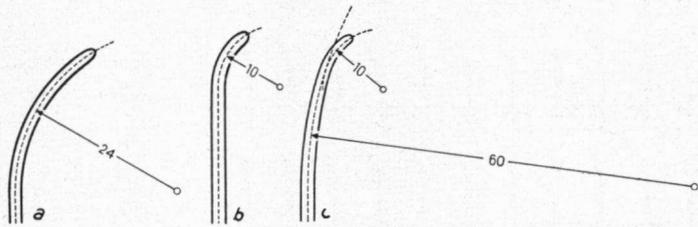


Fig. 4. Types of geotropic response curves found after preillumination with a) small, b) large and c) intermediate dosages of red light.

Fig. 5 shows reproductions of part of the shadowgraphs made in the course of the experiment. Sub a, b and c show stages of the development of the curves at points of time that are chosen in such a way that all curvatures amount to about the same values (from 35 to 40 degrees). Fig. 5d shows curves of dark controls developed in two hours. Fig. 5e and f show the coleoptiles of Fig. 5b and c after $3\frac{1}{2}$ hours. As can be seen from these pictures, coleoptiles that received the larger dosages of red light curve in a way that differs markedly from that of the others, in an early stage already. Fig. 5g shows examples of the type of curve that is found in coleoptiles that have received intermediate dosages. Their shape is schematically drawn in Fig. 4c.

With the help of a photographic enlarger and a curvimeter the growth rate of the convex and of the concave side of the coleoptiles was measured. The growth of the concave side was always less, that of the convex side always more than that of the dark control. In the coleoptiles to which the larger dosages of red light were administered, the growth rate of the concave side proved to be even significantly negative between the 30th and the 90th minute after the beginning of the geotropic stimulation. As, however, no measurements were made of the growth rate of different zones of the coleoptiles, these data may be of little use.

DISCUSSION

The enhancement of the geotropic response—measured in degrees—after preillumination with red light is far less dosage dependent than is the influence on the shape of the developed curves. With large dosages of red light the difference in growth of the convex and concave sides of the coleoptile is located in the topmost centimeter or so only, the base remaining perfectly straight (Fig. 4a), whereas with small dosages a less or almost equal difference occurs over the full length of the coleoptile (Fig. 4b). As with intermediate irradiancies intermediate shapes arise (Fig. 4c), it appears that small irradiancies only

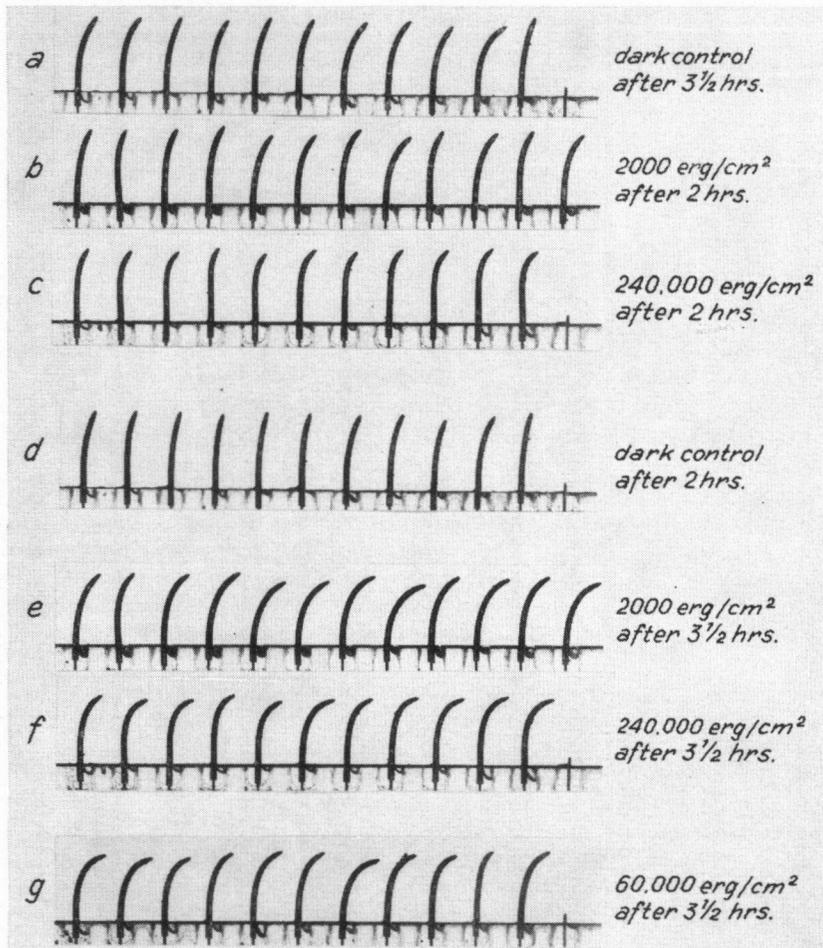


Fig. 5. Some prints of shadowgraphs showing development of bends.

cause a general enhancement of the geotropic response, whereas with larger irradiancies a dosage-dependent further enhancement of the geotropic response of the topmost centimeter of the coleoptile and a dosage-dependent diminishing of the geotropic response of the base can be observed. These two effects compensate each other to a great extent when the number of degrees curvature is considered.

The situation recalls the observation of CURRY, THIMANN and RAY (1956), who found that red light accelerates the growth of the topmost centimeter of the coleoptile, and inhibits—to a smaller extent—the growth of the basal part. A red-induced acceleration of the growth of the topmost centimeter was also found by the present author (BLAAUW, 1961).

It is likely that the mechanism of red light induced alterations is connected with the endogenous auxin relations in the coleoptile. From several investigations it is known, that in some instances red light diminishes the endogenous auxin contents of the tip. BRIGGS, (1963) noted the following phenomena, when measuring the amount of auxin diffusing from excised tips:

- 1) After two hours' illumination of intact seedlings with red light, less auxin diffuses from their tips than from the tips of the dark controls.
- 2) When tips are illuminated after having been cut from the seedlings, the same amount of auxin diffuses from them as from the tips of the dark controls.

BLAAUW-JANSEN (1959) found:

When one hour after the illumination of the seedlings with red light, the tips are solvent-extracted, less auxin is found than from tips of dark controls.

From these results it may be concluded that red light does not influence the rate of auxin synthesis or decomposition in the tip, but that it accelerates the basipetal transport. $1\frac{1}{2}$ hours after the red illumination (Blaauw-Jansen) or two hours after the beginning of the red illumination (Briggs) a large part of the auxin contained in the non-irradiated tip appears to have migrated to basal parts of the coleoptile. In consequence, the endogenous auxin concentration in the upper part of the coleoptile has possibly become more favourable for a geotropic response—assuming for the moment, the lateral transport system not being affected by the illumination. Then the observation that with large dosages of red light, the base of the coleoptile remains straight, could be explained with the assumption that the enhanced drainage of auxin from the upper part causes an accumulation of auxin in the base, so that in that region the situation becomes less favourable for geotropic response (cf. ANKER, 1956).

At first sight the observations on red induced growth alterations mentioned above, are not in keeping with this image. Blaauw-Jansen's work, however, opens the opportunity to explain these alterations as occurring in the concentration range in which her "red light factor" plays its part. This substance effectuates a pronounced beak in the auxin concentration-growth curve in the concentration range from 10^{-9} to 10^{-8} mg/ml IAA. So, in the presence of the "red light factor" a lowering of the endogenous auxin contents might go with an enhancement of growth and inversely. The occurrence of a similar beak in the IAA concentration vs. epinastic curvature response in petioles of *Coleus* was reported by SOEKARJO (1961).

Attempts to produce the red light-induced effects in decapitated coleoptiles supplied with extracts from red-illuminated plants or with growth controlling substances may shed more light on our problems. Investigations in this direction are in progress.

At this moment what we can say with certainty is only that red

light alters the condition of the coleoptile in such a way that its response to a unilateral geo-stimulus is markedly influenced. It therefore seems to be highly probable that such a change of condition will cause an altered response to a phototropic stimulus as well. The concept of red light influencing the pigment system active in phototropism, therefore is not evidently needed.

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