THE PHOTOTROPIC RESPONSES OF AVENA COLEOPTILES

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SUMMARY

The various phototropic curvatures of the oat coleoptile are presented in a three-dimensional diagram. Three positive responses can be distinguished, separated by areas of negative or at least decreased curvatures. The reciprocity law is valid for two of the positive responses and one negative response. A third positive response is initiated over a large range of intensities at a fixed stimulus duration. The slope of its increase with stimulus duration, however, depends on light intensity.

1. INTRODUCTION

The extent of the second positive curvature is said to be dependent on stimulus duration (THIMANN & CURRY 1960; ZIMMERMAN & BRIGGS 1963a, b; EVERETT & THIMAN 1968; PICKARD 1969). In view of a planned analysis of this phenomenon we re-examined the relations between positive and negative curvatures of the *Avena* coleoptile. The dependence of these curvatures on light intensity and duration of stimulus and the influnece of a red pre-irradiation were investigated.

2. MATERIAL AND METHODS

Avena coleoptiles were raised and isolated as described by BLAAUW-JANSEN & BLAAUW (1968). The coleoptiles were placed on pins that were fixed in cavities filled with tap water. At 1 to 2 hours from the time of isolating the coleoptiles were exposed to 2 hours of the same red light that was used to inhibit mesocotyl growth (light from a 40-Watt Philips red fluorescent tube filtered by red selenium glass, the intensity being approximately 400 erg/cm^2 sec). In experiments in which the effect of red light was studied this treatment was omitted. Phototropic stimulation followed the red light period by irradiation of the narrow sides of the coleoptiles with blue light. Monochromatic blue light of an intensity lower than 10^4 erg/cm^2 sec was obtained from an incandescent iodine lamp with an appropriate system of lenses, a layer of 5 cm of water, a Calflex filter (Balzer, Liechtenstein) and a 458 nm double band filter ("Depal", Schott u. Gen., Mainz). In some of the experiments the 458 nm filter was replaced by 2 Broad-Band blue Filtraflex-DT filters (Balzer) cutting off wavelengths longer than 500 nm and thus transmitting light of an intensity of about 10^4 erg/cm^2 sec.

The light intensity was varied by changing the distance of the holders from the light source and by the use of NG (type neutral) filters (Schott u. Gen., Mainz). For exposure times of less than 1 second the shutter mechanism was used, for longer exposure times the shutter was operated by hand and timed with a stopwatch.

The double shadowgraph technique was applied, which records initial and final (post 100 min) position. The shadowgraphs were taken with safelight of 560 nm (about 0.01 erg/cm^2 each time) and measured with a goniometer. Each value in the plotted data is based on measurements of at least 12 coleoptiles.

3. RESULTS

3.1. Positive and negative responses

Two series of experiments were performed. In the first series the dose-response curves for 13 fixed durations of stimulus were determined. The length of exposure ranged from 1/100 sec to 40 min. In each experiment the coleoptiles were stimulated with blue light at 16 intensities (from 10^{-2} erg/cm^2 sec up to 10^3 erg/cm^2 sec). *Fig. 1* shows the dose-response curve resulting from one of these experiments (exposure duration was 30 sec).

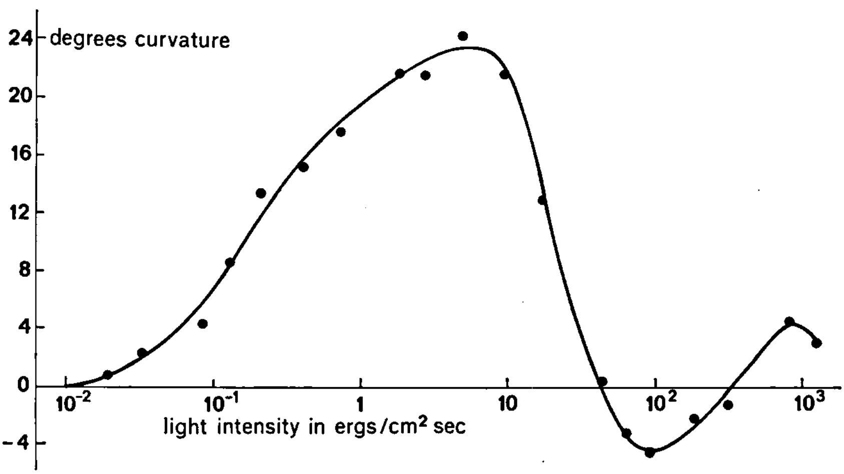

Fig. 1. Dose-response curve for phototropism in oat coleoptiles. Exposure duration was 30 seconds.

In the second series of experiments plants were irradiated with a fixed light intensity for intervals from 1 sec to 40 min. The result of an experiment in which the plants were exposed to $10^{1.27}$ erg/cm^2 sec is presented in *fig. 2*. In other experiments light intensities of $\log I = 0.4, 1.6, 2.9, 3.1, 3.5$ and 4.1 (erg/cm^2 sec) were used.

The dose-response curves resulting from these two series of experiments were normalized and composed to the diagram of *fig. 3*. Two ridges of positive curvatures traverse the diagram. The first (A) is a ridge with a peak height of about

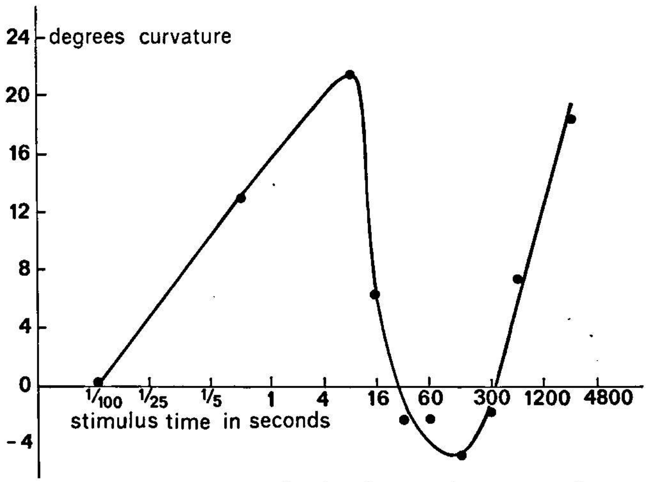

Fig. 2. Dose-response curve for the phototropic response of oat coleoptiles. Light intensity: $10^{1.27}$ erg/cm² sec.

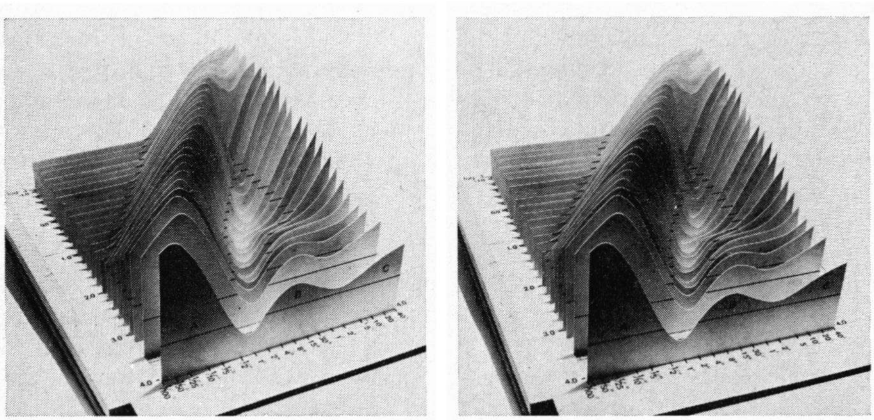

Fig. 3. Three-dimensional graph of the various phototropic responses of *Avena* coleoptiles, combining the series of experimental graphs. (Left to right: increasing exposure time; Front to rear: decreasing intensity; Vertical axis: curvature.) A three-dimensional view can be obtained by treating these two frames as a stereoscopic pair.

25°, running along a line of equal light quantities of about 120 erg/cm². These curvatures have the characteristics of the first positive curvature for which the reciprocity law is valid. The presence of the maximum curvature at about 120 erg/cm² is in accordance with the data of DU BUY & NUERNBERGK (1934), ZIMMERMAN & BRIGGS (1963), EVERETT & THIMANN (1968), and MEYER (1969).

Another positive ridge (B)[1] runs parallel to the first separated by the elonga-

[1] Ridge B is identical with the second positive curvature of DU BUY and NUERNBERGK (1934) and is missing in recent dose-response curves. Area C is identical with the second positive curvature as distinguished by recent authors, with system III of ZIMMERMANN and BRIGGS (1963) and with the third positive curvature of DU BUY and NUERNBERGK (1934). See discussion.

ted valley of negative curvatures. Its maximum value is only about 8 degrees and it is restricted to the region of light intensities above 100 erg/cm² sec. At lower intensities and longer exposure times this ridge evanesces into the indifferent zone described by CLARK (1913) and noted by THIMANN & CURRY (1957). This indifferent zone is thus the first indication of the appearance of the high intensity ridge B.

The above scenery of the "positive" ridges A and B and a "negative" valley is bordered towards longer exposure times by the slope of a "positive" elevated area (C)[1]. It extends over the full range of light intensities and is initiated at an exposition time of about 4 min. Its appearance seems to depend on the duration of the light stimulus and not on its intensity, but its slope depends on light intensity.

The valley of negative curvatures follows a line of equal light quantities at about 3×10^3 erg/cm² so the reciprocity law is valid. It comes to a dead end against the slope of area C and is limited along its sides by the two ridges A and B. Penetrating into the part of the diagram where low light intensities are combined with long exposure times, the end of the valley curves away somewhat to light quantities too low to conform with the reciprocity law.

A second depression, although not a negative one, is interposed between ridge B and area C. Probably the high-intensity ridge B too obeys the Bunsen-Roscoe law as demonstrated in the diagram. This shows that the maxima of the ridge occur at equal light quantities of about 6×10^4 erg/cm² (provided the stimulation does not exceed 4 minutes and the light intensity is not lower than 100 erg/cm² sec).

3.2. The effect of a red pre-irradiation

Whereas 2 min of red light ($I = 400$ erg/cm² sec) followed by 90 min of darkness are sufficient to saturate the red light effect on the first positive curvature (ridge A), it is necessary to irradiate the plants during a full period of two hours with the same intensity to increase the positive curvatures of ridge B. *Table 1* indicates that without this pre-irradiation hardly any positive curvature can be demonstrated.

Table 1. Influence of a red pre-irradiation ($I = 400$ erg/cm² sec) on positive curvatures B. Phototropic stimulation by blue light of 10^4 erg/cm² sec (broad band filters). Curvatures in degrees.

Exp.	Pretreatment	Duration phototropic stimulus									
		1″	2″	4″	8″	16″	30″	1′	2′	4′	8′
1	none		−4.9	−4.3	0	0.	0.7	−0.5			
	2′ red, 2 hrs dark		−1.4	0.9	1.8	1.8	1.6	3.5			
	2 hrs red		2.5	2.2	6.4	4.6	2.9	1.6			
2	2′ red, 2 hrs dark	1.5	−2.3	0.3	1.3	1.1	1.3	0.9	−1.2	−1.0	−1.0
	2 hrs red	−4.9	−2.3	2	4.5	3.8	2.0	0.1	2.1	3.0	3.1

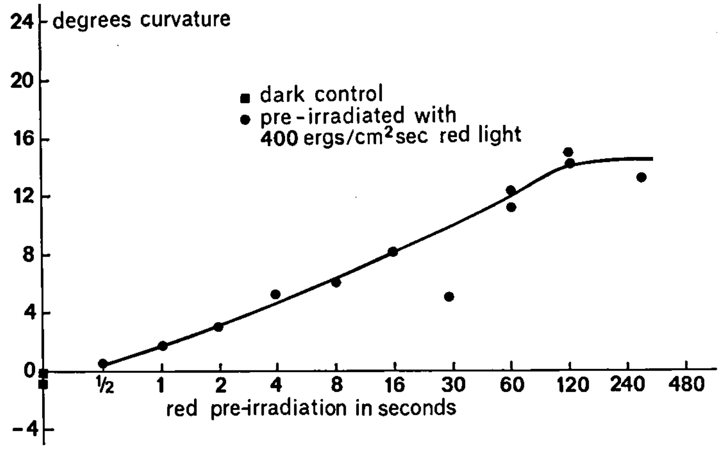

Fig. 4. Effect of increasing doses of red light on the time-dependent phototropic curvature (C). Intensity of red light 400 erg/cm^2 sec. Phototropic stimulation 1½ hours after the red irradiation with 20 minutes of blue light (λ = 458 nm, I = $10^{1.6}$ erg/cm^2 sec).

The effect of a red pre-irradiation on "positive" area C, however, reaches its saturation level with a stimulus duration of 2 minutes (I = 400 erg/cm^2 sec). This is shown in *fig. 4*. The same applies to the effect of red light on the extent of the first positive curvature (BLAAUW & BLAAUW-JANSSEN 1965) and of the geotropic curvature (BLAAUW 1961).

The effect of red light on area C is developed to its full extent when a period of 1½ or 2 hours is inserted between the red irradiation and the beginning of phototropic stimulation. With shorter intervals curvatures are increased to a lesser extent (*fig. 5*). The same applies to the red light effect on the first positive curvature (BLAAUW & BLAAUW-JANSEN 1965) and on the geotropic curvature (to the published, at variance with the results of BLAAUW 1961 and WILKINS 1965).

Without a red pre-irradiation the "positive" area C starts with a stimulus duration longer than 15 minutes (*fig. 6*), once again, within wide limits, independent of light intensity. This is in agreement with the results of ZIMMERMANN & BRIGGS 1963a). As a consequence the negative valley without a pre-irradiation is not only wider but also longer than indicated in the diagram.

4. DISCUSSION

In 1934 DU BUY & NUERNBERGK distinguished between three types of positive curvatures in the phototropic response of the *Avena* coleoptile. WENT & THIMANN (1937) presented these data in graphic form which can also be found in GALSTON (1959). A third positive curvature, however, failed to come into evidence in more recent dose-response curves for oat coleoptiles (CURRY 1957;

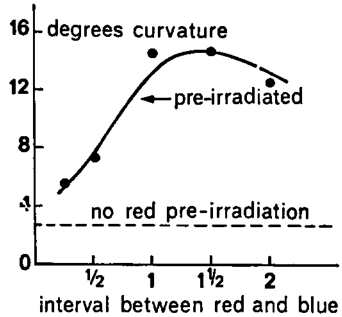

Fig. 5. Influence of the interval between red and blue irradiation. Red irradiation: during 2 minutes, I = 400 erg/cm² sec. Blue irradiation: during 20 minutes, I = $10^{1.6}$ erg/cm² sec.

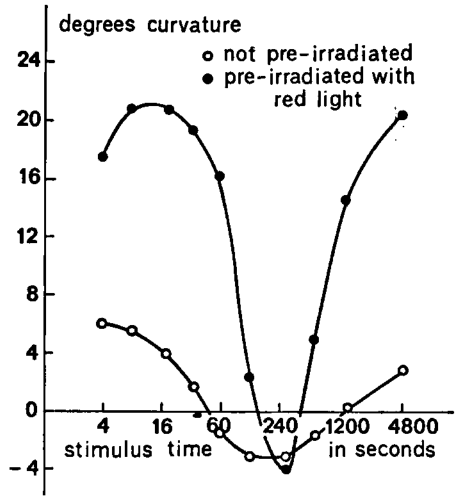

Fig. 6. Effect of red preirradiation on the width of the negative valley. Intensity of the blue light: $10^{1.6}$ erg/cm² sec. Intensity of the red light: 400 erg/cm² sec.

ZIMMERMAN & BRIGGS 1963a; EVERETT & THIMANN 1968; PICKARD 1969).

If we traverse the diagram of *fig. 3* at an intensity of 840 erg/cm² sec (as DU BUY & NUERNBERGK did) from short to long exposure times, the cross-section of *fig. 7* is obtained. We pass successively the ridge of large positive curvatures (A), the valley of negative curvatures, the ridge of small positive curvatures (B), a second depression, and the slope of the positive area (C) with exposure times longer than 4 minutes. The agreement of this figure with the graph of WENT &

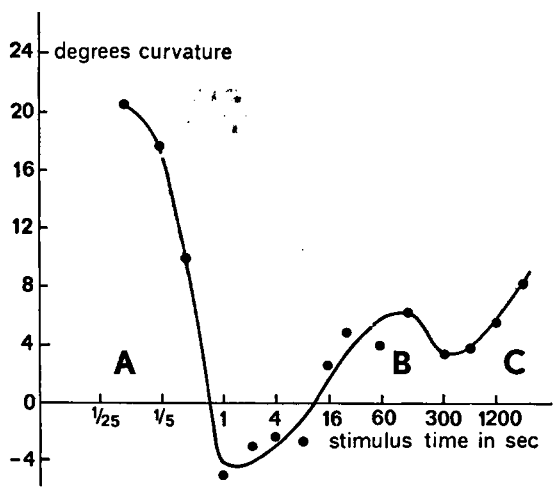

Fig. 7. Dose-response curve at an intensity of $10^{2.9}$ erg/cm² sec

Thimann is obvious. The maximum of ridge B is much lower than the maximum of ridge A, in accordance with the data of du Buy and Nuernbergk, but contrary to the graph based on their data by Went and Thimann.

We conclude that ridge B is identical with the second positive curvature of du Buy and Nuernbergk, the "positive" area C corresponding with their third positive curvature. So it is not the third positive curvature of du Buy and Nuernbergk that could not be reproduced but their second one. In recent work it did not show up because either the light intensity was too low (EVERETT & THIMANN 1968), or the duration of stimulus was too long (PICKARD 1969). Only in the graphs of CLARK (1913) and in a diagram of THIMANN & CURRY (1957), based on data of many workers, it can be seen as an indifferent zone, interposed between the negative curvature and the subsequent positive curvature. ARISZ (1919) did not discern a second positive curvature from a third one, but his tables do not disagree with the supposition that in his experiments at low light intensities the negative curvature was followed up by the time-dependent positive curvature C, at high intensities, however, by ridge B.

In recent papers (EVERETT & THIMANN 1968; PICKARD 1969) attention was drawn to the intensity dependence of the second positive curvature (C). The results of this paper are in accordance with their evidence.

In our diagram the curvatures in area C increase linearly with log duration of stimulus and are initiated by a stimulus duration of about 4 minutes. This is in agreement with Curry's estimation: about 240 seconds would be required for initiation of second positive curvature. If replotted on a logarithmic time-axis (*fig. 8*) the stimulus-duration curves for a series of light intensities given by Everett & Thimann coincide on the abscissa at a stimulus duration of about 220 seconds. A fairly precise fit of the data points, obtained if the data are replotted in this manner, justifies this approach.

The duration-of-exposure versus response curves of Pickard indicate a minimum stimulus duration in the range of 6 to 60 seconds. She found that the lower the slope of the curve, the longer was the minimum effective stimulation period.

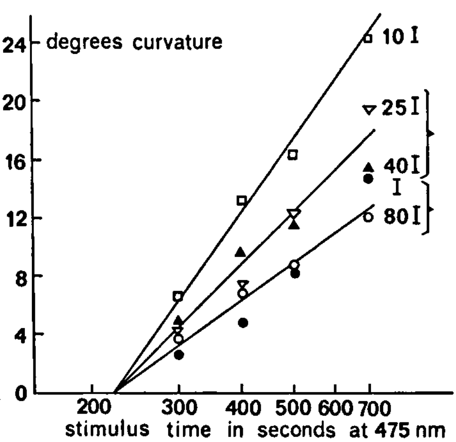

Fig. 8. Second positive responses of oat coleoptiles plotted against log stimulus duration. I = 0.6 erg/cm² sec. Data after Everett and Thimann.

If replotted on a logarithmic time axis, her curves appear to be made up of two parts: up to a stimulus duration of about 25 minutes the data points fall on a line with a low slope; with stimulus durations of more than 25 minutes the points can be fitted on a straight line of much steeper slope (*fig. 9*). The x-intercept indicating the minimum duration of exposure for this type of positive response is 210 seconds. The break in the curve at 25 minutes exposure time can perhaps be correlated with the phenomenon of recovery of the phototropic system after a pre-irradiation of the coleoptiles with unilateral or vertical blue light of large intensity. By such a pre-irradiation the capacity for curving phototropically is minimized, but after 20–25 minutes the sensitivity of the phototropic system appears to be re-activated (ARISZ 1915; MEYER 1969).

If it is assumed on the basis of the foregoing evidence that the second positive curvature (C) increases linearly with the logarithm of stimulus duration and is initiated with an exposure time of about 240 seconds, the phototropic response curves of ZIMMERMAN & BRIGGS (1963b) for system III (second positive curvature) have to be reconsidered. The data points for these curves were obtained by subtracting the curvatures predicted for the first positive curvature and the negative system from total experimental curvature. In view of the foregoing evidence it may be doubted whether their method of plotting these points as a linear function of exposure time with an x-intercept of zero is permittable. By that manipulation the interesting feature of a constant minimum stimulus duration of 4 minutes for red-pre irradiated plants and a minimum stimulus duration of 15 minutes for dark controls is quite obscured.

From *fig. 3* it can be seen that at low intensites (about 0.5 erg/cm^2 sec) and long stimulus durations (more than 3 minutes) the "positive" ridge A meets the "positive" area C. At higher light intensities area C is separated by a dip or a valley from the ridges A and B. So at intensities above 1 erg/cm^2 sec it is im-

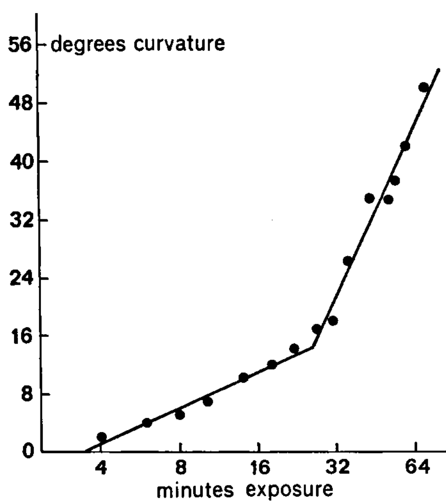

Fig. 9. Dependence of response on log duration of exposure to 4 × 10^4 erg/cm^2 sec "white" light. Data after Pickard.

possible to pass from the area of positive curvatures C to the ridges of positive curvature A or B without passing an area in which the curvature is decreased. This has been the starting-point of our experiments on the nature of the time-dependent positive curvature of the *Avena* coleoptile.

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