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THE INFLUENCE OF RED LIGHT ON THE PHOTOTROPISM OF AVENA COLEOPTILES

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Abstract

The shift of phototropic dosage-response curves by red light appears to be dependent on the time interval between red light treatment and phototropic induction. Red light enhances the maximum of the first positive curvature when administered before phototropic induction. Its effect, when given after induction, is only evident with plants exposed in addition to red light the day before the experiment.

Introduction

Conflicting reports are published on the effect of red light on the phototropic curvatures of oat coleoptiles.

Curry (1957) observed that red light shifts the curve for dosage versus response of the first positive curvature to the right.[1])

Blaauw-Jansen (1959) reported an enhancement of the maximum of the first positive curvature by red light pretreatment. Her fig. 2 does not exclude a slight shift of this maximum in the direction of larger dosages.

Asomaning and Galston (1961) found an increase or a decrease of the first positive curvature depending on the red light regime applied. Briggs (1963a, b) deduces from their fig. 3 a shift of the dosage-response curve for first positive curvature along the log dosage axis after a 15 minutes red light treatment.

Briggs (1963b) and Zimmerman and Briggs (1963), however, report only a considerable shift of the log dosage response curve to the right, not accompanied by any enhancement of the maximum of the curvature.

Zimmerman and Briggs (1963) summarize this disagreement in results as follows: "Some authors reported an increase in phototropic sensitivity while others reported a decrease following exposure to red light."

Briggs (1963b) supposes that the duration of the red light exposure as applied by Blaauw-Jansen was too short (15 min) to yield a significant shift of the curve (Briggs needs red light exposures of at least an hour for maximum effect). He doubts the significance of the increase found by Asomaning and Galston for lack of statistical work.

The study by Blaauw (1961) on the influence of red light on the

[1]) By courtesy of Prof. Thimann, we were able to consult G. M. Curry's thesis in which most of the phenomena worked out quantitatively in the present paper proved to be mentioned in outline.

geotropic curvature of *Avena* coleoptiles showed that the enhancement of the curvature by red light is only observed after a "waiting time" of about 30 minutes. With "waiting time" was meant: the interval of time between the beginning of the illumination and the moment at which the plants were placed in a horizontal position. Now, BRIGGS' table 10 (1963a) shows that four short red light treatments ranging from 1 second to 2 minutes and administered at half-hour intervals, induce the same shift in phototropic sensitivity as 100 minutes continuous red light before induction. This result suggests, but does not prove, that also in the case of red influenced phototropic curvatures the "waiting time" (in the meaning of: the time interval between the beginning of the red light treatment and the beginning of the phototropic induction) plays a most important part.

The present authors thought it possible that a short red light treatment followed by a dark "waiting time" of 100 minutes should affect phototropic curvature to the same degree as a continuous red light treatment of 100 minutes. The first flash of the continuous illumination might already be fully effective, the rest of the exposure time functioning as "waiting time". If this hypothesis should fit in, the discrepancy between the results of Briggs and Blaauw-Jansen could be reduced to the interposing of different "waiting times" (resp. from 100 to 120 min, and 15 min) at least in so far as the shift is concerned.

On account of these considerations experiments on the relations between the influence of the "waiting time" and red light-affected phototropic curvatures were carried out.

MATERIALS AND METHODS

After the husks of seeds of *Avena sativa* (cultivar "Victory") had been removed, the seeds were moistened by shaking them in tap water, and put to germinate on glass strips measuring 19 × 3 cm, wrapped in two layers of filter paper [1]). The seeds were put 0.8 cm apart, their lower ends 1.3 cm above the level of the liquid. Two strips with seeds were inclined at an angle of 30 degrees from the vertical in a zinc tray with dimensions of 20 × 20 × 3 cm into which 30 ml of tap water was poured. The trays were covered with glass plates to maintain maximal humidity and kept in a dark room at 23° C. During a period of 21 hours the seeds were exposed to red light produced by a 40 W Philips red fluorescent tube filtered by red selenium glass. Fig. 1 shows the emission spectrum of the tube, fig. 2 the transmission spectrum of the red glass. The intensity at the level of the germinating seeds was ca 40 μW cm^{-2}.

After this, the trays were put in absolute darkness at a temperature of 23° C and a relative humidity of about 85 % for 72 hours during which period (after ca. 24 hours) the cover plates were removed in

[1]) This method for growing coleoptiles was, adapted to our purpose and means, designed in analogy with that of WIEGAND and SCHRANK (1959).

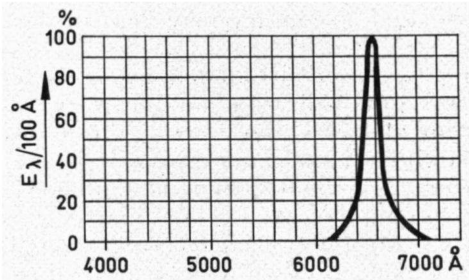

Fig. 1. Emission spectrum of the red fluorescent tube (supplied by Philips, Eindhoven).

darkness. Seedlings obtained in this way have first internodes 12–20 mm and coleoptiles 20–30 mm long.

Working with very dim green light (intensity at the level of the plants 0.01 μW cm^{-2}) and for the greater part by touch, coleoptiles were excised and after removal of their primary leaves put upon pins with their narrow sides facing each other. The green light (λ 560 nm) was obtained from an incandescent lamp with an appropriate system of lenses and filtered through a Schott double band interference filter plus a Balzer interference filter (the transmission spectrum of this combination is shown in fig. 2).

The pins are placed in small depressions cut in perspex holders (Blaauw 1963), each holder containing 12 pins. The depressions are filled with water as a supply for the coleoptiles. After these ma-

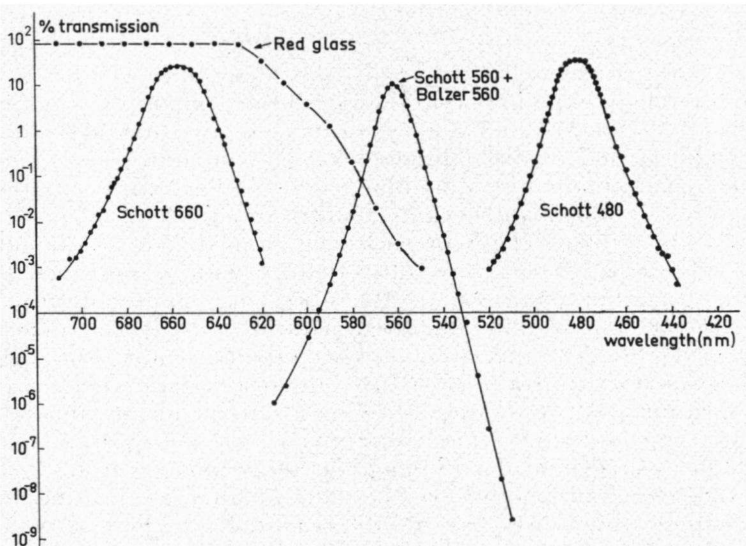

Fig. 2. Transmission spectra of the red selenium glass, the interference filters and the combination of interference filters used.

nipulations the isolated coleoptiles were kept in darkness for several hours.

The coleoptiles then were irradiated from above with red light. The red light was obtained from an incandescent lamp with an appropriate system of lenses and filtered through a Schott double band interference filter (λ 660 nm; transmission spectrum in fig. 2). In this way an area of about 20 cm in diameter was illuminated with an intensity of 100 μW $cm^{-2} = 5.6 \times 10^{-10}$ einstein cm^{-2} sec^{-1}. 20 holders with 12 coleoptiles each received the red light treatment in abtches of two holders. After the "waiting time" the unilateral illumination with blue light was administered to the narrow side of the coleoptiles. For each dosage of blue light a batch of two holders was used. The source of blue light was the same as that of the red light but the light was filtered through a Schott double band interference filter with the transmission centered at 482 nm (fig. 2). The intensity at the level of the plants was 35 μW cm^{-2} = 14.35 einstein cm^{-2} sec^{-}/. The light dosages were increased by increasing the exposure time. From each holder two shadowgraphs were made, the first immediately after phototropic induction, the second 100 minutes after the phototropic induction. To this end light of 560 nm was used. The sensitivity of the film being 1250 ASA (32 DIN), extremely little green light was sufficient to produce a satisfactory blackening (ca 0.01 μW sec cm^{-2}).

Curvatures were measured by means of a goniometer. Standard errors seldom exceeded $\pm$ 1.5° and averaged about $\pm$ 0.8°.

Experimental

1. *Preliminary experiments*

First an experiment was carried out to determine whether a continuous red light exposure of 120 minutes before phototropic induction can be replaced by a red light treatment of two minutes followed by a dark period of 118 minutes. In fig. 3 phototropic response is plotted against quantity of blue light. The curve represents curvatures obtained with plants that received 120 min (72×10^4 μW sec cm^{-2}) of red light pretreatment immediately before phototropic induction, as well as results obtained with plants given a red light treatment of 2 min (12×10^3 μW sed cm^{-2}) and phototropically induced 118 minutes after this treatment. It is clear that these two red light treatments affect phototropic response to the same degree.

The next experiment was carried out to ascertain whether a red light treatment of two minutes is the most suitable for maximal effect. Rows of coleoptiles, therefore, were given a progressively increasing irradiance of red light over a range of energy dosages from 1 to 10^5 μW sec cm^{-2} and phototropically induced after a "waiting time" of 90 minutes by 35 μW sec cm^{-2} blue light. From the results shown in fig. 4 it is clear that an irradiation period of two minutes is suitable to obtain maximal effect.

However, it must be pointed out that, indeed, the curvature in

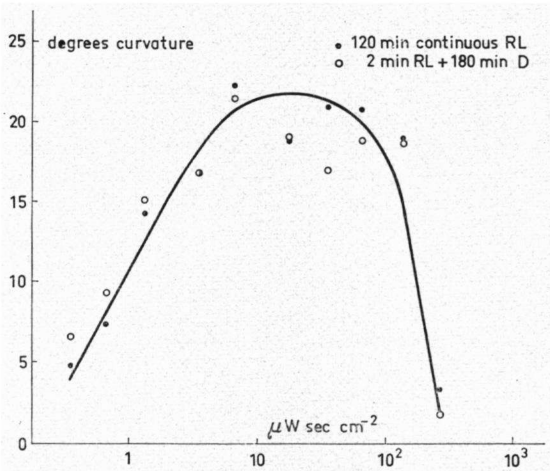

Fig. 3. Dosage-response curve for oat coleoptiles pretreated with a continuous red light exposure of 2 hours, or with a 2 min red light exposure followed by a dark period of 118 min.

degrees from 32 seconds red light treatment upwards remains the same, but that the shape of the curve of the coleoptiles is dosage dependent in this range of light dosages. As in the case of the red light influenced geotropic response (BLAAUW, 1963) the curvature is more confined to the topmost centimeter of the coleoptile as larger dosages of red light are administered.[1])

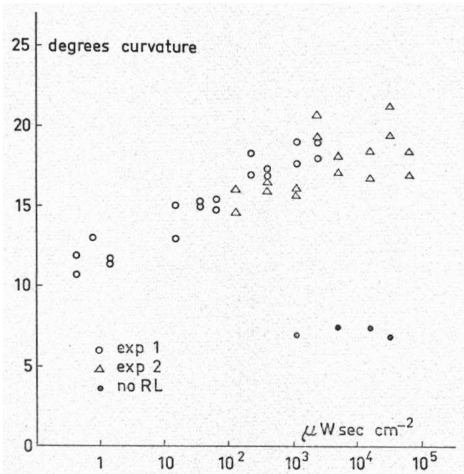

Fig. 4. Effect of progressively increasing irradiances of red light on phototropic curvature. Curvatures induced by 35 μW sec cm^{-2} blue light.

[1]) Curry's supposition that the enhancement of the curvature after red light treatment can be accounted for by the phenomenon of the differences in growth of front and back sides becoming more restricted to the tip is untenable for planimetrical reasons.

2. *Influence of the "waiting time"*

The next series of experiments was conducted to investigate the effect of the duration of the "waiting time".

Fig. 5 (a–f) illustrates the influence of "waiting times" varying from 0 to 120 minutes on subsequent first positive and negative curvatures. For each "waiting time" a pair of dosage response curves was obtained, the upper curve representing the curvatures of plants with a red light pretreatment of two minutes, the lower one representing the curvatures of the dark controls. With a "waiting time" of 0 minutes, red

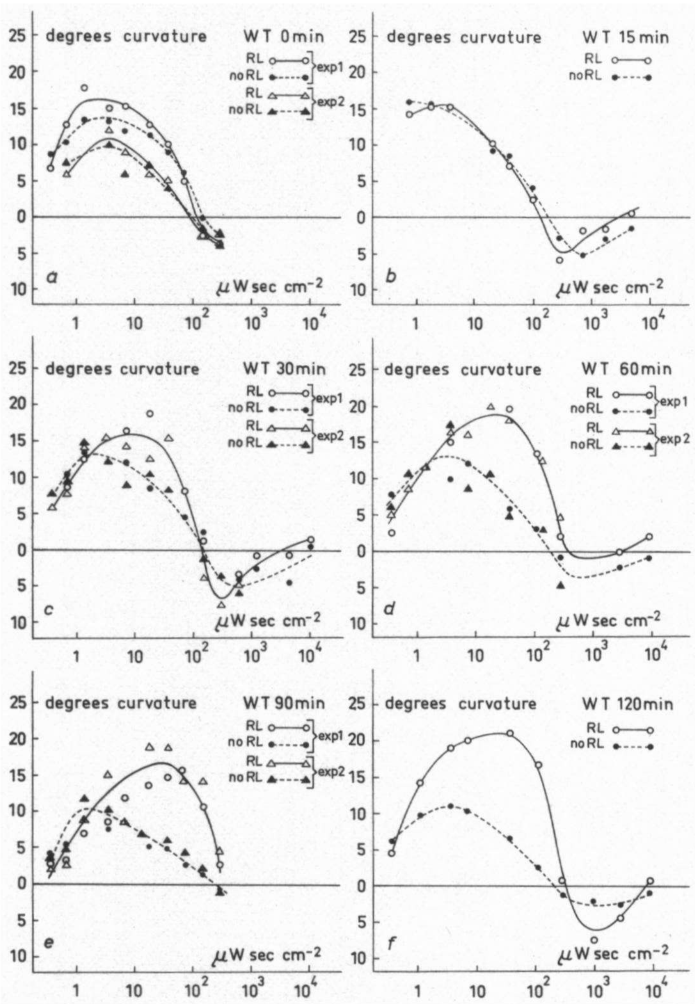

Fig. 5. Phototropic dosage-response curves as influenced by a fixed dosage of red light and a number of "waiting times".

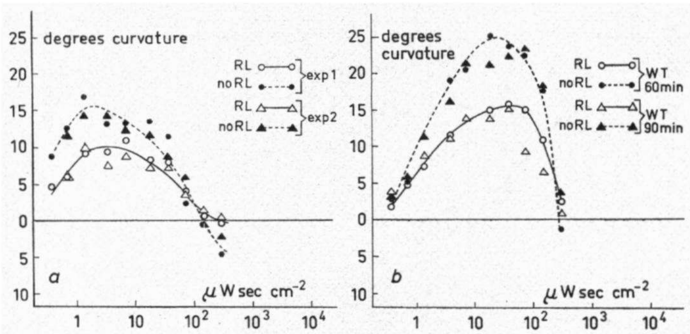

Fig. 6. Influence of an exposure to red light 16 hours before phototropic induction on dosage-response curves. a. Lower curve: plants given red light 16 hours before induction. Upper curve: dark controls. b. The same, but, in addition, all plants exposed to red light 60 resp. 90 min before phototropic induction.

light only enhances the maximum of the first positive curvature. As the "waiting time" is raised to 120 minutes this enhancement of the maximum is more pronounced, and moreover, the maximum is shifted towards higher dosages of blue light.

In our experiments red light does not influence that quantity of blue light at which the first positive curvature changes into a negative one. The possibility of red light increasing the negative curvatures is not excluded by our results.

3. *Influence of red light given 16 hours before induction*

Asomaning and Galston found a decrease of phototropic curvature responses when the plants had been given a red light pretreatment of 15 minutes, administered in three 5-minute doses at 6, 30 and 54 hours after moistening (i.e. resp. ca 64, 40 and 16 hours before phototropic induction). Their results with plants irradiated only at 6 or at 6 and 30 hours after moistening do not exclude the possibility that this decrease of phototropic response is caused by the red light exposure at 16 hours before phototropic induction only.

Therefore, a series of experiments as described under 2) was carried out with plants irradiated for 2 minutes with red light from the red fluorescent tube, 16 hours before phototropic induction.

Within the range of blue intensities used, the plants that were irradiated 16 hours before, did, indeed, produce smaller curvatures than the dark controls (fig. 6) .This red light exposure affects only the degree of curvature. The maximum of the first positive curvature is not shifted by this pretreatment, as is shown in fig. 6a and b.

For the rest, these plants react to a red irradiation administered within two hours before phototropic induction in the same way as the plants under 2) do (fig. 7).

4. *Red light after phototropic induction*

Once more, the influence of red light administered after photo-

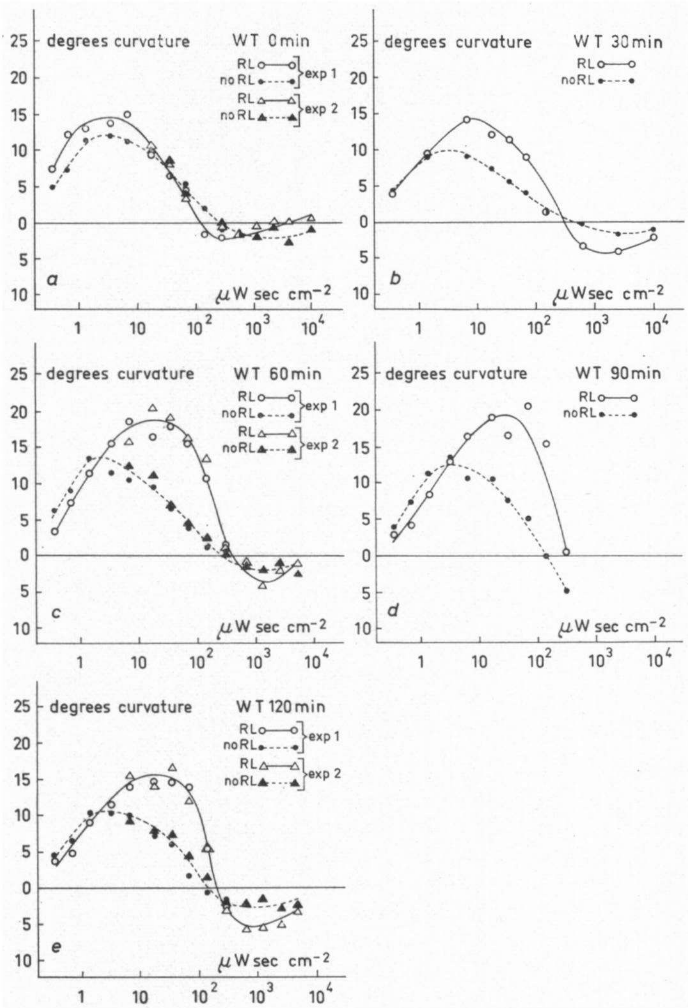

Fig. 7. Phototropic dosage response curves as influenced by a fixed dosage of red light and a number of "waiting times". Coleoptiles irradiated in addition with red light 16 hours before phototropic induction.

tropic induction was investigated. The experiments illustrated in fig. 8A were done with plants kept in darkness till phototropic induction; the plants of fig. 8B were given 2 minutes red light 16 hours before they were exposed to unilateral blue light. The upper curve of both figures represents curvatures of plants that were given two minutes red light immediately after phototropic induction, the lower curves represent plants kept in darkness after the blue irradiation. It is evident that only the red pretreated coleoptiles are sensitive to red light given after phototropic induction. This was confirmed

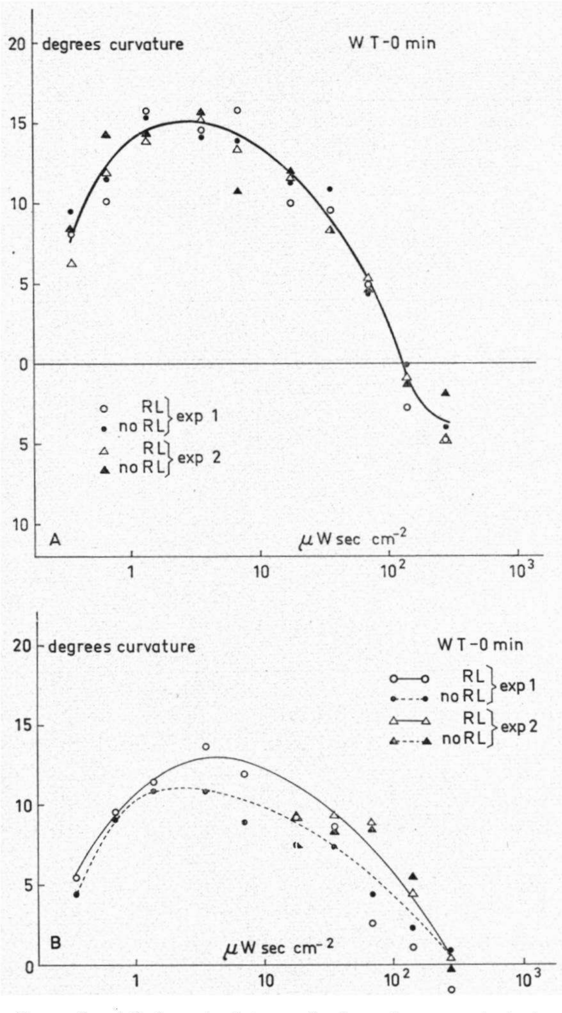

Fig. 8. The effect of red light administered after phototropic induction ("waiting time" = —0) on the dosage response curve.
A. Coleoptiles not pretreated with red light.
B. Coleoptiles pretreated with red light 16 hours before phototropic induction.

by the data of Table 1. Each set of coleoptiles was given 35 μW sec cm^{-2} blue light with or without 2 minutes red light after the induction, but, in addition, the coleoptiles of exp. 1 received 2 minutes red light 16 hours before unilateral blue light exposure.

Discussion

The experiments described above clearly demonstrate the relation between the length of the "waiting time" and the occurrence of the

TABLE 1.

(Numbers of plants in brackets.)

A 2 min red light 16 hours before induction

	no RL after induction	2 min RL after induction
	8.9 ± 0.82 (11)	13.1 ± 1.14 (12)
	10.4 ± 0.96 (11)	13.8 ± 0.97 (11)
	8.8 ± 0.71 (12)	14.9 ± 0.81 (12)
	8.5 ± 0.39 (12)	12.6 ± 0.98 (10)
	11.2 ± 0.68 (11)	15.5 ± 0.79 (12)
Average	9.5 ± 0.33 (57)	14.0 ± 0.41 (57)

B. No red light before induction

	no RL after induction	2 min RL after induction
	14.0 ± 1.31 (12)	15.8 ± 0.88 (8)
	13.9 ± 1.55 (11)	13.4 ± 1.40 (10)
	13.0 ± 1.12 (11)	14.2 ± 1.47 (10)
	12.7 ± 1.42 (10)	15.9 ± 0.96 (10)
	11.8 ± 1.16 (10)	16.7 ± 1.05 (9)
	14.0 ± 1.12 (10)	17.0 ± 1.42 (10)
	16.8 ± 0.66 (12)	13.2 ± 1.22 (11)
	15.1 ± 0.77 (11)	14.1 ± 1.84 (12)
	12.2 ± 1.20 (12)	11.1 ± 1.80 (10)
	13.3 ± 1.30 (10)	14.7 ± 1.08 (11)
Average	13.7 ± 0.36 (109)	14.6 ± 0.41 (101)

red light induced shift in the dosage response curve. Thus, the conflicting statements of BLAAUW-JANSEN (1959) and Briggs (1963 a, b) on this point are reconciled, the former interposing a "waiting time" of 15 minutes, the latter "waiting times" of from 100 to 120 minutes between the beginning of the red light treatment and the phototropic induction.

The decrease in phototropic sensitivity reported by ASOMANING and GALSTON (1961) appears to be due to the third of their three red light treatments, about 16 hours before phototropic induction. This decrease not being accompanied by a shift in the dosage response curve is inconsistent with the views of BRIGGS (1963b) who states: "When Asomaning and Galston administered all three of their red light treatments they obtained a clear decrease in phototropic sensitivity represented by a shift of the dosage-response curve to the right" and (ZIMMERMAN and BRIGGS, 1963): "It is clear from their fig. 3 that red light shifts the log dosage-response curve for first positive curvature towards higher dosages". This fig. 3 is reproduced in the present paper (our fig. 9). As neither the curve representing the dark controls nor the curve for the plants with the red light treatment of 15 minutes reaches its maximum, no conclusion can be drawn from this graph as to the occurrence of a shift of the red light curve.

The present paper confirms the results of BLAAUW-JANSEN (1959) on the enhancement of the maximum of the first positive curvature

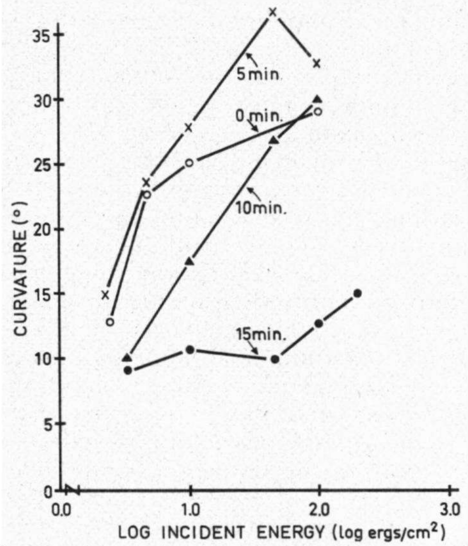

Fig. 9. (From ASOMANING and GALSTON, 1961).

by red light. BRIGGS' failure to find this enhancement is possibly due to the impurities of his green light ("if extreme care was not taken even with this latter illumination, enough irregularity and curvature occured to produce large variability from plant to plant in a given phototropic experiment", ZIMMERMAN and BRIGGS, 1963) or to the effect of pure green light itself. In our experiments green light was only used during the isolation of the coleoptiles, whereas BLAAUW-JANSEN (1959) worked in complete darkness. From BRIGGS' papers no certainty can be obtained whether the plants were exposed to green light before and during manipulations, and if so, to what extent.

Experiments designed to investigate the influence of extremely pure green light on subsequent phototropic curvatures have been started by the present authors.

As to the third point of difference: the influence of red light administered after phototropic induction, it appears that its effect is dependent on the pretreatment of the plants the day before the experiment is done. It is likely that the plants used by BLAAUW-JANSEN (1959) for these experiments caught small quantities of red light the day before they were phototropically excited. In accordance with this hypothesis is that in her memory the plants were quite straight, whereas plants grown in complete darkness use to show fairly pronounced autotropic curvatures.

A new discrepancy stands out from the graphs of fig. 5c, e, f and fig. 7a, b, c, e: in our experiments red light does not shift negative curvatures to larger light quantities. ZIMMERMAN and BRIGGS (1963), however, report an approximately fourfold decrease of the sensitivity for negative curvature, whereas the sensitivity shift for first positive curvature is tenfold. Possibly this discrepancy, too, can be accounted for by scrutinizing the composition of the green light he used or the manipulation of it.

In BRIGGS' papers (1963a) on the influence of short lasting red light exposures at 30-minute intervals on phototropic curvature two statements have been made:

1. "The experiments involving very brief exposures of red light suggest that phytochrome is probably converted to the far-red-absorbing form within a few seconds of the inception of illumination.[1]) Under conditions of continuous red illlumination the phytochrome must all be converted to the far-red-absorbing form within a few seconds. Yet phototropic sensitivity continues to change for a full hour. Apparently, not only must the pigment be converted, but it must remain in the far-red-absorbing form for a significant period of time for sensitivity to undergo maximum change. (BRIGGS, 1963a).

2. ". . . . both CURRY and BRIGGS reported that brief red light treatment followed by a dark period was as effective as continuous light treatment in shifting the dosage-response curve . . . ." (BRIGGS 1963b).

BRIGGS' experiments described in his table 10 (1963a) do not justify this second statement although it proved to be correct. However, it contradicts the first one. As the experiments described in this paper clearly show that one flash of red light at the beginning of a 2-hour dark period is quite sufficient to obtain the effect of a continuous red exposure of 2 hours, the first statement should be regarded as incorrect.

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[1]) In close agreement with our findings on red light influences in geotropism are Curry's experiments in which is demonstrated that all the concerning red light effects can be initiated with far red light as well.