

# INFLUENCE OF LIGHT ON GROWTH, GEOTROPISM AND GUTTATION OF AVENA SEEDLINGS GROWN IN TOTAL DARKNESS

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## ABSTRACT

Investigations were conducted with etiolated seedlings of *Avena* on the influence of red, far red and blue light on:

- a. the growth of sections of coleoptile and mesocotyl
- b. the geotropic reactions of coleoptile and mesocotyl
- c. guttation of the seedlings.

The experiments were carried out in darkness, the experimental irradiations excepted.

Two systems of reactions to light appear to be present in the seedlings. One of them shows a very low saturation value of about  $1 \text{ erg cm}^{-2}$  for red and far red light, the sensitivity to blue light being much lower than for the former wavelengths. This system was found in section growth and the geotropic reaction. The other system shows a dependency on the amount of light energy applied up to at least  $10^5 \text{ erg cm}^{-2}$  for red, far red and blue light. In this system the sensitivity to far red light is much lower than to red light and about as high as to blue light. This system was found to affect guttation and the shape of the geotropic curves of the seedlings.

The latter system appears to exert its influence through changes in transport rates of water and solutes, as is indicated by its influence on guttation.

From the results is concluded that this change in the rate of water transport influences the transport rates of auxins and, consequently, the relative growth rates of different parts of the seedlings.

Connections of the conclusions drawn with some data from the literature on phototropism are discussed.

## CHAPTER I

### INTRODUCTION

Since the discovery of growth substances by WENT (1928) and his postulate that no growth occurs without growth substance, the physiology of the seedling of *Avena sativa* has been studied intensively.

Especially the influence of growth substances and that of light on the growth of the coleoptile have been thoroughly investigated. Two aspects of the physiology of the *Avena* seedling, however, have received relatively little attention as yet, namely the influence of light on seedlings cultivated in total darkness, and the correlation between the growth of the coleoptile and that of the mesocotyl.

Since it had been shown that, by growing *Avena* seedlings in red light during part of the cultivation period, these develop longer and straighter coleoptiles whereas mesocotyl growth is inhibited at the same time, most investigations have been executed with seedlings cultivated in this way. When it was necessary to manipulate these seedlings, this was done in red or orange light because it was known that this light has no phototropic activity.

For many years there has even been a tendency to consider red light as "safelight". In recent years, however, red light has been shown to have a strong effect on many physiological processes in plants. BLAAUW-JANSEN (1959) showed, for the *Avena* coleoptile, that red light has a strong influence on the phototropic behaviour and on the reactivity to auxin in the standard *Avena* curvature test and in the coleoptile section test.

She found that red light can even cause a reversed concentration dependence of growth on auxin in the concentration range of  $10^{-9}$  to  $10^{-8}$ . She further showed that a "red light factor" is formed after irradiation with red light and that this factor is responsible for this phenomenon.

Among those who investigated the influence of light on seedlings that had received no other light than the experimental irradiation, WEINTRAUB and McALISTER (1942), WEINTRAUB and PRICE (1947), GOODWIN and OWENS (1948), and SCHNEIDER (1941) are mentioned here.

Schneider worked out a method to investigate the growth of sections of dark-grown *Avena* seedlings in total darkness. He found an inhibiting influence of red and of green light on the growth of mesocotyl sections. The amounts of energy of red light applied by him were rather large. He used a light intensity of about  $8 \text{ erg cm}^{-2} \text{ sec}^{-1}$  and left the sections continuously in this light. The energy radiated on the sections during the growth period of 30 hours and more was at least  $8.10^7 \text{ erg cm}^{-2}$ . The energy of green light used was not mentioned.

Weintraub and McAlister, and Weintraub and Price placed germinating seeds in total darkness or in light from the moment they were moistened till the seedlings had reached their final length. They applied amounts of energy up to  $4.5 \times 10^4 \text{ erg cm}^{-2}$ , using light of different wave lengths. From the results they calculated an action spectrum for mesocotyl inhibition with a peak at about  $\lambda = 660 \text{ nm}$  and values of  $\pm 10\%$  of the main peak value at about  $\lambda = 580 \text{ nm}$  and  $\lambda = 710 \text{ nm}$ .

GOODWIN and OWENS (1948) obtained the same results with short irradiation times. From these investigations the conclusion

can be drawn that no light, irrespective of its wave length, can be regarded as "safe".

The correlation between the growth of the coleoptile and that of the mesocotyl of *Avena* seedlings has been investigated mainly by MER (1951, 1953, 1959, 1962).

As already mentioned, irradiation with red light decreases the growth rate of the mesocotyl. At the same time, the coleoptile shows an enhanced growth rate. MER (1959) found that, when plants are placed in air with a carbon dioxide content of 5 %, the growth rate of the mesocotyls is enhanced and that of the coleoptiles decreased. The reverse could be observed when seedlings were grown in air of a low relative humidity and at a high rate of air flow. He tried to explain the influence of carbon dioxide on growth through an action of this gas on the uptake of nutrients by the seedlings from the endosperm, but could not find conclusive experimental confirmation of this hypothesis.

Investigations on the influence of light of different wave lengths on growth and tropism of the *Avena* seedling mainly done with seedlings, of which the growth of the mesocotyl had been strongly inhibited by red light, have as yet not yielded a theory capable of correlating the phenomena found. On the contrary, most investigations seem to yield more new problems than solutions.

The state of things described above induced the present author to study the effect of light on *Avena* seedlings, investigating especially the reactions of both the coleoptiles and the mesocotyls without any use of light, but for the experimental irradiations, and comparing these reactions with those of seedlings grown in darkness throughout.

The phenomena studied were:

a. The straight growth of sections, because it seemed worthwhile to investigate reactions of the coleoptile and of the mesocotyl separately without the possibility of their influencing each other.

b. The geotropic curvature. Blaauw showed that the geotropic curvature of the coleoptile is modified by light, in magnitude (BLAAUW 1961), as well as in shape of the curve (BLAAUW 1963).

c. Guttation. ENGEL and FRIEDRICHSEN (1951) have shown that guttation of *Avena* seedlings is influenced by irradiation with light.

In this paper will be shown that it is probable that, apart from a direct effect of light on coleoptile and mesocotyl, also an influence of light on transport systems in the seedlings determines the relative growth rates of the coleoptile and the mesocotyl.

## CHAPTER II

### MATERIAL AND METHODS

Seedlings of *Avena sativa* cultivar *Victory oats* were used for the experiments. Seeds without glumes were wetted by shaking them in tap water. They were planted in moist vermiculite, and placed in a dark room in which a constant temperature of  $\pm 22^{\circ}$  C and a relative

humidity of about 95 % were maintained. After about  $3\frac{1}{2}$ –4 days the plants had attained a length of about 3– $3\frac{1}{2}$  cm and were then used in the experiments.

The light used for irradiations was obtained from an incandescent lamp (12 V, 100 W) and passed through an optical arrangement in which interference filters could be placed. The maximal deviation of the beam from the optical axis was  $7^\circ$ . The light was deflected vertically downwards on the plants. The interference filters used were made by BALZER (Liechtenstein) and SCHOTT und GEN. (Jena).

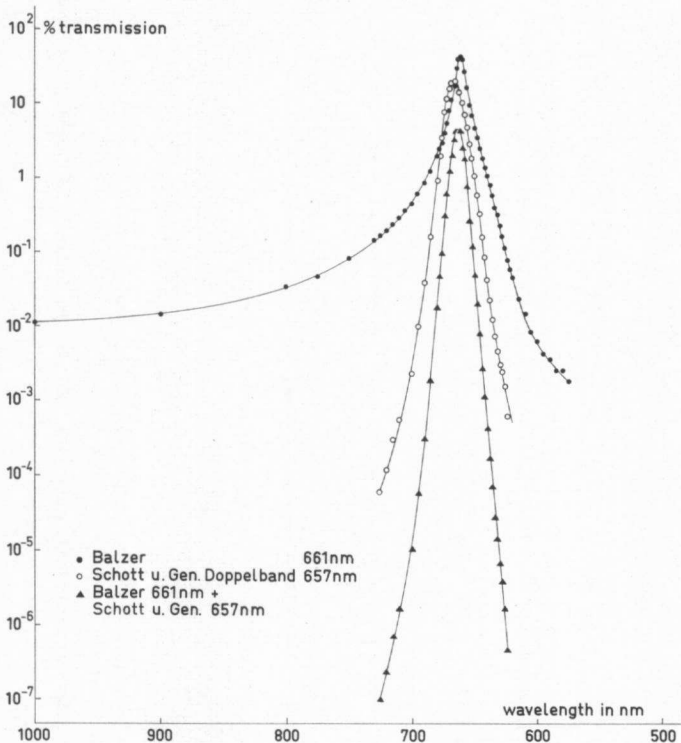


Fig. 1. Transmission curves of a Balzer filter, a Schott u. Gen. filter and the combination of these filters.

The wavelengths used were approximately  $\lambda = 730$  nm,  $\lambda = 660$  nm,  $\lambda = 560$  nm and  $\lambda = 480$  nm. An example of the transmission of the filters used is given in Fig. 1.

The purity of the light used can be judged from this graph. For instance, in light of  $\lambda = 660$  nm having passed through two Balzer filters, light of  $\lambda = 720$  nm does not exceed  $4 \times 10^{-6}$  of the incident light. In light of  $\lambda = 660$  nm obtained by using one Schottfilter together with one Balzerfilter light of  $\lambda = 720$  nm does not exceed  $5 \times 10^{-9}$  of the incident light.



For convenience sake we mention here the relations between different units used in papers on the influence of light on plants.

$$\begin{aligned} 1 \text{ erg cm}^{-2} &= 0.1 \text{ watt sec cm}^{-2} \\ 1 \text{ erg} &= 0.85 \times 10^{-15} \times \lambda \text{ einstein} \end{aligned} \quad (\lambda \text{ in nm.})$$

The light energies given to the plants and mentioned in the tables and graphs were all administered in irradiations of one hour or less.

In a number of cases, more than one experiment was carried out with the same amounts of energy but with different filtercombinations. To obtain the same amounts of energy different irradiation times had to be used. The present author has never been able to detect any deviation from the reciprocity law.

*To avoid repetition and misunderstanding it is stated here that from the moment the seeds were placed in darkness all manipulations of the plants were executed in total darkness.*

The only irradiations the plants received were the experimental irradiations and the irradiations that were used to make shadowgraphs.

In most experiments only one shadowgraph was made at the end of the experiment. In some experiments two or more shadowgraphs were made in order to obtain records of the course of a curving process. In these cases this will be mentioned where the experiments are described.

In the presentation of the results of the experiments (Chapters III, IV, V, VI) are given, together with the means, the standard errors of the means:

$$s = \sqrt{\frac{\sum (x - \bar{x})^2}{n(n-1)}}$$

$n$  = number of values measured  
 $x$  = value measured  
 $\bar{x}$  =  $\Sigma x/n$  = mean

In some tables values of  $P$  are given.  $P$  stands for the probability that the difference of the means of two groups is incidental.

#### GROWTH OF SECTIONS

Plants were selected by touch and placed in a section cutting tool as described by VAN DER WEIJ (1932).

Sections 3 mm in length were cut from the coleoptiles and from the mesocotyls of the plants. The tip, 2 mm in length, was discarded. Between the coleoptile sections and the mesocotyl sections a section of 5 mm was discarded. In this piece the node was located (Fig. 2).

Owing to the fact that the length of the coleoptiles varied, the mesocotyl sections were not cut at equal distances from the node, the mean distance from the node being about 2 mm.

To be able to place the plants in the Van der Weij tool in total darkness, a block of perspex with twelve grooves in which the seedlings

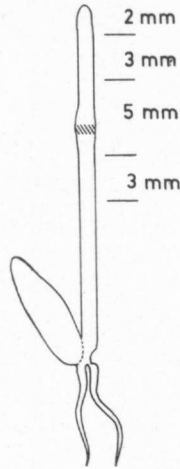


Fig. 2.

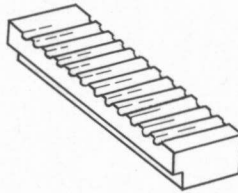


Fig. 3.

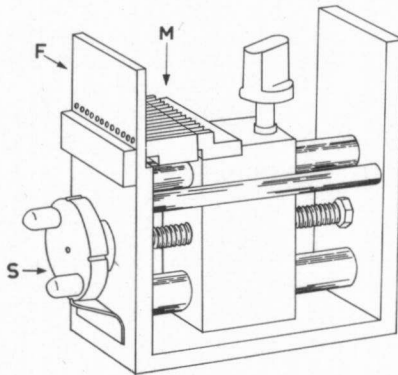


Fig. 4.

fitted (Fig. 3), was fixed in the tool (Fig. 4), by turning the screw S, bridging the gap between the frontplate F (Fig. 4) and the moving block M. After adjusting twelve plants in the tool and fixing them by block B (Fig. 5), the screw S was turned 180° counterclock-wise

to loosen the perspex block which then was taken away. The screw S was then turned back to its former position and the cutting procedure could start.

To that end the cutting tool was turned so that the plants were in a vertical position, the screw S was rotated the appropriate number of turns and a razor blade R (Fig. 5) was slid forward over the front-plate F (Fig. 4 and 5) to cut the sections (Fig. 5). The razor blade was then removed with the sections adhering to it (Fig. 6).

The cut sections were then either floated on a buffer solution or they were stuck to a microscope slide on which narrow strips of lanolin had been applied in advance. These slides were placed in a buffer solution. The buffer solutions used were made up of  $K_2HPO_4$  and  $KH_2PO_4$  in tap water to a final concentration of 0.01 M and a pH of 6.0.

Henceforward the procedures described are called either "method with floating sections", or in the latter case "halved slides method".

These methods will now be described in detail.

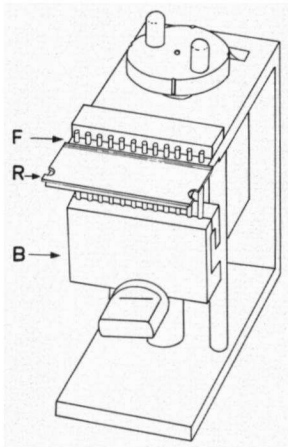


Fig. 5.

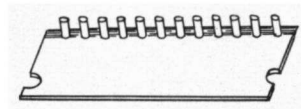


Fig. 6.

#### METHOD WITH FLOATING SECTIONS

The sections were put into the solution in which they were to grow, on the surface of which they floated by surface tension. After the period of time, in which they were to grow, the sections were taken into the light, dried superficially with filterpaper and placed on a photographic film to be shadowgraphed.

#### HALVED SLIDES METHOD

Slides for microscopy were cut into two parts on which two narrow strips of lanoline were applied. The halved slides were then placed in a holder (Fig. 7, S). On the basal plate of this holder, two plates,

A and B, could be placed each having a shallow groove, in which the razorblade (Fig. 8) could be placed and slid forward until the sections, standing up on it, touched the lanolin strips. The sections were then pressed into the lanolin by sliding a block C over the razorblade. Lateral movements of blocks A, B and C were prevented by ridges and grooves. Blocks C and B and the razorblade were then removed. The procedure of cutting and transferring sections was then repeated to fix them on the lower lanolin strip. (Fig. 8).

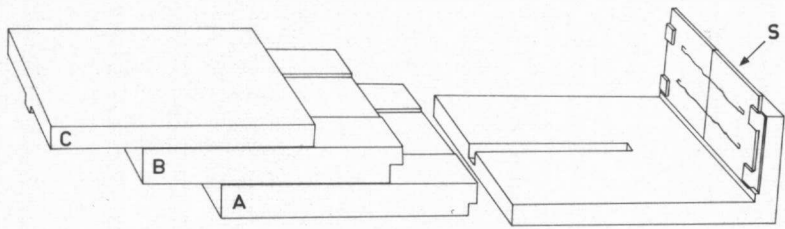


Fig. 7.

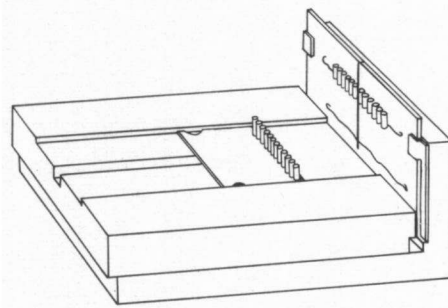


Fig. 8.

In the experiments sections were cut from the coleoptiles and from the mesocotyls of the same plants. The coleoptile sections were stuck to the upper lanolin strip, the mesocotyl sections were stuck on the lower strip on the same slide.

The experimental irradiation was administered to one half of a slide. The other half was kept in total darkness. Both halves of the slides were then placed in the buffer solution in which the sections were allowed to grow. The influence due to contamination in the vessels, which may not be avoided entirely in total darkness, was reduced in this way, because irradiated sections and dark controls were in the same vessel.

A certain amount of the buffer solution in which the sections were to grow was put into a perspex vessel (Fig. 9). This container had been provided with a slot in which a piece of photographic film could be placed and with a device to raise or to lower the

level of the fluid. This was done by removing (Fig. 10) or inserting (Fig. 11) a block of perspex in the deep part of the container. The shallow part contained the halved slides with the sections. The level of the fluid was chosen so that the sections were just breaking the surface of the fluid (block removed). The sections were allowed to grow when the fluid was at the lower level (block removed).

To make shadowgraphs the fluid level was raised so that the sections were submerged and no distortion of the image on the film could be caused by the menisci around the sections. In the slot (Fig. 9, 10, 11) a piece of photographic film was placed and a shadowgraph was made.

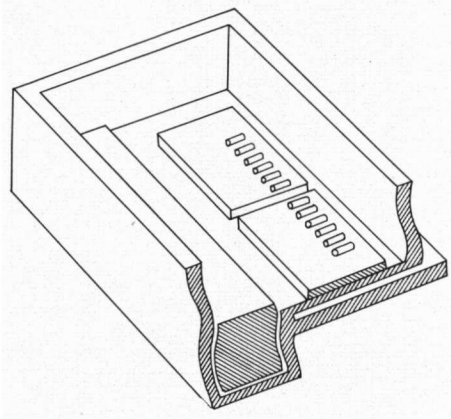


Fig. 9.

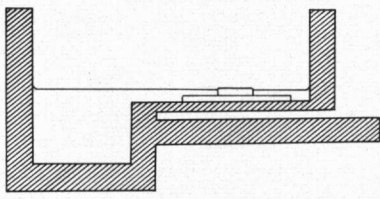


Fig. 10.

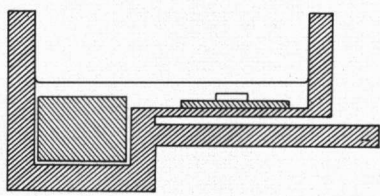


Fig. 11.

## EXPERIMENTS ON GEOTROPISM

*Entire plants*

Plants were cultivated in vermiculite as described.

Care was being taken that the plane in which the two vascular bundles of the coleoptile are located was the same as that in which the plants were to curve. After 4 days they were put in a horizontal position. After they had been allowed to curve a shadowgraph was made.

For reasons explained below, a method was devised for using excised plants.

*Excised plants*

Plants were selected by touch and cut off from roots and seed at approximately equal distances from their tips. They were placed with the cut end in a holder (Fig. 12). In order to secure an adequate

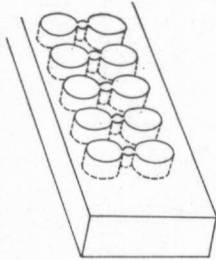


Fig. 12.

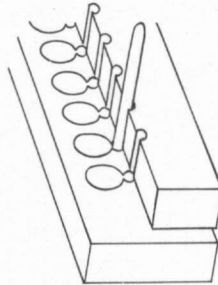


Fig. 13.

water supply each cavity containing a plant communicated with a small depression at each side, filled with water. When the plants were in a horizontal position this water was kept by capillary forces. The plane through the two vascular bundles of the coleoptile was located so as to ensure the curvature to develop in this plane.

To make it feasible to place the plants into the cavities in total darkness a grooved block of perspex (Fig. 13) was placed on the holder. By feeling along the edge of the block a groove was found and the plant was slid downwards along the groove. The plant then slipped easily into the cavity that had been made a bit wider at its upper end. After all the cavities had been filled the grooved block was removed. (Fig. 14). The experimental irradiations were administered and the holders were placed in a rack. The rack was turned  $90^\circ$  so that the plants were in a horizontal position (Fig. 15). Records were made by means of shadowgraphs.

After the shadowgraphs were made the films were immediately processed and dried, which took about 15 minutes. The plants were taken into the light and checked minutely for damage, because even small lesions impair the growth of the seedling causing unwanted curvatures. The shadowgraphs of damaged plants were not taken

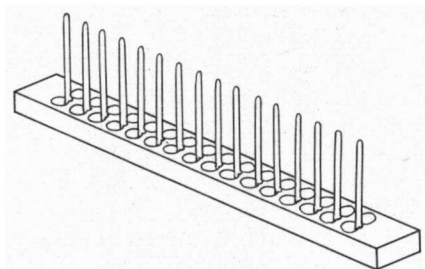


Fig. 14.

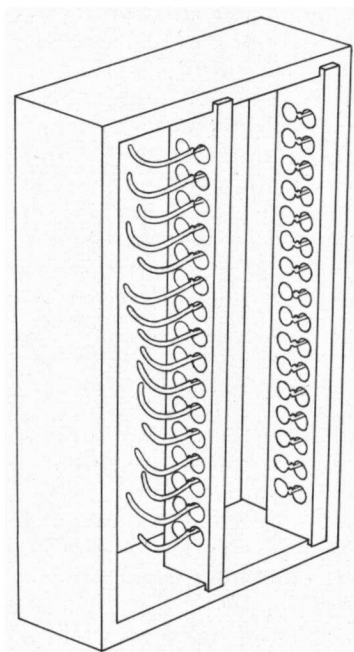


Fig. 15.

into account. The plants were then placed on their shadowgraphs and the place of their node was marked on the film. Measurements of curvatures were made by means of a goniometer.

### CHAPTER III

#### GROWTH OF SECTIONS OF COLEOPTILES AND MESOCOTYLS

In the literature on growth of sections of *Avena* seedlings but few papers deal with the influence of light on the growth of sections. Only LIVERMAN and BONNER (1953), SCHNEIDER (1941) and BLAAUW-

JANSEN (1959) investigated the influence of red light on the growth of sections.

Liverman and Bonner used sections of plants which probably had been grown in darkness and which were manipulated in green safe-light. They found that red light (i.e. light of wave lengths longer than 660 nm) causes an increase in the growth rate of coleoptile sections. They further stated that far red light did not have an effect on sections of plants that had been grown in darkness (and had probably been cut in darkness) but that it cancels the effect of red light given previously.

Blaauw-Jansen cut coleoptile sections from plants that had been grown part of the time under an orange light source and thereafter in darkness. The sections were cut under green light of  $\lambda = 560$  nm.  $1\frac{1}{2}$  hours prior to cutting, part of the plants received 700 erg  $\text{cm}^{-2}$  of red light of  $\lambda = 660$  nm. She allowed the sections to grow for 3 hours in a maleate buffer with 3 % sucrose. The dark controls showed a slightly faster growth than the irradiated sections if no auxin was present in the solution. She states that she could not reproduce the findings of Liverman and Bonner.

Schneider (1941) kept his plant material in total darkness and found with irradiations of at least  $8 \cdot 10^7$  erg  $\text{cm}^{-2}$  of red light given during the whole growth period, a decrease of the growth of sections of the mesocotyl.

#### THE TIME-GROWTH RELATION

In order to ensure that the sections kept their ability to grow during the whole experiment, experiments were done in which the sections were photographed several times over a period of ca 20 hours. The light used for photographing had a wave length of 560 nm and the energy required for each exposure was about 0.01 erg  $\text{cm}^{-2}$ . This light had passed through one filter from Schott und Gen. and through two Balzer filters.

In the same experiments the effect of a relatively small dosis of red light of  $\lambda = 660$  nm i.e. 125 erg  $\text{cm}^{-2}$ , administered at the start of the experiment, has been studied. This light quantity is many times lower than that used by previous authors. The method used was that of the "halved slides". This method provides the possibility of making shadowgraphs of the sections repeatedly without disturbing them. The result of an experiment is presented in Fig. 16. The growth is expressed as the total length at time  $t$  ( $L_t$ ) minus the initial length of the sections ( $L_0$ ). It can be seen that the sections retain a considerable growth rate even after a 19 hours' stay in a buffer solution. Furthermore, it is clear that 125 erg  $\text{cm}^{-2}$  of red light ( $\lambda = 660$  nm) notably enhances the growth rate of the coleoptile sections and decreases that of the mesocotyl sections.

Comparison between the curves for the dark controls and those of the irradiated sections clearly shows that the effect of the irradiation does not disappear in the course of time.



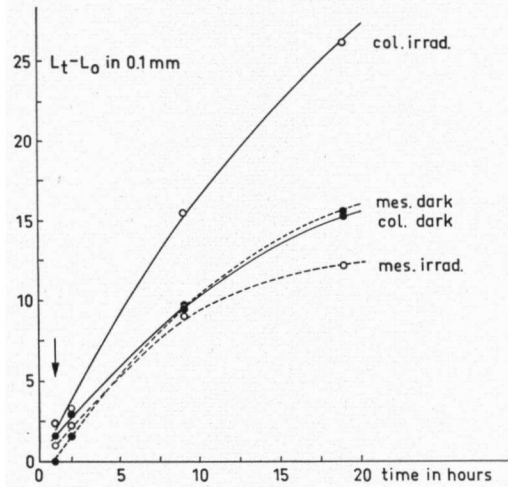


Fig. 16. The influence of red light on the growth of sections of coleoptiles and mesocotyls in the course of time. Irradiated sections (irrad.) received  $125 \text{ erg cm}^{-2}$  of  $\lambda = 660 \text{ nm}$  at the time indicated by the arrow.

#### EXPERIMENTS WITH VERY SMALL QUANTITIES OF RED AND FAR RED LIGHT

The pronounced effect of  $125 \text{ erg cm}^{-2}$  of  $\lambda = 660 \text{ nm}$  on the growth rate of coleoptile and mesocotyl sections induced the present author to investigate the effect of very small quantities of light of  $\lambda = 660 \text{ nm}$  and of  $\lambda = 720 \text{ nm}$  on sections of plants that previously had been grown and manipulated in absolute darkness. The method used was that of the "floating sections". The light used had passed through two Balzer filters, each with the maximum transmission at about the same wave length. No shadowgraphs could be made at the start of these experiments. For that reason the differences in final length between the irradiated sections ( $L_t$ ) and the dark controls ( $L_d$ ) is plotted in the graphs. The duration of the experiments was 17 hours from the moment of irradiation. The amount of light energy has been plotted on the abscissa. The results are presented in Fig. 17, A and B.

#### EXPERIMENTS WITH A LARGER QUANTITY OF RED LIGHT

It seemed interesting to know whether much larger amounts of energy still would not cause a greater reaction. Therefore, experiments were performed with  $\lambda = 660 \text{ nm}$  and an energy of  $13\,000 \text{ erg cm}^{-2}$ . The results are shown in Table 1.

The results of the latter experiments show that up to  $13\,000 \text{ erg cm}^{-2}$  the reaction does not increase as the energy increases.

From the experiments presented may be concluded that the dosage response of the reaction of sections of coleoptiles and mesocotyls can be rendered as shown in Fig. 18.

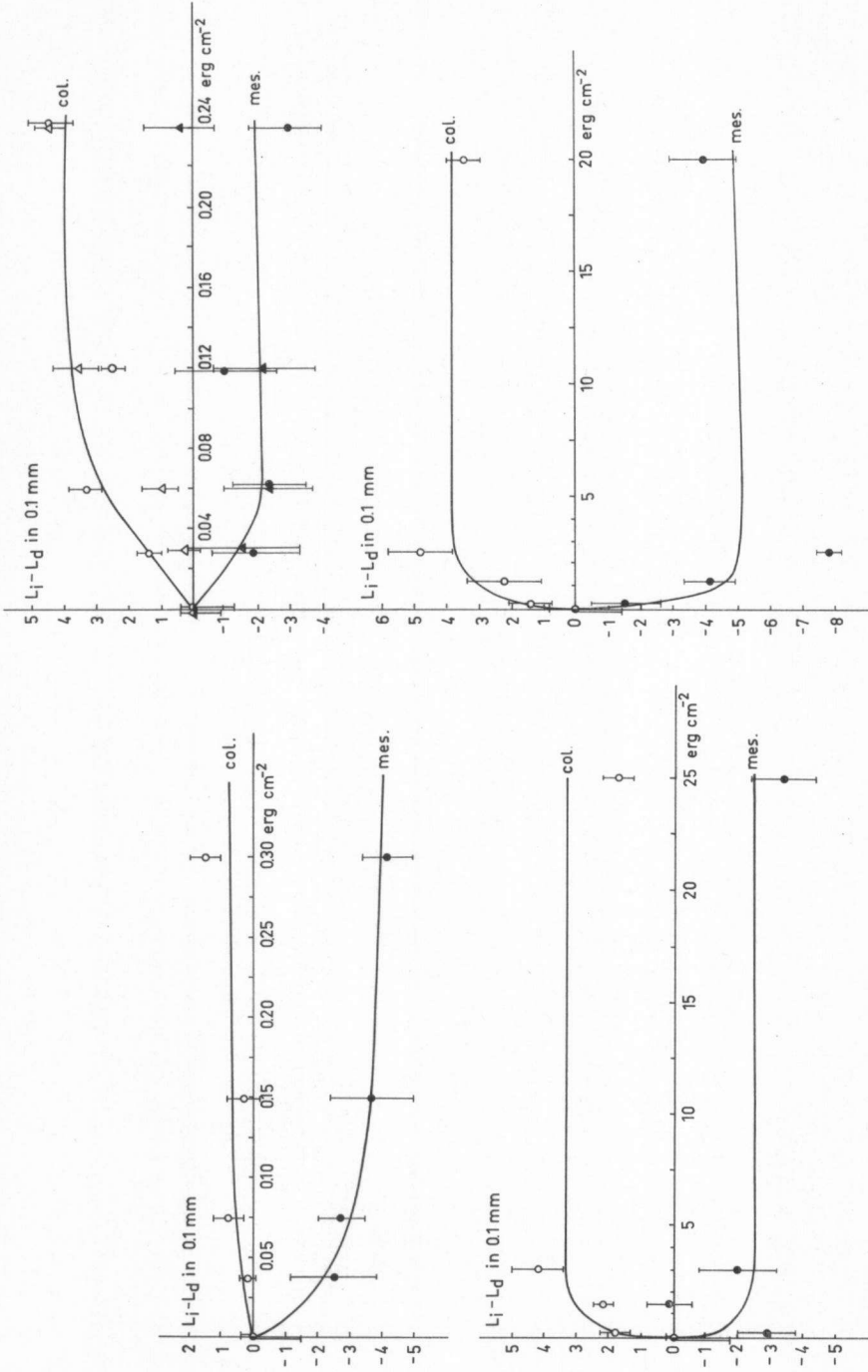


Fig. 17. Differences in length of irradiated sections ( $L_1$ ) and dark controls ( $L_d$ ) after a growth period of 17 hours.  
A. Influence of different amounts of  $\lambda = 660$  nm.  
B. Influence of different amounts of  $\lambda = 720$  nm.

TABLE 1

*Lengths of coleoptile- and mesocotyl-sections in mm after 20 hours.*The influence of  $13\,000 \text{ erg cm}^{-2}$  of  $\lambda = 660 \text{ nm}$ . The numbers between brackets are numbers of plants.

	<i>a</i> irrad.	standard error	<i>b</i> dark	standard error	<i>a-b</i>
col.	3.75 (11)	0.04	3.52 (10)	0.05	0.23
	3.96 (10)	0.06	3.72 (9)	0.03	0.24
mes.	3.27 (11)	0.05	3.46 (10)	0.07	- 0.19
	3.48 (7)	0.08	3.60 (9)	0.05	- 0.12

These results are substantially in agreement with those of SCHNEIDER (1941) who found a slightly greater decrease of mesocotyl growth, which may be due to differences in method (he let sections grow continuously in red light).

The results of LIVERMAN and BONNER are partly at variance with these results; with red light ( $\lambda = 660 \text{ nm}$ ) the increase of growth of coleoptiles found by us is less than that in their experiments. The reactions to far red light ( $\lambda = 730 \text{ nm}$ ) observed by us are not found by Liverman and Bonner.

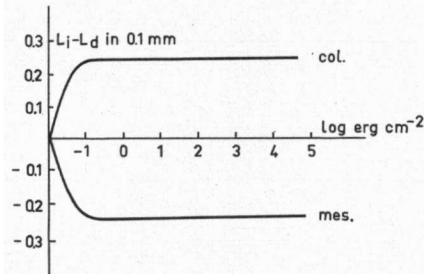


Fig. 18.

In a number of experiments an attempt was made to find an antagonism as indicated by Liverman and Bonner. We were not able to detect an antagonistic effect. Liverman and Bonner state that the antagonistic action of far red to red light only occurs in the presence of auxin. The discrepancy between our results and theirs can possibly be attributed to the fact that we did not add auxin. The fact that the sections in our experiments did grow without added auxin i.e. only on the auxin contained in the sections does not make this explanation very probable.

Our results do not agree with those of BLAAUW-JANSEN, who found a decrease of the growth of coleoptile sections when red light was applied prior to the cutting of the sections. We irradiated after the sections had been cut.

Blaauw-Jansen, moreover, added sucrose to the medium and used a different buffer. The disagreement between her results and ours is probably due to these differences in method.

## EXPERIMENTS WITH GREEN AND BLUE LIGHT

Experiments with blue light ( $\lambda = 480$  nm, energies up to 500 erg cm<sup>-2</sup>) using the "floating sections method" did not show an effect of this light on the growth of either the coleoptiles or the mesocotyls. To obtain a greater certainty that blue light did indeed have no effect on the growth of sections, experiments were done with the "halved slides method". Also some experiments with green light ( $\lambda = 560$  nm) were done.

As in the previous experiments with the "floating sections", no records were made at the start of the experiments. Differences in the mean initial length between different sets of sections, however, could not be avoided. These differences are due to inaccuracies in the manipulations during the cutting procedure. In the "halved slides method" half of the sections of one batch serves as control for the other, irradiated, half. So, differences in initial length have lost their influence on the results. Also, differences in growth could occur due to possible impurities in the culture fluid, which can rather easily be introduced during the manipulations in the dark. The effects of

TABLE 2  
*Lengths of coleoptile- and mesocotyl-sections in mm after 20 hours.*  
The influence of irradiation with 480 nm and 560 nm.

wavelength	Each number represents the mean of:					
	4 sections			4 sections		
	<i>a</i> 50 erg cm <sup>-2</sup>	<i>b</i> none	<i>a-b</i>	<i>a</i> 500 erg cm <sup>-2</sup>	<i>b</i> none	<i>a-b</i>
480 nm	3.58	3.40	0.18	3.56	3.53	0.03
	3.64	3.49	0.15	3.53	3.60	-0.07
col.	3.90	3.88	0.02			
	$P = 0.1$			$P = 0.2$		
mes.	3.80	3.84	-0.04	3.75	3.96	-0.21
	3.70	3.81	0.11	3.79	3.74	0.05
	3.80	3.63	0.17			
	$P = 0.3$			$P = 0.6$		
wavelength	4 sections			5 sections		
	<i>a</i> 20 erg cm <sup>-2</sup>	<i>b</i> none	<i>a-b</i>	<i>a</i> 500 erg cm <sup>-2</sup>	<i>b</i> none	<i>a-b</i>
	<i>a</i>	<i>b</i>	<i>a-b</i>	<i>a</i>	<i>b</i>	<i>a-b</i>
560 nm	3.64	3.47	0.17	3.85	3.69	0.16
	3.83	3.68	0.15	4.08	3.83	0.25
col.						
	$P = 0.01$			$P = 0.02$		
mes.	3.21	3.40	-0.19	3.66	3.92	-0.26
	3.35	3.65	-0.30	3.64	3.88	-0.24
	$0.02 > P > 0.05$			$0.02 > P > 0.05$		

these impurities are reduced by placing the irradiated and control sections in the same container. This is also the case when using the "halved slides method".

The results of these experiments are presented in Table 2. From this table can be seen that the plants react to 20 erg cm<sup>-2</sup> of green light of  $\lambda = 560$  nm, showing that even the use of this wavelength as a safe light is dangerous. With blue light of  $\lambda = 480$  nm it was not possible to detect a reaction with irradiations up to 500 erg cm<sup>-2</sup>.

A number of experiments was done with the "halved slides method", using irradiations with  $\lambda = 660$  nm and  $\lambda = 720$  nm. The results were substantially the same as those found with the method with floating sections and are therefore not presented.

From Fig. 17, A and B and Table 1 can be seen that both coleoptiles and mesocotyls react in the same way to irradiations with red and far red light, the coleoptiles becoming longer than the controls, the mesocotyls shorter.

The sensitivity of the plants to energy of the wave lengths mentioned above is very high, 0.1 erg cm<sup>-2</sup> being sufficient to make the plants react.

It was not attempted to estimate the lower limit of energy causing a reaction at  $\lambda = 560$  nm. The reactions at 20 and 200 erg cm<sup>-2</sup> suffice to show the same energy dependence as in the case of  $\lambda = 660$  nm or  $\lambda = 730$  nm, although the lower limit may be higher than for the latter wave lengths.

The plants do not react to radiation of  $\lambda = 480$  nm up to 500 erg cm<sup>-2</sup>.

The accuracy of the estimations of sensitivity is low, which is due to the shape of the graph: a. the maximum reaction does not exceed 0.4 mm, b. the reactions do not increase with increasing energies above  $\pm 1$  erg cm<sup>-2</sup> and c. the rather great variability of the material.

## CHAPTER IV

### THE GEOTROPIC REACTION

The geotropic reaction in *Avena* is known to be influenced by light of different wave lengths.

BLAAUW (1961), using plants that had been cultivated the first 24 hours under orange light and manipulating them under dim green light, found that red light ( $\lambda = 660$  nm) and far red light ( $\lambda = 735$  nm) had the same influence on the geotropic curvature of coleoptiles of *Avena sativa*, both wave lengths causing an increase of these curvatures. The dependence of the geotropic response on the amount of energy of red light is not a very strong one. His graphs show that even by small doses (about 10 erg cm<sup>-2</sup>) of red light an increase of the curvature is caused, but up to about 1000 erg cm<sup>-2</sup> no evident dosage dependence has been found. Above 1000 erg cm<sup>-2</sup> a slight increase in curvature is evident. Experiments with irradiations with blue light show an

increase or a decrease of the subsequent geotropic reaction, depending on the amount of energy applied and the length of time during which the plants were left vertical between irradiating them and putting them in a horizontal position. If this lapse in time was not introduced, the results were comparable with those with red light.

In a second communication BLAAUW (1963), working with excised coleoptiles, found the same energy response to red light as in 1961. He describes, moreover, that the shape of the curves developed in the coleoptiles is altered by the irradiation with red light i.e. with high dosage ( $24 \times 10^5$  erg  $\text{cm}^{-2}$ ) the curve appeared only in the topmost centimeter of the coleoptile. In the dark controls the curves extended over the whole length of the coleoptile. This phenomenon appeared to be more dosage dependent than the magnitude of the curvature. BLAAUW (1961) measured the elongation of the topmost part of about 1 cm of coleoptiles. He could not find a consistent correlation, at the wave lengths used, between the growth of this part of the coleoptile and the curvatures, and concluded that there exists no simple relation between the magnitude of the curvature and the growth rate.

It seemed interesting to investigate whether a correlation could be found between the rate of the straight growth of sections and the geotropic reaction, using dark grown plants. The very low dosage dependence when irradiating with red light of the geotropic curvature as found by Blaauw and of the growth rate of sections as shown in the previous chapter seems to indicate such a correlation.

#### THE GEOTROPIC REACTION OF INTACT PLANTS

As a first orientation plants were put in a horizontal position after treating them with different amounts of light of  $\lambda = 660$  nm, the controls receiving no irradiation at all. After 7 hours the curvatures of coleoptiles and of mesocotyls were measured. The results are shown in Fig. 19. The curves in this graph show that the curvatures of the coleoptiles increase with increasing amounts of energy, those of the mesocotyls decrease, so that the sum of both measurements, being the total curvature, does not change with increasing energies. After 7 hours there is no increase in total curvature, because after this period the curvatures are approaching  $90^\circ$ , the transversal geotropic component for the plants curved most strongly being less than that for the least curved ones (c.f. the next chapter). It can easily be concluded from the shape of the curves, that the location of the bend shifts towards the tip by the irradiation.

In the experiments with intact plants difficulties arose because of the tendency of the plants to extend the curving zone of the plant into the vermiculite. To eliminate this difficulty the method with excised plants was adopted. This had the added advantage that the results with the geotropically curving plants could be compared more safely with the results of the straight growth experiments because the seed and roots were removed, so that the two kinds of experiments were comparable in view of food supply.

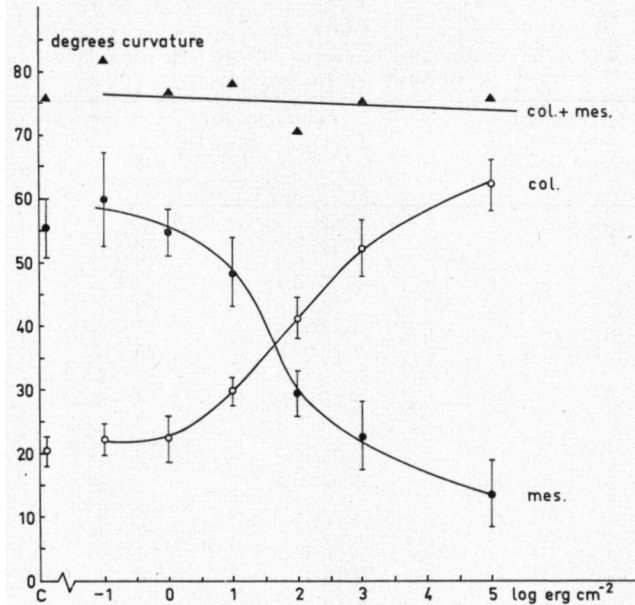


Fig. 19. Geotropic curvatures of coleoptiles (col.) and mesocotyls (mes.) and total curvatures (col. + mes.) of intact plants after irradiations with different amounts of  $\lambda = 660$  nm. Measurements after 7 hours.

#### THE GEOTROPIC REACTION OF EXCISED PLANTS

Experiments were conducted with the method with excised plants to investigate the relation between energy and reaction.

Plants were irradiated with different amounts of light. The light was filtered through a combination of one filter from Schott und Gen. and one filter from Balzer. Only for the irradiations with  $10^5$  erg cm<sup>-2</sup> of blue light ( $\lambda = 480$  nm) the light was filtered through one filter from Schott und Gen. only.

Table 3 shows the curvatures developed in 7 hours and the differences between the curvatures after irradiation (a), and the curvatures of the corresponding dark controls (b). From Table 3 can be concluded that for red ( $\lambda = 660$  nm) and far red ( $\lambda = 735$  nm and  $\lambda = 718$  nm) light the relation between the amount of energy and the total reaction does not differ much, if at all, from the relation found in the straight growth experiments. The great variability in the curvatures is for the major part due to difficulties in selecting the plants in total darkness.

In contradistinction to the results with section growth the total curvatures are influenced by blue light of  $\lambda = 480$  nm. The increase of the total curvatures is, however, smaller than that for light of  $\lambda = 660$  nm or  $\lambda = 735$  nm. It may be that the geotropic curvature is a more sensitive detector of an effect of irradiation with blue light than the straight growth as measured in the sections. In that case

TABLE 3  
The influence of different amounts of energy of different wave lengths on the total curvature of excised plants.

Irradiated		Total curvatures in degrees after 7 hours		
wave length	erg cm <sup>-2</sup>	<i>a</i>	<i>b</i> dark control	<i>a-b</i>
$\lambda = 660$ nm	1	56.5	46.9	9.6
	10	66.0	46.9	19.1
	13	26.4	7.4	19.0
	18	34.6	36.3	— 1.7
	100	67.8	37.6	30.2
		60.8	20.3	40.5
		49.2	37.0	12.1
		20.6	14.1	6.5
		56.5	35.1	21.4
	1 000	23.7	6.1	17.6
		36.7	22.3	14.4
		50.8	19.3	31.5
	1 300	43.5	17.5	26.0
6 000	47.7	30.9	16.8	
	80.2	18.9	61.3	
$\lambda = 735$ nm	1	14.5	23.2	— 8.7
	10	43.0	34.2	8.8
		20.8	23.2	— 2.4
	100	37.9	34.2	3.7
$\lambda = 718$ nm	100	47.6	35.1	12.5
	1 000	52.0	19.3	32.7
		47.6	17.5	30.1
	1 400	48.5	20.3	28.2
34.4		37.0	— 2.6	
31.7		6.1	25.6	
28 800	39.8	22.3	17.5	
	27.9	14.2	13.7	
$\lambda = 560$ nm	2	41.5	32.4	9.1
	20	51.5	32.4	19.1
	200	37.8	32.4	5.4
		28.5	14.5	14.0
	26.4	16.4	10.0	
$\lambda = 480$ nm	100	35.7	29.3	6.4
		59.8	45.6	14.2
		39.7	22.3	17.4
	1 000	56.6	29.3	27.3
		44.6	45.6	—1.0
		28.9	27.7	1.2
		46.1	36.5	9.6
		31.3	22.3	9.0
	2 000	46.4	29.3	17.1
		36.4	45.6	— 9.2
		36.9	27.7	9.2
	10 000	38.8	36.5	2.3
		26.1	22.3	3.8
29.9		27.7	2.2	
32.8		36.5	— 3.7	



the latter method then is apparently not delicate enough to detect a very slight effect of light.

In the graphs in Fig. 20 curvatures found in a number of experiments with different wave lengths and different amounts of energy are presented. The measurements were taken 17 hours after the start of the experiment.

Of each set of plants the curvatures of the coleoptiles and the mesocotyls were measured separately. Also their sums i.e. the total curvatures were calculated. In several instances these curvatures were measured and compared with the sums of coleoptiles and mesocotyls. The two results were consistently the same.

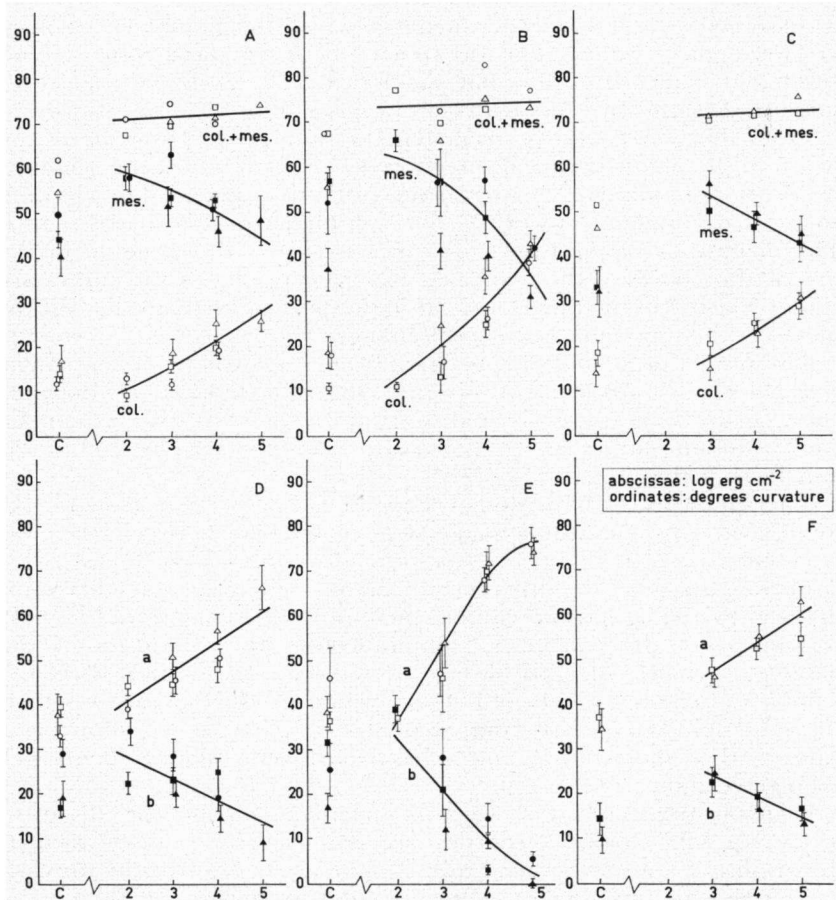


Fig. 20. The influence of different amounts of  $\lambda = 480$  nm (graphs A and D),  $\lambda = 660$  nm (graphs B and E) and  $\lambda = 735$  nm (graphs C and F) of the geotropic curvatures of excised plants. Measurements after 17 hours. Curvatures of coleoptiles (col.), mesocotyls (mes.) and total curvatures (col. + mes.) are shown in graphs A, B, C. Of the same plants curvatures were measured with a reference point 6 mm below the node: apical parts (a) and basal parts (b) (Graphs D, E, F).

In addition, measurements were taken of the upper and lower parts of the plant, a point 6 mm below the node taken as reference point. In this way a shift of the bend in the plants could be detected even if this bend did not reach the coleoptile.

After 7 hours also, a shadowgraph was made using  $0.01 \text{ erg cm}^{-2}$  of green light ( $\lambda = 560 \text{ nm}$ , 1 Schott and 2 Balzer filters) except in one of the experiments presented in Fig. 20, B and E. No influence of  $0.01 \text{ erg cm}^{-2}$  of  $\lambda = 560 \text{ nm}$  has been detected. The results of these shadowgraphs are part of the data presented in Table 3.

In these graphs (Fig. 20), showing the results of experiments in which the plants were allowed to curve for 17 hours, the same relation between the amount of energy and the total geotropic reaction can be observed as found in experiments in which the plants were allowed to curve for 7 hours. The differences between dark controls and irradiated plants are somewhat smaller after 17–20 hours than after 7 hours, because the curvatures are approaching  $90^\circ$ .

From the graphs can be seen that the same shift of the site of the bends in the plants in apical direction occurs with increasing amounts of energy as found in the experiments with intact plants. The absolute energy requirements, however, are much higher in the excised plants. The magnitude of the total curvatures is clearly independent of the site of the curves in the plants. At lower energy levels the curves are chiefly located in the mesocotyls, at higher levels chiefly in the coleoptiles. Yet the total curvature is not changed.

The results presented in this chapter are, as a whole, in agreement with the results of BLAAUW (1961 and 1963). The light dosage dependence of the curvatures for red and far red light is low, as can be seen from the curves for total curvatures in the graphs in Fig. 20 and from Table 3.

The similarity of light dosage dependence of section growth and total geotropic curvatures suggests a correlation between the two phenomena.

Blaauw describes the difference in shape of the geotropic curvature induced by red light as an enhancement of the curving of the topmost centimeter of the coleoptile; simultaneously the curvature of the basal part of the coleoptile would be reduced. Irradiation with low amounts of energy of light of  $660 \text{ nm}$  would produce plants in which the geotropic curves are composed of two parts: a tip showing a curve with a short radius and a basal part being slightly bent over a longer distance.

From our results it can be seen that an enhancement of the curvature in the tip zone of the coleoptile need not occur. As can be seen in the graphs of Fig. 20 the bend in the plants shifts towards the tip with increasing amounts of light energy without an increase of the curvature of the coleoptile.

To show the development of the curves in the plants an experiment was carried out in which excised plants were irradiated with  $41\,000 \text{ erg cm}^{-2}$  of red light of  $\lambda = 660 \text{ nm}$  or left in the dark (control). Immediately after this the plants were placed in a horizontal position

and a photograph was made with  $0.01 \text{ erg cm}^{-2}$  with  $\lambda = 560 \text{ nm}$ . After this the plants were photographed with intervals of one hour up to 6 hours. Drawings of the projected negatives were made and superposed. The result is shown in Fig. 21.

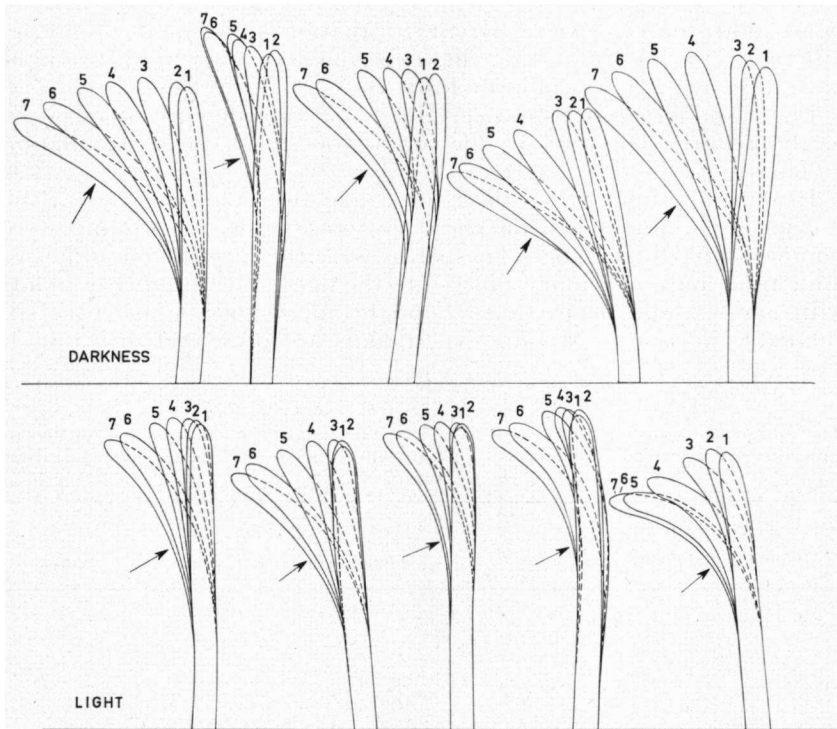


Fig. 21. The development of curvatures in excised etiolated seedlings in darkness and after irradiation with  $41\,000 \text{ erg cm}^{-2}$  of  $\lambda = 660 \text{ nm}$ . The shadowgraphs were taken at intervals of one hour. The arrows indicate the place of the node.

## CHAPTER V

### GUTTATION

During the experiments on geotropism of dark-grown *Avena* seedlings it was noted that a correlation seemed to exist between irradiation and the number of plants producing a drop of liquid at the tip of the coleoptile.

A survey of the literature revealed some communications on the influence of light on guttation of which that of ENGEL and FRIEDERICHSEN (1951) is of interest for the investigation at hand.

Engel and Friederichsen cultivated *Avena sativa* seedlings in darkness for about 4 days. After the plants had been placed in light the guttation increased. After 4 hours a maximum was reached and a decrease of

guttation set in. The phenomenon was observed with red, green, blue and "white" light filtered through glass filters of Schott und Gen. No intensities of light energies were mentioned, but it is clear from the description of the light sources used that the amounts of energy applied were large. In some of their experiments they used, with the same light source, two different irradiation times and found no difference in the results of the experiments. This indicates light saturation at the lower light quantity.

Engel and Friederichsen ascertained that no influence of variation of the relative humidity of the air and of mechanical stimulation on guttation did occur.

In our experiments the plants used had been cultivated as in the previous experiments. In darkness any possible drops of liquid were removed with filter paper. The plants were then irradiated and after some time, mostly 4 hours, taken into the light. The number of plants with and without drops were counted and the percentage was calculated. The results of some experiments are presented in Table 4.

TABLE 4

Percentages of plants with a drop of fluid 4 or 9 hours after irradiation with different amounts of various wave lengths. The numbers between brackets are numbers of plants.

wave length in nm	time in hours	irradiation in erg cm <sup>-2</sup>			
		0	100	1000	2000
735	4	4 (50)	19 (58)	24 (42)	19 (64)
	4	0 (64)		33 (54)	
660	9	3 (79)	13 (47)		
	4	5 (62)	10 (40)	33 (54)	
	4	0 (64)		45 (49)	
560	4	5 (62)	8 (53)	31 (62)	
	4	0 (64)		5 (38)	
480	4	4 (50)	8 (50)	30 (60)	
	4	0 (64)		35 (57)	

As can be seen in Table 4 the plants react to irradiations with all wave lengths used. The reaction seems to increase with increasing amounts of energy and the sensitivity of the reaction for red light seems greater than for blue, green and far red light.

The variations in the results were large as was to be expected since Engel and Friederichsen mentioned that in their material the variability was also very large.

Because of this the experiments were repeated after our culture method had been modified: the pH of the culture medium, which had been tap water in the previous experiments, was changed to pH = 6, because, by adding a phosphate buffer 1/100 M, in some experiments, it was noted that the pH had influence on the guttation

and pH = 6 seemed to be about optimal. Instead of placing the seeds in vermiculite they were placed on sloping glass plates covered with filter paper, the lower end of which was in contact with the solution.

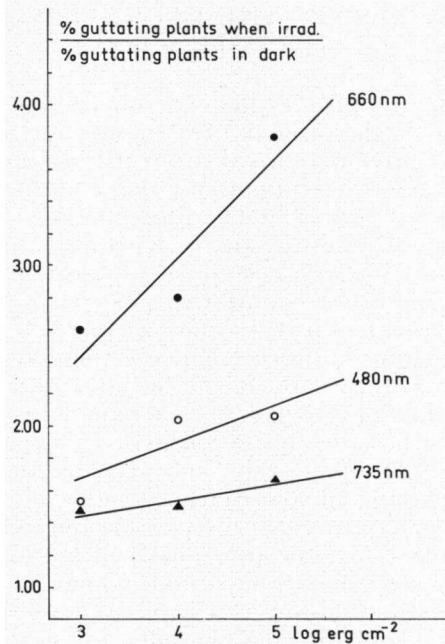


Fig. 22. The influence of different amounts of light of  $\lambda = 480$  nm,  $\lambda = 660$  nm and  $\lambda = 735$  nm on the proportion of seedlings showing guttation, as compared to the dark controls.

The variability, however, was still very large. The result of this series of experiments, which was done with higher amounts of energy, is shown in Fig. 22. Because at this pH the dark controls also show an appreciable guttation, and the guttation shows a rather large variation between different experiments, the results are expressed as the ratio of the proportion of guttating plants to the total number of plants for plants that were irradiated, and for plants kept in darkness.

The results confirm the trends indicated in Table 4:

- the sensitivity for red light (660 nm) is higher than that for blue (480 nm) and that for far red light (735 nm),
- the reaction is clearly dependent on the amount of light energy.

## CHAPTER VI

THE INFLUENCE OF THE COLEOPTILE AND OF  
APPLIED AUXIN ON THE GEOTROPIC RESPONSE  
OF THE MESOCOTYL

Considering the results of the experiments on geotropism and especially of those on the place of the curves in the seedlings, it is not improbable that transport of auxin from the coleoptile to the mesocotyl might be a factor determining the shape of the geotropic curvature of the seedlings. ANKER (1962) propounded a hypothesis based on relative transport rates of auxin explaining the differences in geotropic reaction of *Avena* coleoptiles in normal and in inverse position as well as on the clinostat.

BLAAUW (1963) suggested that transport might be involved in the mechanism causing the different shapes he observed in the geotropically induced curvatures of coleoptiles after different irradiations with red light. (His plants had been irradiated with orange light during the first part of the cultivation period, so that the mesocotyls had a length of 12–20 mm and the coleoptiles of 20–30 mm.)

MER (1951), working in total darkness, showed that it is possible to reduce the growth of the mesocotyl by decapitating the coleoptile. Removal of the tip of the coleoptile only had not much influence on the subsequent growth of the mesocotyl. Removal of the coleoptile including the node had, however, a strongly inhibiting effect on the growth of the mesocotyl. Besides the node he also removed part of the mesocotyl. He concluded that growth of the mesocotyls might, for the major part, be due to auxin that is not supplied by the coleoptile but produced in the node or the shoot apex, or due to the removal of the meristematic zone of the mesocotyl. According to the lateral distribution theory of geotropism the geotropic stimulation would cause a transversal asymmetry which is, among other things, shown by an asymmetrical distribution of auxins in the coleoptile (CHOLODNY 1926; WENT 1926; WENT and THIMANN 1937).

It seemed, however, hardly probable to us, that in so short a piece of tissue as e.g. the node, an asymmetric distribution could be effected. To investigate this matter the following experiments were carried out. From excised plants pieces of different length were removed from the apical end. In one group also the nodes were removed. They were placed in a horizontal position for 17 hours.

In contradistinction to all former experiments the seedlings were manipulated under green light of  $\lambda = 560$  nm. The energy irradiated on the plants was for all groups  $18\,000$  erg  $\text{cm}^{-2}$ .

The results of the experiments were: no curvatures occurred except in plants of which the coleoptiles had been left intact. A similar experiment was done in total darkness: the result was the same as that of the former experiments.

This suggests that of the two alternative interpretations of his

experiments on the removal of the node, suggested by Mer, the one based on the effect of the removal of the apical part of the mesocotyl should be the correct one.

To investigate the influence of auxin on the geotropical behaviour of mesocotyls that had been deprived of the node and coleoptile we did the following experiments.

Agar blocks containing different concentrations of indole-3-acetic acid (I.A.A.) were prepared as is usual in the standard *Avena* curvature test.

In a number of experiments agar blocks were placed on the cut surface of the mesocotyls so that they were in contact with the whole of that surface. The plants were then placed in a horizontal position and shadowgraphed. After four hours a second shadowgraph was made.

In none of our experiments, applying auxin concentrations of  $5 \times 10^{-6}$ ,  $10^{-6}$ ,  $5 \times 10^{-7}$ ,  $10^{-7}$ ,  $5 \times 10^{-8}$  and 0, did we observe any curvatures.

In other experiments agar blocks were placed on the apical cut surfaces in such a way that they were only in contact with half of the cut surface. It was, however, not feasible to place all the blocks so that exactly half of the surface was covered. This is, for the major part,

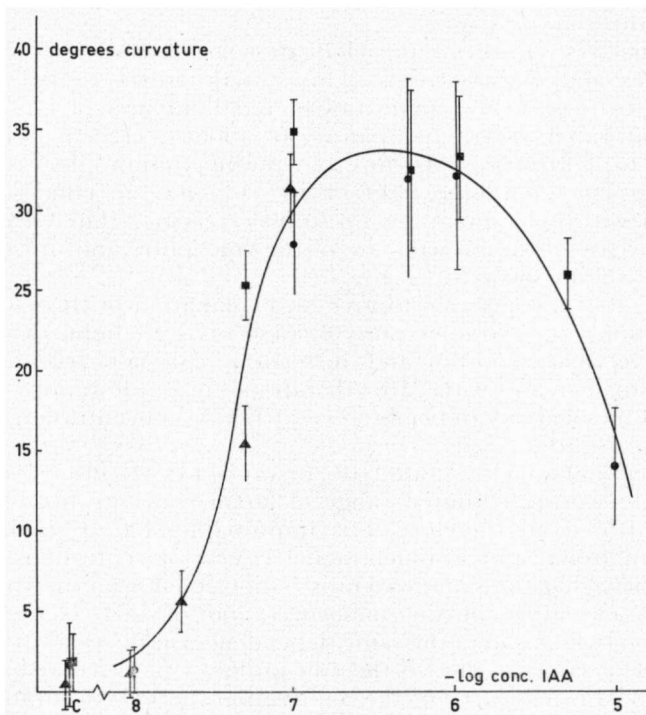


Fig. 23. Curvatures of isolated mesocotyls after unilateral application of I.A.A. in different concentrations at the apical surface. (Preparation in green light  $\lambda = 560 \text{ nm}$ ,  $18\,000 \text{ erg cm}^{-2}$ .)

the reason of the variability in the curvatures observed. These experiments also were done in light of  $\lambda = 560$  nm.

The plants were shadowgraphed at the beginning of the experiment and after 4 hours. They were left vertical during the whole experiment.

The results of such experiments, presented in Fig. 23, clearly show that unilateral application of auxin causes curvatures in a wide range of concentrations.

From our experimental results we have concluded that the inability of the mesocotyl to curve geotropically, if the whole or part of the coleoptile has been removed, is due to the fact that auxin produced in the tip of the coleoptile is necessary for the growth of the mesocotyl and that this auxin, in horizontal seedlings, is distributed asymmetrically in the coleoptile. The mesocotyl lacks the faculty to effect such an asymmetrical distribution.

## CHAPTER VII

### DISCUSSION

From the experimental results reported it is apparent that irradiation with light acts upon two different systems in plants developed in absolute darkness.

The first system affects the elongation as measured in sections of coleoptiles and of mesocotyls. This system proves to be very sensitive to extremely low quantities of red light and of far red light and is saturated at a light quantity of about  $1 \text{ erg cm}^{-2}$ . It is also sensitive to light of  $\lambda = 560$  nm, but the maximum effect is reached at  $20 \text{ erg cm}^{-2}$ . In blue light of  $\lambda = 480$  nm no effect could be obtained with light quantities up to  $500 \text{ erg cm}^{-2}$ . The effect results in an increase of the growth rate of the coleoptiles and in a decrease of that of the mesocotyls.

As yet, it is not possible to give an explanation of this contrast in the reaction of coleoptiles and of mesocotyls to light. A different auxin level in coleoptiles and mesocotyls can not account for it, because an increase of the growth rate of coleoptiles and a decrease of that of mesocotyls can be observed at I.A.A. concentrations ranging from  $10^{-4}$  to  $10^{-9}$ .

The red light factor, found by BLAAUW-JANSEN (1959) shows its effect only within a limited range of auxin concentrations of about  $10^{-8}$  to  $10^{-9}$ , and therefore it is improbable that it should cause changes in growth rate at much higher I.A.A. concentrations.

It is likely that the same system is responsible for an increase of the geotropic curvature of the mesocotyl and of the coleoptile after irradiation, which shows the same dependence on the quantity of light energy. The same applies to the coleoptiles of plants that have been cultivated during part of the cultivation period in orange light (BLAAUW 1961 and 1963). It is noteworthy that the increase of the geotropic curvature or, rather, of the rate of curving, is not directly correlated with the absolute growth rate that is increased in the



coleoptile and decreased in the mesocotyl. As yet no further research has been done by the present author to investigate the mechanisms involved in this light system. This system will be indicated as "section growth system".

The second system is correlated with the shape of the geotropically generated bend and with the guttation of the coleoptiles. It is affected by light but this influence shows not only a dependence on the wave length but also an appreciable dependence on the amount of light energy; for each wave length it increases with increasing amounts of radiant energy. The sensitivity of this system is highest for  $\lambda = 660$  nm. The sensitivities for  $\lambda = 480$  nm and for  $\lambda = 735$  nm are of about the same order of magnitude and much lower than that for  $\lambda = 660$  nm. In the geotropic response the place of the curve is shifted by irradiation towards the tip and the higher the amount of light energy applied, the more the geotropic curve shifts toward the tip. Also guttation is affected by light: the sensitivity to different wave lengths and the dosage dependence is the same as for the reaction mentioned above.

As yet, it is impossible to give a theoretical evaluation of the phenomena elicited by the second system. As a preliminary hypothesis it could be assumed that an enhanced water excretion is caused by a change of the permeability of the protoplasmic membranes by the irradiation. Changes of this permeability under influence of light have been shown to occur. A review of the older literature can be found in JÄRVENKYLÄ (1937). All investigations mentioned, however, have been conducted with techniques which differed so strongly from ours, notably with respect to the light and dark regime, that the results can hardly be compared with ours. VIRGIN (1951) investigated the influence of light on the protoplasmic viscosity in *Elodea*. He found that relatively small amounts of red light decreased the viscosity of the protoplasm. Blue light had a much stronger effect than red light.

A change in permeability caused by irradiation would affect the transport of water and other substances through the tissues in longitudinal direction (and possibly in transversal directions). When plants are placed in horizontal position an asymmetrical distribution of auxin is effected (lateral distribution theory, CHOLODNY 1926; WENT 1926; WENT and THIMANN 1937). This transversal auxin gradient, induced by geotropical stimulation, would travel through the plant from tip to base.

When passing through the coleoptile, this transversal gradient would grow steeper as it proceeds towards the base of the plant. On its way the amount of auxin would be diminished both by inactivation and by absorption by the tissue passed. To induce a visible curvature the difference in concentration of auxins would have to surpass a certain value and the over-all concentration should neither be too high nor too low (ANKER 1956). This value would be reached a certain time after the auxin is released from the tip, irrespective of the rate of the longitudinal transport. As a consequence, the distance from the tip, at which the arising curve starts, would be determined by the

rate of the transport in longitudinal direction; another factor is the different capacity of the tissues, through which the gradient passes, to react on the difference between the auxin concentration at the two sides of the plant: a same given concentration difference may give rise to different curvatures in different parts of the plant.

If in this system the longitudinal rate of transport in basal direction would decrease, the developing curve would start at a point nearer to the tip of the plant and the curve would extend less far towards the base of the plant. Parts of the plant near the apex that normally would not be exposed to a sufficiently steep auxin gradient will now produce a curve. As a consequence of the diminishing amount of auxin reaching the lower parts of the plant, the growth of those parts might be decreased. This hypothesis is supported by the experimental results: irradiation with red light, and to a less degree, with blue light and far red light, shifts the geotropically induced bend towards the tip, the basal parts of the plants curve less or, with high doses of energy, not at all.

CURRY, THIMANN and RAY (1956) using *Avena* seedlings that had been cultivated so as to reduce the elongation of the mesocotyls, showed that irradiation with red light enhanced the growth of the apical parts of the coleoptile while the growth of the basal parts was decreased.

The decrease of the growth of the more basal parts of the plants, viz. the mesocotyl, is shown quite clearly in the experiments of SCHNEIDER (1941), WEINTRAUB and McALISTER (1942), WEINTRAUB and PRICE (1947) and GOODWIN and OWENS (1948). The concurrence between the relative efficiencies of the wave lengths used in our experiments and the action spectrum found by the authors mentioned above indicates that the same basic system may influence the location of the bend in the plant as well as the ultimate length reached by the plant. It is, moreover, as already mentioned in Chapter I, common practice when cultivating *Avena* seedlings, to inhibit mesocotyl growth by exposing them to red or orange light during part of the cultivation period. This suppression can not be caused by the "section growth system" only, because:

- a. it is evidently dependent on amounts of energy far above  $1 \text{ erg cm}^{-2}$
- b. the sensitivity to  $\lambda = 735 \text{ nm}$  is relatively much lower than to  $\lambda = 660 \text{ nm}$
- c. the inhibition is too strong to be accounted for by the inhibition of growth as found in mesocotyl sections.

Inhibition by a reduced supply of auxin in addition to the direct inhibition could cause this strong reduction of growth. Also an influence of light on cell division should be mentioned as a mechanism inhibiting the growth of the mesocotyl. This influence will be discussed below.

A consequence of this hypothesis is that the coleoptile should show an enhanced growth, because less auxin passes down to the mesocotyl in addition to the increase in growth as found for the straight growth of the sections. This also fits in well with the facts mentioned above.

SCHNEIDER (1941) shows a graph of plants cultivated in darkness in comparison to plants cultivated continuously in red light. From this graph it is clear that the growth of the coleoptiles is enhanced by red light, during the first four days of cultivation.

After four days, however, the irradiated coleoptiles do not grow anymore. The coleoptiles of the dark grown plants grow up to the 8th day; the ultimate length of these coleoptiles is greater than of those grown in red light. Thus it appears that, in addition to the influence on the growth rate, the changed conditions in the plants caused by irradiation make the growth stop sooner. THOMSON (1959), reporting on results obtained with peas, described a red light effect that could be reversed by far red light, and postulated that irradiation with red light causes a shortening of the time needed to reach maturation, whatever that may be. In connection with this the influence of light on cell division should be kept in mind.

The dependence on the amount of light energy as well as the sensitivity to different wave lengths are similar for the growth of intact plants, for guttation and for the effects of light on the place of the geotropical curves in the seedlings.

The excretion of liquid of guttating plants must be correlated with an enhanced transport of water and solutes in apical direction. This leads to the notion that an enhanced water transport through the living cells in apical direction may be the cause of the decrease of the transport rate in basal direction. This idea finds some support in a comparison of the influence of red light on the curving of intact plants (Fig. 19) with that on excised plants (Fig. 20). In intact plants having a greater capacity to transport water in apical direction by the presence of roots (which is shown by the fact that excised plants do not guttate) the effect of red light upon the site of the curve is more pronounced than in excised plants. From these speculations on the migration of auxin and similar substances through an etiolated *Avena* seedling a hypothetical mechanism can be pictured.

Auxin is supposed to be produced in the tip of the coleoptile and transported in basal direction. In dark-grown plants the rate of this transport is high. On its way towards the base of the plant the auxin concentration decreases, more or less independent of the rate of transport.

If a plant is placed in a horizontal position, auxin is also transported in lateral direction towards the lower side of the seedling, this lateral transport being, for the major part, independent of the rate of transport in longitudinal direction.

The rate of transport of water (and solutes) from the base in apical direction, now, may have a strong influence on the rate of the migration of auxin towards the base. An increase resp. decrease of water transport in apical direction could decrease resp. increase the rate of the auxin transport in basal direction. These changes in the rate of auxin transport can be deduced from the site of the geotropic curves in the seedlings.

Consequences of this mechanism would be:

- a. The concentration of auxin in the upper parts of the seedling should be higher resp. lower when the rate of the water transport in apical direction is higher resp. lower. The reverse should be the case for the lower parts of the plant.
- b. It follows from a. that the growth rate of the upper parts of the plant should be increased by an increase of water transport in apical direction; the lower parts should show a decreased growth rate. The reverse should be caused by a decrease of the rate of the water transport.
- c. This mechanism should be independent from the cause of the change in the rate of the water transport.
- d. When the phototropic curvature is effected through a lateral migration of auxin (as has recently been shown again by BRIGGS and co-workers) the location of the curve should shift in the same sense as the geotropic curvature for the same reason.

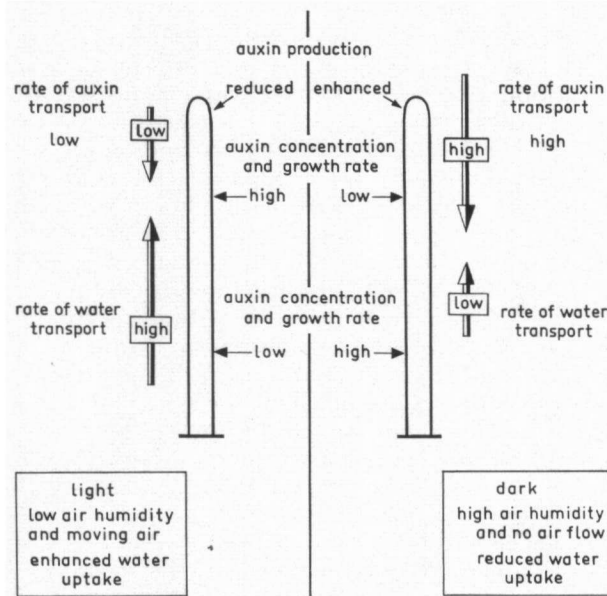


Fig. 24.

The mechanism as described above is shown in schematic form in Fig. 24. In this scheme are included conclusions drawn from literature that will be treated in the following pages.

We will now confront this hypothesis, that for convenience sake will be called the "transport hypothesis", with some data from the literature. We will, however, not mention all publications that could have a bearing on the present communication. We will only try to

connect those that we thought most important, with the work at hand.

EVERY, BURKHOLDER and CREIGHTON (1937) investigated the influence of light on the development of *Avena* seedlings. They cultivated the plants either in darkness or in continuous light. They found that both enlargement of the cells and the number of the cells was affected by the irradiation. The length of the cells was increased in the coleoptiles and decreased in the mesocotyls. In both coleoptile and mesocotyl the cell number was decreased by irradiation. The decrease in cell number in the mesocotyl was larger than in the coleoptile, which indicates that light reduces the amount of a (hypothetical) substance influencing cell division, but that most of the reduced amount remained in the coleoptile. This could happen through the same mechanism as described above for auxin.

MER (1953) investigated the influence of an air flow and of the air humidity on the growth of the coleoptile and of the mesocotyl of *Avena*. From his experimental data can be seen that conditions favourable for evaporation i.e. low humidity and, more important, a high rate of air flow, reduce the mesocotyl length. The same conditions show a tendency to enhance slightly the growth of the coleoptile, the latter effect, however, being less than that on mesocotyls.

MER and RICHARDS (1950) and MER (1959) investigating the influence of 5% carbon dioxide in the air surrounding seedlings of *Avena*, found a reduction of the growth of the coleoptile and an enhancement of that of the mesocotyl. Mer tried to explain this influence of carbon dioxide by correlating it with a reduction of the uptake of nutrients from the endosperm, caused by carbon dioxide enrichment of the atmosphere (MER 1957).

To check this hypothesis, he placed the root systems of plants in solutions of 2% glucose, sucrose or mannitol and observed against expectation, again a reduction of the growth of the coleoptile and an enhancement of that of the mesocotyl. Inhibition of the growth of the mesocotyl by a heat treatment did not change this effect. The use of mannitol as a metabolically almost inactive substrate was aimed to check possible osmotic influences of the sugar concentration used. With mannitol he obtained the same results as with glucose and sucrose and concludes that mannitol is metabolised with the same ease as sucrose and glucose.

MER (1958 and 1959b) added to the root system of seedlings of *Avena* among other compounds ethylalcohol in a concentration of 0.2%. This caused changes in the growth rate of the coleoptile and of the mesocotyl in the same way as those produced by carbon dioxide. He tried to connect this influence with that of carbon dioxide by tentatively assuming that alcohol might increase the carbon dioxide production in the cells. He could not give a satisfactory explanation of the effect of carbon dioxide on both coleoptile and mesocotyl. The transport hypothesis might account for these results. The influence of sugars is to be found in their osmotic activity, as is indicated by the results with mannitol, i.e. a decrease of water uptake and

transport in apical direction, which causes an increase of the auxin supply to the mesocotyl and a decrease of that of auxin useful to the coleoptile. Enrichment of the atmosphere with 5% carbon dioxide, and alcohol in the root medium could conceivably act on the transport system of the plants, either through a reduction of the capacity of the roots to take up water or directly on the transport system in the mesocotyl and the coleoptile.

The influence of environmental factors favourable for transpiration can also be explained with our transport hypothesis, because an enhanced transpiration causes an increase of the water transport in apical direction.

MER (1959a) describes a number of experiments done to investigate the influence of high temperatures on the reduction of the growth of the mesocotyl. The effect of high temperatures (40° C during 3 hours after two days' normal growth) is for the major part the same as the influence of red light: the growth of the coleoptile is enhanced and that of the mesocotyl is reduced.

Departing from the "transport hypothesis" we tentatively state that the effect of high temperature in the experiments of Mer could at least partly be caused by a transitory lowering of relative air humidity by the increase of the temperature.

The trend that in general the effect of a change in the rate of the auxin transport is greater on the growth of the mesocotyl than on that of the coleoptile, may partly be explained by the somewhat greater reactivity of the growth of the mesocotyl on changes in the auxin concentration than that of the coleoptile (NITTSCH and NITTSCH 1956).

We will now try to connect the "section growth system" and the "transport hypothesis" with some results of research on phototropism. To make this connection, it would be easiest to start from a hypothesis of phototropism that is based principally on changes in auxin concentrations in the plant. Such a hypothesis is given by BLAAUW-JANSEN (1959).

She had found that the auxin concentration/growth curve shows a peak at very low auxin concentrations. She published a graph with two tentative auxin concentration/growth curves, one for plants with and one for plants without pre-irradiation with red light. This graph is reproduced in Fig. 25.

To explain the occurrence of the different phototropic curvatures BLAAUW-JANSEN (1959 p. 28) reasons as follows: "Let it be assumed, with OPPENOORTH (1941), that the auxin content of the coleoptile decreases with increasing quantities of blue light. As the irradiated side of the coleoptile receives larger amounts of blue light than the shade side, the illuminated side will contain less auxin than the shade side. Now with increasing amounts of unilateral light energy the I.A.A. content of the light side as well as that of the shade side can be imagined to move along the graph of Fig. ... (our Fig. 25) to the left, that of the light side preceding that of the shade side. At the outset (at low energy, i.e. at a high but decreasing auxin content of the coleoptile)

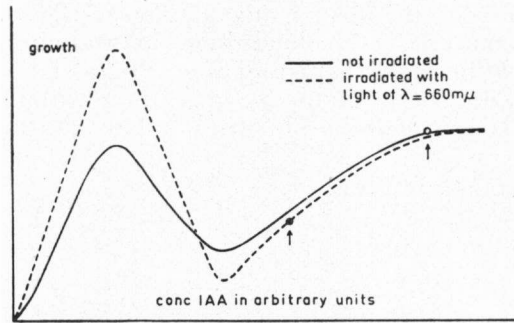


Fig. 25. Tentative course of the growth rate curve of intact coleoptiles at different I.A.A. concentrations. Circle: I.A.A. level of non-irradiated plants; dot: I.A.A. level of plants irradiated with red light. (From BLAAUW-JANSEN 1959).

the growth of the light side may be less than the growth of the shade side (first positive curvature). When mounting the slope towards the first peak from the right side of the graph, however, the growth rate of the light side may surpass the growth rate of the shade side (negative curvature) whereas, when the first peak has been passed, the tables are once more turned (second positive curvature)."

It is, however, not necessary to accept all features of this hypothesis, to show the connection between the "transport system", the "section growth system" and phototropism. The explanation given by Blaauw-Jansen for the first negative curvatures, especially in plants that have not been pre-irradiated with red light, is based on very slight experimental evidence (Fig. 26). BLAAUW-JANSEN (1962) investigating the influence of orange light on the growth of coleoptile sections, was not able to find a peak in the concentration growth curves of the dark controls. In all her experiments the values for sections without added auxin were higher than the values found at the lowest concentrations used (I.A.A. concentrations  $10^{-9}$  and  $10^{-10}$ ). The peaks she found in these experiments with plants that had been pre-irradiated with orange light were, however, much less pronounced than in the experiments of 1959. This might indicate that the section test used in the experiments of 1962 was less sensitive for the detection of this peak.

We will use the hypothesis given by Blaauw-Jansen as a base for showing a connection between the two light systems described in this paper, and phototropism.

It was shown that if tips of *Avena* coleoptiles were extracted (BLAAUW-JANSEN 1959) or tips of corn (BRIGGS 1963) or *Avena* (VAN OVERBEEK 1937) were used to produce diffusates, the amount of auxin found was decreased by irradiating the plants with red light before the tips were cut. Briggs also analyzed separately the auxin diffused from the illuminated and from the shadow halves of tips of corn coleoptiles which had been irradiated unilaterally with "white" light. The dose of this "white" light was chosen so that it lay at the transition

of the first positive to the first negative curvature. This light quantity therefore, does not produce a phototropic curvature in plants kept in the dark before the unilateral irradiation. Plants, receiving an irradiation with red light prior to the irradiation with "white" light, reacted on this light quantity of "white" light with a positive cur-

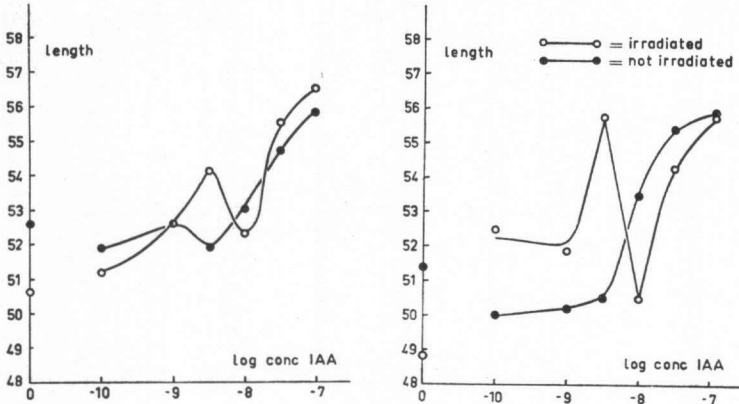


Fig. 26. Final length reached in I.A.A. solutions by coleoptile sections cut from non-irradiated seedlings and from seedlings irradiated with light of  $\lambda = 660$  nm. (From BLAAUW-JANSEN (1959)).

vature. In tips from plants, pre-irradiated with red light before being cut, as well as the corresponding dark controls, more auxin was found in the halves of the shadow side than in the irradiated halves. The total amount of auxin in the plants irradiated with red light, however, was, as in preceding experiments, lower than in the dark controls. The amount of auxin laterally transported in both groups, was the same.

Nevertheless, the plants pre-irradiated with red light showed a positive phototropic curvature, whereas in the dark controls no curves developed. Briggs states that it is paradoxical that, though the unilateral irradiation of the dark controls produced a difference in the auxin concentration between the two halves similar to that in the red pre-irradiated plants, yet no similar curvature occurred. At most, Briggs states, one might expect in the dark controls a somewhat reduced curve, if in this case the auxin concentration lies beyond that range of concentrations in which there is a linear proportionality between the auxin concentration and the response to auxin. He bases this statement on the supposition that the auxin concentration in the growing zone of the coleoptile, in which the curvature arises would be higher in the dark controls than in the red pre-irradiated plants.

In 1963 ZIMMERMAN and BRIGGS published a number of graphs showing the light dosage response curves of phototropism for oat seedlings with and without pre-irradiation with red light. The photo-



tropic stimulations were given with blue light at three different intensities. These graphs are reproduced in Fig. 27.

From the published graphs we will first compare c and f. The authors note that of the phototropic curvatures the first positive and the first negative curvatures have lost sensitivity to blue light by the pre-irradiation with red light. The second positive curvature appears to have an increased sensitivity.

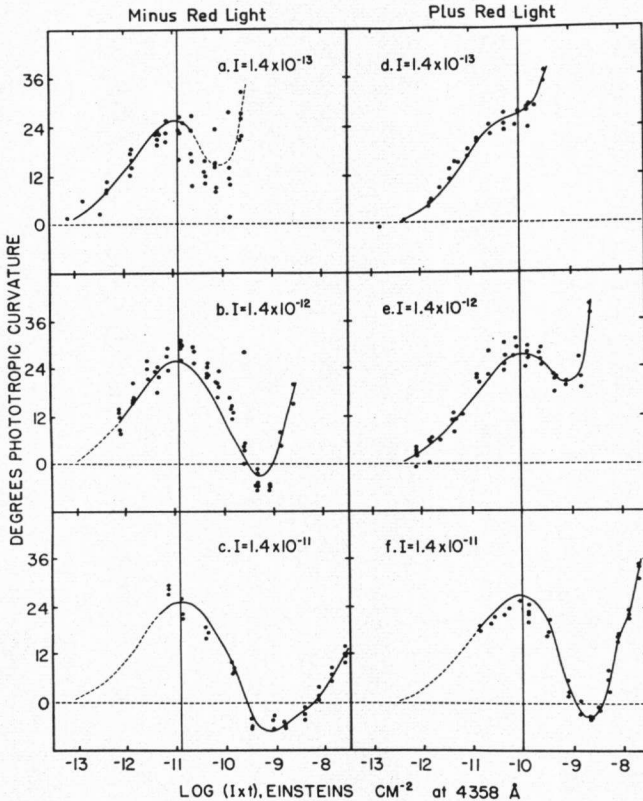


Fig. 27. Phototropic dosage response curves for oat coleoptiles at three intensities of monochromatic light. Left-hand curves (a, b, c), no red light pre-treatment. Right-hand curves (d, e, f) two hours red light pre-treatment. (From ZIMMERMAN and BRIGGS 1963a.)

From graph b of ZIMMERMAN and BRIGGS we have reconstructed an auxin concentration/growth curve as indicated by the theory of BLAAUW-JANSEN. The two graphs are shown in Fig. 28 curves b and 1. The situation in which no curvature is found must coincide with the minimum and maximum in curve 1. The maximum and minimum in the phototropic curvatures of curve b must coincide with the ranges of the steepest slopes of curve 1. A comparison of the curves in Fig. 27 for plants that received no pre-irradiation, with the curves

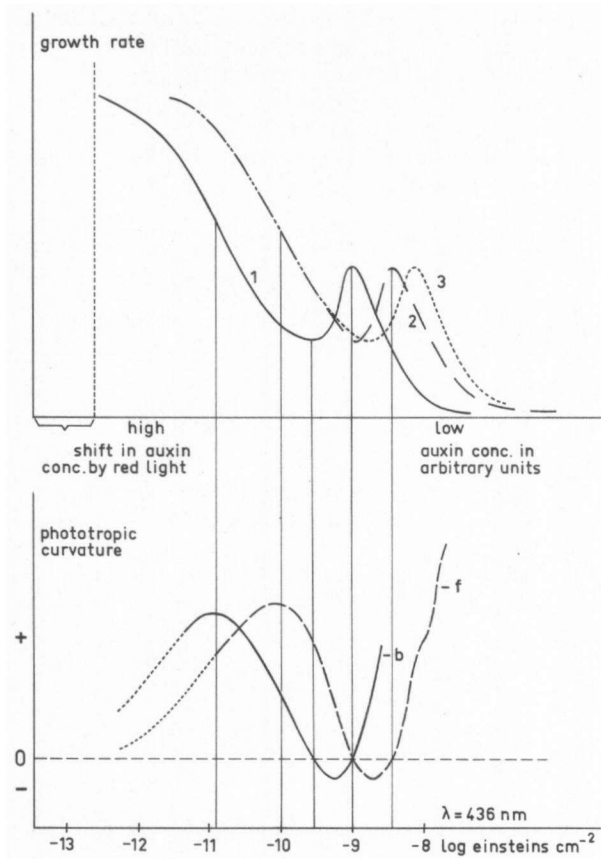


Fig. 28.

for red pre-irradiated plants shows that in all three cases the maximum of the first positive curvature is shifted, by the pre-irradiation with red light, to energies of unilateral light of about 10 times as large as for the dark controls. Translated into terms of curve 1 (Fig. 28): a 10 times larger amount of unilaterally applied light is needed for the pre-irradiated plants to reduce the auxin level to the concentration that is correlated with the maximal positive curvature. According to the "transport hypothesis" red pre-irradiation would increase the auxin concentration, so a larger amount of blue light energy is necessary to reduce the auxin concentration to the same level as before the red pre-irradiation.

From a comparison in the series of curves a, b, c, and e, f, g (Fig. 27), it appears that the intensity, or rather the irradiation time, of the blue light used to produce the phototropic curvatures, has a strong influence on the shape of the curves of Fig. 27.

If we want to compare cases of curvatures from curve b with

similar curvatures in pre-irradiated plants (for instance the maximal first positive curvatures of both) and we want this comparison to be free from a bias due to irradiation time, we must compare curve b with curve f (it is a lucky coincidence that the light energy shift for the maximum of the first positive curvature is about a factor 10). The correctness of this comparison seems to be borne out by the similarity in shape of curves b and f. By the same reasoning curve a should show the greatest similarity to curve e, which again appears to fit in. Curve f has also been drawn in Fig. 28. From curve f an auxin concentration/growth curve has been constructed in the same way as for curve b: this is curve 2. If it is accepted that the maximal first positive curvature in both curve b and f is attained at the same auxin concentration, the abscissa of curve 2 should be shifted to the right over the distance found between the maxima of the first positive curvatures of curves b and f. For comparison curve 1 has been shifted over the same distance (curve 3).

From the considerations put forth above, it follows that the loss of sensitivity of the first positive curvature by pre-irradiation with red light could possibly be related to a change in auxin concentration in the seedlings. The same holds true for the first negative curvature but for a small shift in relation to the maximal first positive curvature, due to the shift of the peak in the concentration/growth curve.

As to the second positive curvature, it has been made plausible by ZIMMERMAN and BRIGGS (1963a and b) that this curvature should be caused by another reaction mechanism than that causing the first positive and first negative curvatures. Its development would be dependent on the length of the irradiation time, rather than on the amount of energy of blue light. The disappearance of the first negative curvature they ascribe to the increase of the second positive curvature by longer irradiation times simultaneous with the development of the first negative curvature.

Returning to the paradoxical results found by BRIGGS (1963): these can be explained quite well with the proposed mechanism. BRIGGS used a phototropic stimulation that induced a positive phototropic curvature in plants that had been pre-irradiated with red light. In plants without pre-irradiation no curvatures were observed. Departing from the "transport hypothesis" the concentration of auxin in the growing zone is higher in plants treated with red light. In the not pre-irradiated plants the concentration of auxins in the growing zone coincides with the minimum in the concentration/growth curve and so no curvature arises. In the red pre-irradiated plants the concentration in the growing zone is higher and so it is in the concentration range of the first positive curvature.

BLAAUW and BLAAUW-JANSEN (1964) published a number of dosage-response curves for phototropism of the *Avena* coleoptile and of plants with and without pre-irradiation with red light. Their results are in some respects at variance with those of Zimmerman and Briggs: a) The curvatures of pre-irradiated and not pre-irradiated plants do not show a large difference in energy requirement as they

do in the curves of Zimmerman and Briggs. b) The maximum for the first positive curvatures of the red pre-irradiated plants shows a shift to higher energies and also the maximal values are larger than those of the maximal values of the dark controls. c) In both the pre-irradiated and the control plants the transition from first positive to first negative curvature is attained at the same energy of blue light. d) The maximal first negative curvature in pre-irradiated plants is larger than the corresponding maximal value in the control plants.

The increase of the response found in the red pre-irradiated plants as compared with the controls might be attributed to the "section growth system". It would seem probable that the plants of Zimmerman and Briggs had received enough light energy, before the start of the experiments proper, to saturate this system so that no difference between control plants and pre-irradiated plants could result. The "transport system" would, however, still react to a larger amount of energy causing the shift of the response curve to higher energies. If we may assume that the increase of response by the "section growth system" is proportionally the same for all curvatures obtained, we could compute from the curvatures of the dark controls, in the curves given by Blaauw and Blaauw-Jansen, the corresponding values as if only the "section growth system" would have had influence on the curvatures. The curves then obtained will show approximately the same relation to the curve of the red pre-irradiated plants as was found by Zimmerman and Briggs. From the curves given by Blaauw and Blaauw-Jansen it would appear, that a larger shift of the peak in the corresponding concentration growth curve has been effected by the pre-irradiation with red light, than that observed in the curves given by Zimmerman and Briggs. Again, this may indicate that the plants of Zimmerman and Briggs had received a certain amount of light energy prior to the experiments.

An investigation on the influence of red light on the shape of the bend in phototropically curving plants has been done by BLAAUW (to be published). He found, indeed, an influence of red light on the shape of the curves in phototropic curvatures.

Concluding, we may state that it seems probable that in phototropism both the "section growth system" and the "transport system" are influencing the response of the seedlings.

From VAN OVERBEEK (1936), BLAAUW-JANSEN (1959) and ZIMMERMAN and BRIGGS (1963) it is clear that irradiation of seedlings with red light causes a decrease in the amount of auxin found in the tip of the coleoptile, either by extraction (BLAAUW-JANSEN, *Avena*) or by diffusion (ZIMMERMAN and BRIGGS, corn, VAN OVERBEEK, *Avena*). This reduction of the amount of auxin has been regarded up to now as a primary effect of red light on the seedling. We want to put forward tentatively that this decrease could be a consequence of a slower basipetal transport caused by red light and resulting in an increase of the auxin concentration in the growing region by red light. This increase of the auxin concentration in the region below

the tip might affect the equilibrium between the production of auxin and its concentration gradient in lower tissues and so reduce its production. This view finds some support in an experiment of Zimmerman and Briggs who could not find an influence of red light on the auxin production of coleoptile tips of corn seedlings if the irradiation with red light was given after the tips had been cut from the plants.

BALL (1962) immersed *Avena* seedlings intermittently in a solution of  $10^{-4}$  M I.A.A. over periods of one hour or three hours. He measured the growth of the seedlings. From his graphs can be seen that the increase of auxin concentration temporarily caused an increase of growth which thereafter decreased till below the control values. This indicates an inhibition of the auxin production caused by the externally applied auxin. The concentration of I.A.A. was, however, so high that the possibility is not excluded that the effect observed is caused by injury.

This view might be checked by estimation of the auxin content of tips of plants that have been grown under different conditions as to air humidity and air flow, because a high transpiration rate, according to the "transport hypothesis", should have the same influence as red light.

In her experiments BLAAUW-JANSEN (1959) used tips one centimeter long. In these tips part of the growing zone was included. The fact that yet she found a decrease in the amount of auxin extracted, can not be explained without an analysis of the relative amounts of auxin present in different zones of the coleoptile.

Another explanation might be that the amount of auxin either found by extraction or with diffusion methods is not directly correlated with the amount of auxin actually acting in the plants. More experimental data are required to choose between the two explanations mentioned above.

To avoid a confusion of the issues, we have thus far intentionally not included the possibility of an influence of light on the lateral transport in the discussion on the "transport hypothesis". From the results of the experiments on the effect of light on the guttation, we concluded that light might affect the permeability of the protoplasmic membranes. If this holds true, this change of permeability may have a similar effect on the transport in transversal as well as in longitudinal direction. An enhancement of lateral transport by red light would strengthen the effect of the influence of light via the acropetal transport of water on the place of the curvature in the plants. Differences in time needed for each of the processes to cause an observable effect could possibly explain some observations that are not accounted for in the "transport hypothesis". One of these instances is the observation by BLAAUW (1963) that red light seems to have more effect on the topmost centimeter of the coleoptiles than on more basal regions. This may be caused by an enhancement of the transversal transport of auxin by red light, which might need less time to take effect, than the influence on auxin migration via the acropetal

water transport. Consequently, there would be an earlier and more pronounced effect in the region below the tip than in more basal parts of the seedling.

Another phenomenon studied by BLAAUW (1961) is the influence of the time between the irradiation of seedlings and the moment they are placed in a horizontal position to curve geotropically. Here again, different processes seem to show their influence. They might be:

- a. Influence of light via the rate of water transport
- b. Influence of light on the rate of lateral distribution
- c. Influence of light (especially blue light) on the auxin concentration.

Different times for these processes to take effect could possibly explain the effects observed. Without a further analysis of the times involved, however, speculations seem inopportune because with three variable time effects unknown in magnitude it is possible to conjecture almost any time/effect curve.

Although the experimental arguments are scanty and there are many confusing data that obscure a clear view on the mechanisms involved in geotropical and phototropical reactions, the views presented in this discussion could be useful as a basis for future research.

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