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THE INFLUENCE OF BLUE, RED AND FAR RED LIGHT ON GEOTROPISM AND GROWTH OF THE AVENA COLEOPTILE

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CONTENTS

ABSTRACT

The influence of short lasting pre-irradiations from above with monochromatic light on the geotropic reaction of the *Avena* seedling is dependent on wave length and quantity of the radiation used, on the interval of time inserted between pre-irradiation and unilateral exposure to gravity (termed "waiting time"), and on the length of the period during which the plants are kept in the horizontal position (termed "exposure time").

With red or far red pre-irradiation merely an increase of the geotropic curvature is observed, but only when a "waiting time" of 30 minutes is inserted and the "exposure time" is about 60 minutes.

With blue (or violet) light is observed a decrease in curvature with 1000–10,000 erg cm^{-2}, a "waiting time" of 30 minutes and an "exposure time" of c. 60 minutes, an increase with 100,000 or more ergs cm^{-2}, a "waiting time" of 0 minutes or of 75 minutes and an "exposure time" of c. 60 minutes.

Any preirradiation influences the growth rate of the coleoptile in some way; this applies to plants in the horizontal as well as in the vertical position.

Correlations between growth and geotropic reaction are different in different cases. Correlation between phototropic and geotropic behaviour could not be demonstrated.

No red - far red antagonism could be detected.

CHAPTER I

INTRODUCTION

About fifty years ago A. H. Blaauw (1914, 1915) reduced the problem of the phototropic reaction to the problem of the light-growth response after illumination from all sides. The phototropism occurring after one-sided illumination would be caused by the difference between the light-growth responses of the lighted and the shadow side of the coleoptile. In spite of this early lead the connection between the amount of light energy applied, and the resulting light-growth reaction and phototropic curvature still is not clear. Elucidation of the problem was hampered by the following complications:

a) Different light qualities influence growth and phototropic reaction in different ways.

b) The growth of the mesocotyl is influenced by irradiation with light of any wave length but not in the same way as that of the coleoptile. Therefore it seems opportune to look for connections between phototropic reaction and light-growth reaction of the coleoptile rather than of the whole seedling.

c) Presumably one-sided irradiation has two different effects on *Avena* coleoptiles: the phototropic effect proper and an effect on the phototonus.[1]) These two effects cannot clearly be distinguished when studying phototropism.

Several workers took into account the above mentioned complications. Koningsberger (1922) investigated the influence of a number of wave lengths on the growth of *Avena* seedlings. He measured, however, the growth of the whole plant and therefore supplied no data that could help to solve the problem of the phototropic reaction. Du Buy and Nuernbergk (1929, 1930) measured the growth of the convex and the concave side of the coleoptile, but used white light.

The complication mentioned in c) is distinguished clearly by Arisz (1915). However, he studied this problem using white light. The same

[1]) The term "tonus" is used in this paper as an equivalent of the german "Stimmung". When after a pre-treatment, e. g. by an all-sided preillumination which does not or even cannot (as is the case with red light) cause a phototropic reaction, the phototropic effect of a subsequent unilateral irradiation is altered, this is due to a change of the phototonus. *Mutatis mutandis* the term geotonus is used in the same sense for the geotropic reaction.

was done by BEYER (1927, 1928), PISEK (1928), FILZER (1928) and BREMEKAMP (1915). FRANCK (1951) tried to separate the tropistic from the phototonic effect by allowing coleoptiles irradiated from all sides to curve geotropically instead of phototropically. He also, however, used white light. BLAAUW-JANSEN (1959) investigated the effect of a number of wave lengths on growth and curvation of the coleoptile only, but she irradiated only one side. For that reason, the two effects mentioned in c) always became intermingled.

The present author intends:

1) to work with light "as monochromatic as possible",
2) to irradiate from directly above,
3) to allow the irradiated coleoptiles to curve geotropically,
4) to measure the growth of the curving zone of the coleoptile,
5) to measure the growth of the part of the seedling underneath the curving zone.

The geotropic reaction and the growth thus are used as a measure of the tonic effect of the all-sided illumination.

Experiments, performed according to these principles, may deepen the insight in the mechanism of the phototropic as well as of the geotropic reaction.

CHAPTER II

MATERIAL AND GENERAL METHODS

PLANTS

Husked seeds of *Avena sativa* (cultivar "Siegeshafer") were wetted by shaking them in tap water and put to germinate on moistened filter paper in a closed petri dish in a dark room which was kept at a temperature of 22° C and a relative humidity of about 80 %. In order to suppress mesocotyl growth, the germinating seeds were exposed to orange light for 19 to 20 hours. After this period the seeds were planted in zinc trays filled with moist vermiculite with the embryo pointing vertically downwards and kept in absolute darkness in the dark room mentioned before. At the age of 84 to 85 hours the plants were used for the experiments. In each tray a row of 18 seeds was planted. A short time before the irradiation, all plants that were not straight, or that seemed too tall or too small were discarded. This operation was feasible when working under green light of an intensity of less than 40 erg cm^{-2} sec^{-1}.

LIGHT

The sources of the orange light were incandescent lamps filtered through *Schott OG2* filters.

Monochromatic light was obtained by focussing the light of an incandescent lamp (60 Volt, 3000 Watt) using a condensor lens and filtering the beam through an interference filter made by Balzer

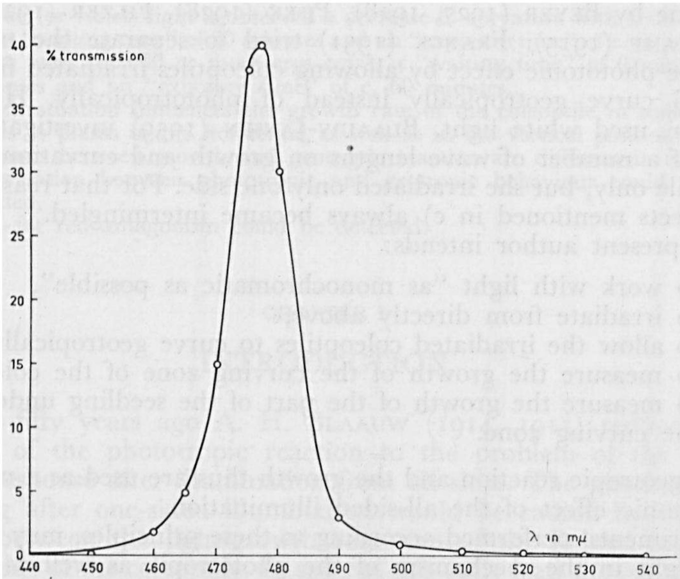


Fig. 1.

(Liechtenstein) of which the tolerance was 0.5 %. A transmission spectrum of one of the interference filters used is shown in Fig. 1.

A mirror reflected the monochromatic light from the filter vertically on top of the plants to be irradiated. As no lens was used to project an image of the condensor lens aperture in the plane of the object, the material was irradiated with a diffused, out of focus image of the light source.

Some of the irregular brightness details of the filament structure of the source were reproduced and the intensity distribution in the irradiated plane was not quite uniform. However, variations never exceeded 15 % and a dependence of the reactions of the irradiated plants on their position in the irradiated plane was never observed. In the light beam a big "*Compur*" shutter was placed.

Whenever the plants were shadowgraphed during the experiments, this was performed with the aid of green light (130 erg cm^{-2} sec^{-1}), produced by filtering a beam of parallel light through an interference filter ($\lambda = 560$ mμ).

The light intensity was measured by means of a thermopile after Moll (Kipp, Delft) and a calibrated Zernike galvanometer (Kipp, Delft).

A summary of the wave lengths and light intensities that were used is given in table 1.

The development and the measuring of curvatures

After irradiation of the coleoptiles with blue, red or far red light the plants were put from the vertical into the horizontal position in such a way that geotropic curvatures would develop in the plane of the

TABLE 1

LIGHT		LIGHT SOURCE		Filter	Light intensity in erg $cm^{-2}sec^{-1}$
Colour	Wavelength in mμ	Watt	Volt		
orange . . .	from 500 upwards	40	220	abs. f.	800
violet . . .	415	3000	60	int. f.	300
blue	479	3000	60	int. f.	900
green . . .	560	100	12	int. f.	40 or 130
red	660	3000	60	int. f.	1400
far red . . .	735	3000	60	int. f.	1850

two vascular bundles. Shadowgraphs were made immediately after the coleoptiles had been placed horizontal and after they had been in this position for 60 minutes. The shadowgraphs were made on Gevaert Document Rapid photographic paper by means of green monochromatic light of an intensity of 130 erg cm^{-2} sec^{-1}. On the shadowgraphs made before any geotropical curvature had developed, the straight and horizontal coleoptiles were selected. Only the curvatures of these selected plants were considered. The curvatures were measured by means of a protractor. Standard errors of the mean, for some experiments, chosen at random calculated by means of the formula

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

where n = the number of coleoptiles,
$\sum (x - \bar{x})^2$ = the sum of the squared deviations from the mean, seldom amounted to values higher than $\pm$ 2° as shown in table 2.

TABLE 2

Number of coleoptiles	Pre-irradiation	"Waiting time"	Curvature in degrees ("Exposure time" = 60 min)	Standard error of the mean in degrees
12	9 min 479 mμ	10 min	28	1.3
9	id.	id.	26	3.7
11	id.	id.	28.5	2.1
11	1 min 479 mμ	30 min	12.5	1.8
12	id.	id.	11.5	1.7
13	id.	id.	14	1.1
13	none	—	18.5	1.1
13	id.	—	16	1.6
11	id.	—	16	1.5
9	id.	—	17	1.9
9	id.	—	17	2.6
7	id.	—	20	1.8
11	id.	—	18	1.6
13	4 sec 660 mμ	0 min	26	1.9
12	30 sec 660 mμ	0 min	32	1.6

*) E.g.: "Exposure period" 30–90 min indicates that the plants have been in the horizontal position from the 30th to the 90th minute after the beginning of the irradiation.

MEASURING OF STRAIGHT GROWTH

In the zinc trays with vermiculite, in which the seedlings had been cultivated, a hair was stretched along the row of plants approximately ½ cm over the surface of the vermiculite. In addition, as a mark, a particle of vermiculite was fixed on the coleoptile at a distance of about 1 cm from the top by means of a trace of lanoline.

After the coleoptiles had been irradiated with blue, red or far red light, shadowgraphs were made every 15 minutes.

On the shadowgraphs the increase in length of the seedlings was measured from hair to mark and from hair to top, to the nearest 0.1 mm. The increase from hair to mark is the increase in the lower part of the seedling, mainly in the mesocotyl. This increase is referred to as "growth of mesocotyl". The growth of the uppermost 1 cm of the coleoptile, in which region the geotropic curvature develops within 60 minutes, is obtained by subtracting the two values measured. This growth is referred to as "growth of coleoptile". Table 3 gives an impression of the standard-errors of the mean in this type of experiment.

TABLE 3

Number of coleoptiles	Pre-irradiation	Growth in mm after three hours	
		of mesocotyl	of coleoptile
12	6 sec 660 mμ	1.4 ± 0.11	2.8 ± 0.19
10	6 sec 660 mμ	1.6 ± 0.09	2.3 ± 0.14
10	4 sec 479 mμ	2.4 ± 0.17	1.2 ± 0.09
12	4 sec 479 mμ	2.0 ± 0.13	1.2 ± 0.15
11	dark controls	2.5 ± 0.22	1.3 ± 0.21
10	dark controls	2.6 ± 0.25	1.5 ± 0.17

MEASURING OF THE ABSOLUTE GROWTH OF CONCAVE AND CONVEX SIDES OF CURVED COLEOPTILES

Coleoptiles were marked by a grain of vermiculite approximately 1 cm from the top. These were shadowgraphed before and after the development of a geotropic curvature. On the shadowgraphs α, d, CD and AB were measured as is indicated in Fig. 2.

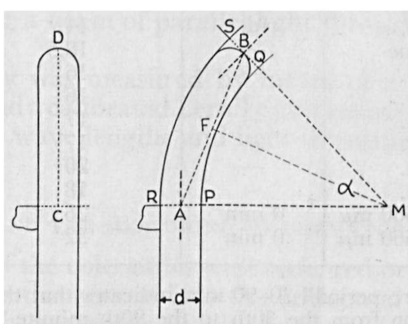


Fig. 2.

Now arc PQ and arc RS can be calculated:

$$\sin \tfrac{1}{2}\alpha = \frac{AE}{AM} \rightarrow AM = \frac{AF}{\sin \tfrac{1}{2}\alpha} = \frac{AB}{2 \sin \tfrac{1}{2}\alpha}$$

$$PM = AM - AP = AM - \tfrac{1}{2}d$$

with $d = 1.6$ mm:

$$PM = AM - 0.8$$

$$PQ = \frac{\alpha}{360} 2\pi PM = \frac{\alpha}{360} 2 \left(\frac{AB}{2 \sin \tfrac{1}{2}\alpha} - 0.8\right) = \frac{\alpha\pi\, AB}{360 \sin \tfrac{1}{2}\alpha} - \frac{1.6\,\pi\alpha}{360}.$$

In analogy:

$$RS = \frac{\alpha\pi\, AB}{360 \sin \tfrac{1}{2}\alpha} + \frac{1.6\,\pi\alpha}{360}.$$

Growth of the convex side = RS — DC.
Growth of the concave side = PQ — DC.

In table 4 the increases in length of convex and concave sides of some sets of coleoptiles, with the standard errors of the mean are shown.

TABLE 4

Number of coleoptiles	Pre-irradiation	Growth in mm after one hour of		Curvature in degrees ("Exposure time" = 60 min)
		concave side	convex side	
12	7000 erg cm^{-2} 660 mμ	0.04 ± 0.03	0.73 ± 0.05	24
9	2700 erg cm^{-2} 479 mμ	0.51 ± 0.05	0.68 ± 0.06	6
10	none	0.10 ± 0.02	0.37 ± 0.02	10

CHAPTER III

INITIAL EXPERIMENTS

a) The necessity of illuminating the seedlings with orange light during the germination period in order to suppress the growth of the mesocotyl and to obtain straight plants is a confusing factor when studying the influence of red and far red light on the geotropic reactions of the coleoptile.

Therefore, the influence of the orange irradiation during the germination on the sensitivity of the coleoptile for red light was examined in experiments 1 and 2.

EXP. 1

The seeds germinated under an orange lamp. They were planted in vermiculite 17, 18, 19, 20, 22 and 23½ hours resp. after having been put on the filter paper. Consequently, the irradiation period also varied from 17 to 23½ hours.

When the seedlings were 84 hours old part of them was irradiated with red light (2800 erg cm^{-2}), the rest was kept in darkness. Thereupon they were placed horizontal and after 75 minutes the curvatures were compared.

At a rough estimate red light enhanced the geotropic curvature of those seedlings that had been pre-illuminated for 19, 20 and 22 hours.

EXP. 2

The seeds were divided into two groups that were pre-illuminated for 16¼ and 19 hours. Of each group two batches of plants were irradiated with red light, two with far red light and four were kept in darkness.

Subsequently all plants were put horizontal and after 75 minutes their curvatures were measured.

The following curvatures had developed (table 5).

TABLE 5
Curvatures developed in 75 minutes

Irridiated with	Pre-illuminated for	
	16¼ hours	19 hours
2700 erg cm^{-2} 660 mμ .	24°	23°
12000 erg cm^{-2} 735 mμ .	25°	28°
dark control	20°.5	20°.5
dark control	20°.5	20°

On account of these experiments it was decided to pre-illuminate the seeds with orange light during a 19 hours germination period.

b) It was thought necessary to find the optimal time interval between the illumination and the beginning of the "exposure time" [1]) to unilateral gravity. Therefore batches of approximately 10 plants

TABLE 6

"Waiting time" in min	Curvature in degrees after irradiation with		Curvature of dark controls in degrees ("Exposure time" = 75 min)
	2800 erg cm^{-2}, 660 mμ	12000 erg cm^{-2}, 735 mμ	
0	31	29	29
30	38	34	26
60	26	26	27
120	29	28	28

[1]) In this paper with "exposure time" is meant: the period during which the plants were kept in the horizontal position.

were irradiated either with 2800 erg cm^{-2} of red light or with 12000 erg cm^{-2} of far red light. Controls were kept in darkness.

The plants were placed horizontal 0, ½, 1 or 2 hours after the irradiation. 75 minutes after that, shadowgraphs were made. Results are shown in table 6.

Consequently for the experiments to come a "waiting time" [1] of 30 minutes was chosen to start with.

c) An experiment was performed to determine the optimal "exposure time". Red light enhances the geotropic reaction. During the first half hour in which the plants are in the horizontal position no curvature can be observed. After a few hours illuminated as well as non-illuminated plants show curvatures of more than 60°.

The greatest percentual difference in rate of curvation between illuminated and non-illuminated plants can be observed after 60 to 70 minutes.

TABLE 7

A			B		
Quantity of light of 660 mμ in erg cm^{-2}	Curvature in degrees ("Exposure time" = 60 min)		Quantity of light of 735 mμ in erg cm^{-2}	Curvature in degrees ("Exposure time" = 60 min)	
	exp. 1	exp. 2		exp. 1	exp. 2
4	18	24	12	21	18.5
8	21	22	30	20	22.5
16	22.5	23	60	23	25
dark	21	17	dark	16	21.5
43	26	24	150	22	24
86	23.5	22	300	22	23
200	24	22	600	18	23
dark	18.5	17	dark	16	22
400	25	28	1,200	24	26
850	22.5	26	2,500	27	24
1,700	23	26	5,000	25	24.5
dark	17		dark		21
3,500	28	32	10,000	24	26
7,000	30	30	20,000	27	29
13,000	25.5	30	40,000	25	32.5
dark	21.5	20	dark	18	20
26,000	26	32	80,000	31	31
50,000	27	31	160,000	26	
100,000	29	30			
dark	18	18	dark	20	19.5
200,000		31	600,000	29	
400,000		28	600,000	29	
800,000		33	600,000	29	
dark		19	dark	21	

[1] In this paper with "waiting time" is meant: the interval of time between the beginning of the illumination and the moment at which the plants are placed into the horizontal position.

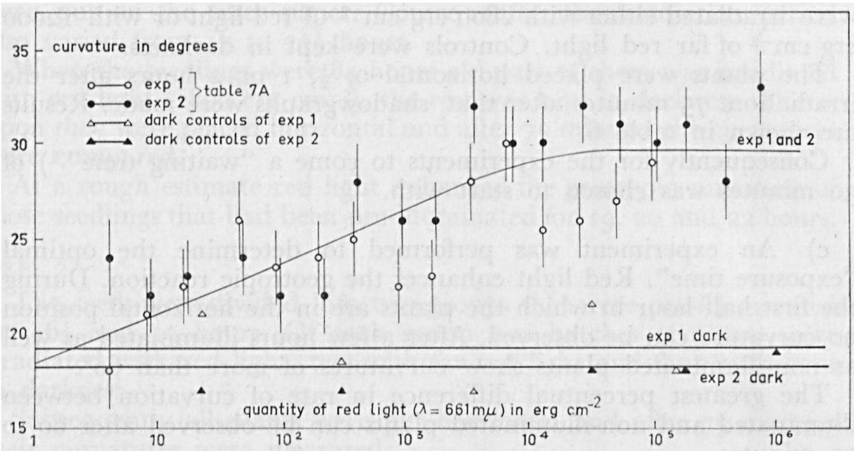


Fig. 3.

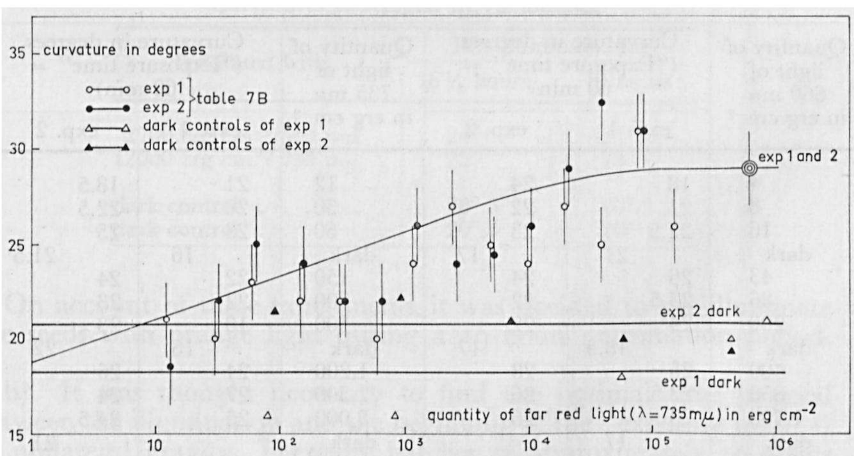


Fig. 4.

This, combined with reasons of convenience, led to the decision to fix the "exposure time" on 60 minutes.

d) In experiments on the influence of red and far red light on the phototropic curvature of the coleoptile (BLAAUW-JANSEN 1959) quantities of 700 erg cm^{-2} of red light and of 6000 erg cm^{-2} of far red light were sufficient to obtain a maximal effect. As it is not beforehand established that the same quantities are required to exert a maximal influence on the geotropic reaction of the coleoptile, a series of trays was irradiated with doses of red and far red light in the range of

4 erg cm^{-2} to 800,000 erg cm^{-2}. This means that the time of irradiation varied from 0.005 sec to 9 min for red, and from 0.005 sec to 5.5 min for far red light. After a "waiting time" of 30 minutes the geotropic curvatures which developed in the next hour were measured (table 7, Fig. 3 and 4). Since the working schemes of these extensive experiments did not allow to administer the irradiations mentioned within a short period of time, it had to be tested whether the quality of the plant material changed during the experiment. For that reason, during the course of an experiment, dark controls which had been horizontal during the same period as the irradiated trays, were measured as a check upon possible aging effects. The values of the dark controls did never indicate any aging effect; it always seemed allowed to represent these values by a straight horizontal line.

Table 7 and Fig. 3 and 4 show that a quantity of about 7000 erg cm^{-2} of red light and about 20,000 erg cm^{-2} of far red light are sufficient to obtain a maximal effect.

Summary of Chapter III

Preliminary experiments are described in which were determined:

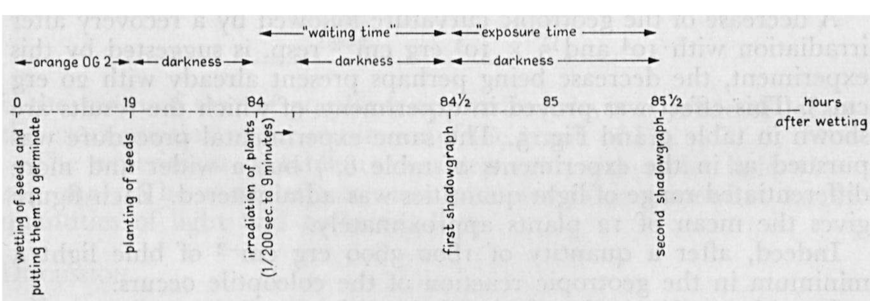
a) The length of the period during which the germinating seeds have to be illuminated with orange light to get a maximal effect on the geotropic reaction (19 hours).

b) The optimal "waiting time" i.e. the interval of time that has to be inserted between the beginning of the pre-irradiation and the beginning of the unilateral exposition to gravity in order to obtain maximal enhancement of the geotropic reaction (30 minutes for red and far red light).

c) The optimal "exposure time", i.e. the period during which the plants should be kept horizontal in order to obtain the greatest difference in the geotropic curvatures between the various treatments (60 minutes).

d) The quantities of red and far red light, sufficient to obtain a maximal effect on the geotropic reaction (7000 and 20,000 erg cm^{-2} resp.).

Working scheme of the experiments from table 7, fig 3 and 4



CHAPTER IV

THE INFLUENCE OF BLUE LIGHT ON THE GEOTROPIC CURVATURE OF THE COLEOPTILE

As a preliminary working hypothesis in this laboratory the phototropic effect of one-sided illumination with blue light is supposed to consist of:

α) a phototonic effect (possibly induced by an all-sided lowering of the indole—3—acetic acid (IAA) level in the coleoptile),
β) the phototropic effect proper (possibly brought about by a destruction of IAA at the irradiated side of the coleoptile or by a shift of the IAA from the irradiated to the shadow side, or by a combination of the two).

The aim of the present author was to separate these two effects (α and β), replacing β by the effect of a geotropic induction and α by irradiation of the coleoptile from right above.

Therefore, coleoptiles were irradiated from above with 20; 10,000 or 50,000 erg cm^{-2} of blue light ($\lambda = 479$ mμ). The experimental working scheme was the same as the one given in the last paragraph of chapter III.

The following geotropic curvatures were observed (table 8):

TABLE 8

Quantity of blue light in erg cm^{-2} ($\lambda = 479$ mμ)	Curvature in degrees ("Exposure time" = 60 min)	
	exp. 1	exp. 2
0 (dark)	17.5	19.0
20	15.7	13.0
10,000	12.0	11.0
50,000	14.5	16.5

A decrease of the geotropic curvature followed by a recovery after irradiation with 10^4 and 5×10^4 erg cm^{-2} resp. is suggested by this experiment, the decrease being perhaps present already with 20 erg cm^{-2}. This effect was proved in experiments of which the results are shown in table 9 and Fig. 5. The same experimental procedure was pursued as in the experiments of table 8,[1]) but a wider and more differentiated range of light quantities was administered. Each figure gives the mean of 12 plants approximately.

Indeed, after a quantity of 1800–3600 erg cm^{-2} of blue light a minimum in the geotropic reaction of the coleoptile occurs.

In table 10 the results of another experiment of the same type are given. This experiment, in which each of the mentioned curvatures

[1]) Working scheme: see p. 407.

TABLE 9

Quantity of blue light in erg cm^{-2} (λ = 479 mμ)	Curvature in degrees ("Exposure time" = 60 min)			
	exp. 1		exp. 2	
4½	25		16	
9	16		19.5	
22	16		11	
dark		25		16
45	20.5		14	
110	11		10	
225	22		11	
dark		27		18
450	10		9	
900	14		8.5	
1,800	5		5.5	
dark		22.5		20
3,600	13		5.5	
7,000	16		13	
14,000	23		17	
dark		22.5		20
27,000	23		20.5	
55,000	21		21.5	
110,000	29		26.5	
dark		25		
220,000			22	
440,000			22	
440,000			24	
dark				19

TABLE 10
"waiting time" = 30 min
"exposure time" = 60 min

Quantity of blue light in erg cm^{-2} (λ = 479 mμ)	Geotropic curvature in degrees
1800	6
2700	2
3600	4
none	22

is the mean of that of 50 plants approximately, was made to locate the exact position of the minimum of the graph of Fig. 5.

The experiment of table 10 was repeated with violet light (λ = 415 mμ). The same effects were obtained, but somewhat larger quantities of light had to be applied.

DISCUSSION

FRANCK (1951), irradiated coleoptiles with white light (150 à 300 lux for 5 or 10 minutes) and placed the plants on the clinostat after they had been stimulated geotropically. He, too, observed that the geotropic curvature was maximally decreased and was sometimes even

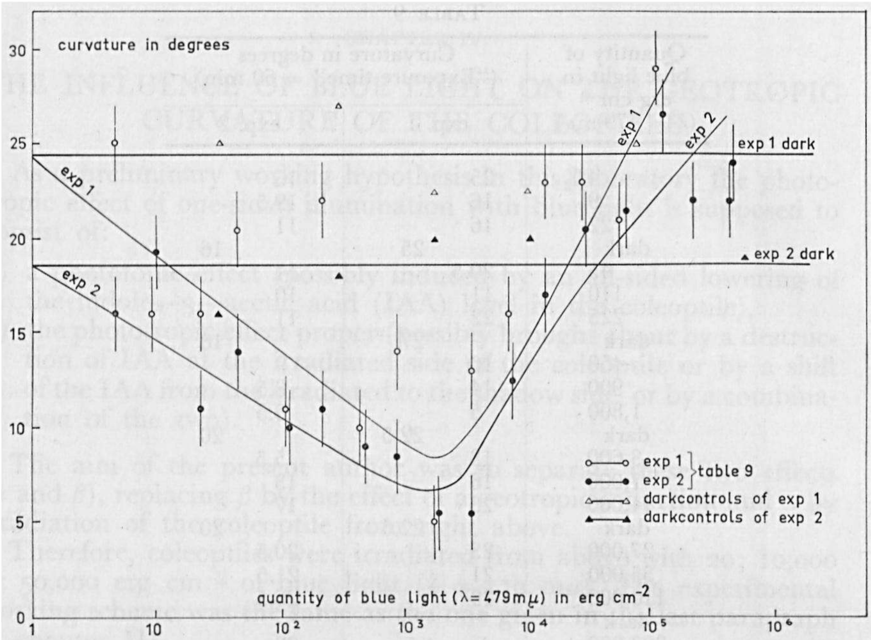


Fig. 5.

negative, when the interval between irradiation and geotropic stimulation amounted to half an hour. The growth of the curving coleoptiles was maximally enhanced under the conditions described.

From experiments yet to be described it will be seen, that these results can possibly be explained by comparing Franck's "white light" with a mixture of blue and red light.

CHAPTER V

COMBINATION OF RED AND BLUE IRRADIATION FROM DIRECTLY ABOVE

The next item to be studied was the influence of combined irradiations with blue and red light on the geotropic reaction of coleoptiles. By doing so it would be possible to determine whether blue and red light affect the geotropic reaction via the same mechanism, the former diminishing, the latter enhancing the geotropic reactions. In that case it should be possible to annihilate the influence of blue light by an irradiation with red or far red light and vice versa. According to BLAAUW-JANSEN (1959), the perception of red light is localized in the primary leaf. This statement can account for the fact that the influence of red light on the coleoptile is not effectuated until 30 minutes have passed, whereas blue light is believed to affect the coleoptile itself and therefore acting immediately.

In chapter XI it will be demonstrated that the statement on the role of the primary leaf is untenable, but this is of no consequence here.

Table 11 shows the working scheme together with the results of an experiment, satisfying the demands stated in the beginning of this chapter.

TABLE 11

1st irradiation	1st lapse (dark) in min	2nd irradiation	2nd lapse (dark) in min	curvature in degrees ("Exposure time" = 60 min)
red (7000 erg cm^{-2})	30	blue (2700 erg cm^{-2})	0	20
none	—	none	—	10
red (7000 erg cm^{-2})	30	none	—	22
none	—	none	—	15.5
red (7000 erg cm^{-2})	30	blue (2700 erg cm^{-2})	0	21
none	—	none	—	12
red (7000 erg cm^{-2})	30	none	—	23.5
none	—	none	—	16
blue (2700 erg cm^{-2})	30	none	—	7
none	—	none	—	16
blue (2700 erg cm^{-2})	0	red (7000 erg cm^{-2})	30	6
none	—	none	—	17
blue (2700 erg cm^{-2})	0	red (7000 erg cm^{-2})	30	5
none	—	none	—	16

From the values given in this table it is clear that the influence of red light is not annihilated by an irradiation with blue light nor vice versa. The experiment of table 11 was completed by the next experiments (tables 12 and 13) in which a red irradiation was immediately followed by an irradiation with blue light.

TABLE 12

1st irradiation	1st lapse (dark) in min	2nd irradiation	2nd lapse (dark) in min	Curvature in degrees ("Exposure time" = 60 min)
red (7000 erg cm^{-2})	30	none	—	26
none	—	none	—	13
red (7000 erg cm^{-2})	30	blue (2700 erg cm^{-2})	0	27
none	—	none	—	15
red (7000 erg cm^{-2})	0	blue (2700 erg cm^{-2})	30	5.5
none	—	none	—	18.5
none	—	blue (2700 erg cm^{-2})	30	4.5
none	—	none	—	18

The results of these three experiments were confusing, as it seemed that

a) a red irradiation immediately followed by a blue one, or vice versa, always produced the same effect as if there had only been an irradiation with blue light.

b) a red or blue irradiation, followed, after 30 minutes, by an

TABLE 13

1st irradiation	1st lapse (dark) in min	2nd irradiation	2nd lapse (dark) in min	Curvature in degrees ("Exposure time" = 60 min)
red (7000 erg cm^{-2})	30	none	—	21.5
none	—	none	—	18
red (7000 erg cm^{-2})	30	blue (2700 erg cm^{-2})	30	10
none	—	none	—	16
red (7000 erg cm^{-2})	0	blue (2700 erg cm^{-2})	30	7
none	—	none	—	16
blue (2700 erg cm^{-2})	30	none	—	4.5
none	—	none	—	20
blue (2700 erg cm^{-2})	30	red (7000 erg cm^{-2})	0	9
none	—	none	—	19
blue (2700 erg cm^{-2})	30	red (7000 erg cm^{-2})	30	13.5
none	—	none	—	17
blue (2700 erg cm^{-2})	0	red (7000 erg cm^{-2})	30	9.5
none	—	none	—	21

irradiation with blue or red light resp. only showed the effect of the first irradiation.

c) when in case b) a second lapse is inserted (i.e. the interval of time between the beginning of the second irradiation and the moment of putting the plants in a horizontal position) always an effect is produced as if there had only been an irradiation with blue light (cf. a).

DISCUSSION

The effect of combined irradiations is incomprehensible at this stage of the investigation. The reader is invited to dismiss the problem till more data will be placed at his disposal.

SUMMARY

("red" means: 7000 erg cm^{-2} of 660 mμ; "blue" means: 2700 erg cm^{-2} of 479 mμ)

a) "red" —lapse of 0 min.—"blue"—lapse of 30 min.
or
"blue"—lapse of 0 min.—"red" —lapse of 30 min.
→ decreased geotropic reaction (the same effect as from "blue" only)

b) "red" —lapse of 30 min.—"blue"—lapse of 0 min. → increased geotropic reaction (the same effect as from "red" only)

"blue"—lapse of 30 min.—"red" —lapse of 0 min. → decreased geotropic re action (the same effect as from "blue" only)

c) "red" —lapse of 30 min.—"blue"—lapse of 30 min.
or
"blue"—lapse of 30 min.—"red" —lapse of 30 min.
→ decreased geotropic reaction (the same effect as from "blue" only, but less pronounced).

CHAPTER VI

FURTHER INVESTIGATION OF THE INTERACTION OF BLUE, RED AND FAR RED LIGHT IN THE GEOTROPIC REACTION

To disentangle the complications risen in Chapter V it was thought necessary to subject the coleoptiles to a *series of quantities* of blue light (as used in the experiments of chapter IV), immediately, or after a lapse of 30 minutes, preceded by an irradiation with red light.

Firstly in chapter V was found
"red"—lapse of 0 min—"blue"—lapse of 30 min → the same effect as that of blue light only, viz. decreased geotropic reaction,
and so it was thought necessary to investigate whether this finding also holds when, instead of 2700 erg cm^{-2} of blue light, other quantities are applied.

Table 14 and Fig. 6 and 7 show working scheme and results of this part of the investigation. (This working scheme will be referred to as "*scheme a*").

It is clear that irradiation with red light enhances the geotropic curvature of the coleoptiles for most quantities of blue light. Thus, under the conditions described, the effects of blue and of red light are additive, at least for the lower quantities of blue light.

TABLE 14

Quantity of blue light in erg cm^{-2}	Curvature in degrees ("exposure time" = 60 min): after irradiation with blue light only		after irradiation with blue light, immediately preceded by 7000 erg cm^{-2} of red light		after irradiation with 7000 erg cm^{-2} of red light only		of dark controls	
	exp. 1	exp. 2	exp. 1	exp. 2	exp. 1	exp. 2	exp. 1	exp. 2
					21	24.5	20	16.5
2	18	17	23	25.5				
9	21	16; 17.5; 15; 17.5	24	24; 23.5; 24; 24.5				
45	14		21					
225	12		10					
900	7		11					
					22	26.5	16	20.5
3,600	9	4.5	13	8				
14,000	9		13					
60,000	19		19					
230,000	20	15; 15.5	17	18.5; 18				
					21	27.5	17	18.5
460,000	18		20					
						22.5	17	19.5
680,000		19; 19.5		18.5; 18.5				

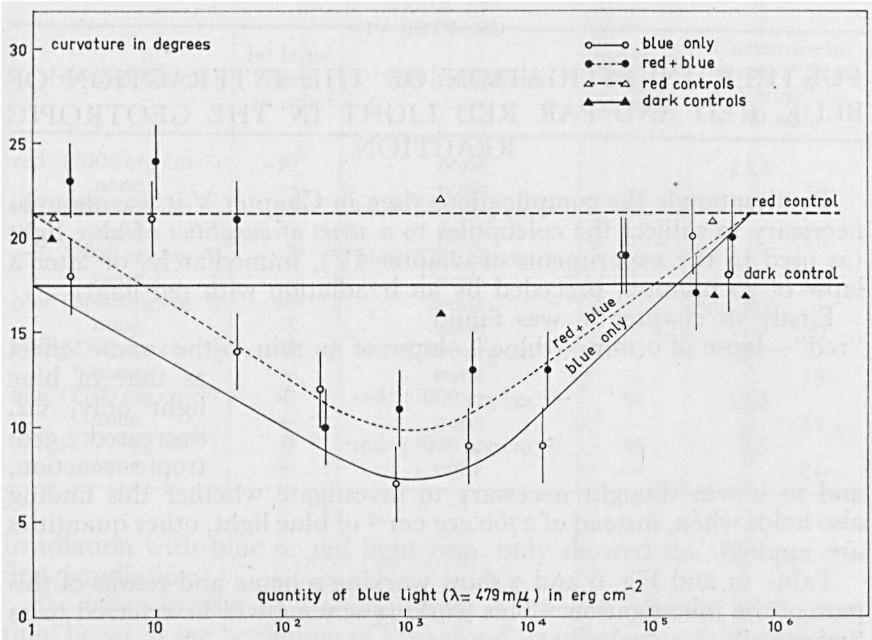

curvature in degrees
blue only
red + blue
red controls
dark controls
red control
dark control
red + blue
blue only
quantity of blue light (λ= 479 mμ) in erg cm⁻²
30
25
20
15
10
5
0
1
10
10 2
10 3
10 4
10 5
10 6

Fig. 6.

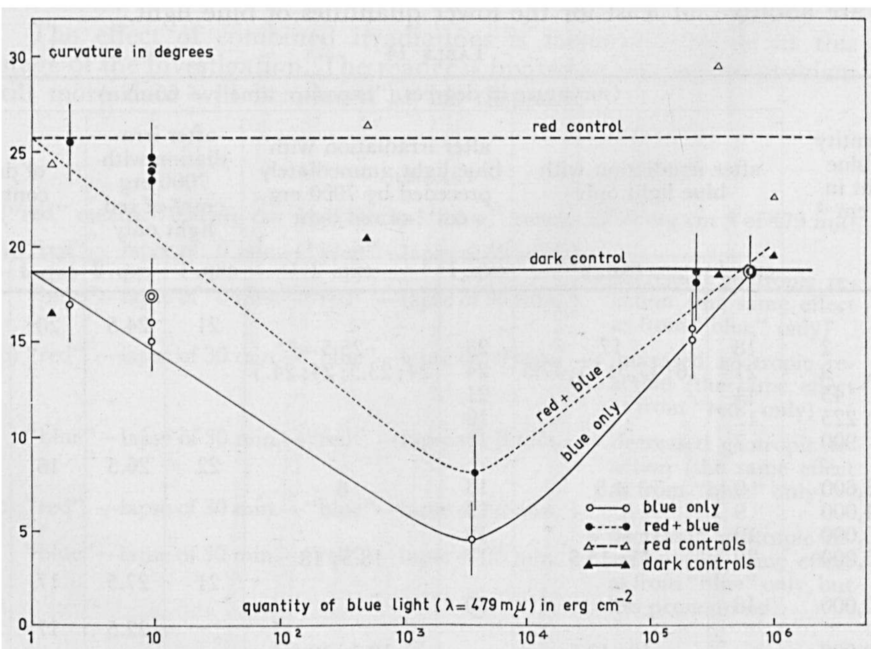

curvature in degrees
red control
dark control
red + blue
blue only
blue only
red + blue
red controls
dark controls
quantity of blue light (λ= 479 mμ) in erg cm⁻²
30
25
20
15
10
5
0
1
10
10 2
10 3
10 4
10 5
10 6

Fig. 7.

Secondly in Chapter V it was stated that
"red"—lapse of 30 min—"blue"—lapse of 0 min → the same effect as that of "red" only, viz. increased geotropic reaction,

and this statement was further examined too.

In this case a difficulty arose, because it is impossible to keep the lapse between red and blue illumination and the lapse between red illumination and the moment of placing the plants horizontal, both equal to 30 minutes, because of the fact that the administration of larger quantities of blue light required up to 15 minutes.

By way of compromise a working scheme was chosen in which the lapse between red irradiation (7000 erg cm^{-2}) and the moment of placing the plants horizontal, was fixed at 50 minutes, and the moment of the beginning of the blue irradiation was chosen in such a way that the plants had to be placed horizontal, immediately after the end of the blue irradiation, so the lapse between red and blue irradiation varied from 50 to 35 minutes.

In this way table 15 and Fig. 8 originated (*scheme b*).

Table 15 and Fig. 8 indicate that neither red + blue nor blue light only had a pronounced effect on the geotropic behaviour of the coleoptiles, possibly with the exception of an increase of the curvature in the range of the high quantities of blue light.

Hence it follows that the influence of red light on the coleoptile in the range of lower quantities of blue light, is independent of the influence of blue light. The experiment according to *scheme b* seems

TABLE 15

Quantity of blue light in erg cm^{-2}	Curvature in degrees ("exposure time" = 60 min)			
	after irradiation with blue light only	after irradiation with 7000 erg cm^{-2} of red light, followed after 50–35 min by blue light	after irradiation with 7000 erg cm^{-2} of red light only	of dark controls
			22	20
			20	18
2	18	—		
9	16	20		
45	17	—		
225	20	18		
900	19	20		
3,600	23	22		
14,000	24	23		
54,000	23	18		
230,000	29	24		
460,000	20	26		
			20	21
				19

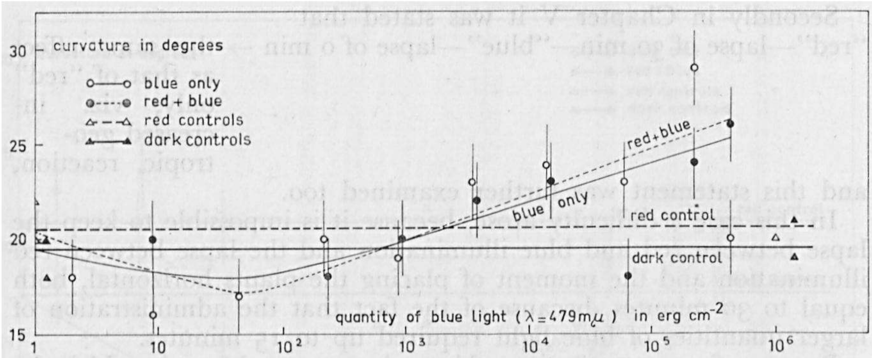


Fig. 8.

not to be conclusive with respect to the interplay of red and blue light because of the unfavourable intervals of time between irradiation and geotropic exposure. Supposing that the coleoptile requires an interval of time of 30 minutes in the vertical position to response on the irradiation either of red or blue light, then *scheme a* meets this requirement for both wave lengths, but in *scheme b* there is not enough time for the coleoptile to respond on blue light and the "waiting time" is too long for red light.

This supposition was tested in the experiment of table 16.

Apparently the effects of red and blue light by themselves, observable when applying a "waiting time" of 30 minutes, have disappeared when this interval is 50 minutes or more (table 16c and h). The blue

TABLE 16

A	1st irradiation	1st lapse in min	2nd irradiation	2nd lapse in min	Curvature (2 series) in degrees ("Exposure time" = 60 min)	
a	none	—	none	—	20	25
b	red 7000 erg cm^{-2}	30	none	—	28	31
c	id.	50	none	—	21	21
d	id.	28	blue 2700 erg cm^{-2}	0	25	26
e	id.	48	id.	0	25	23
f	id.	48	id.	30	18	18
g	none	—	id.	30	8	12
h	none	—	id.	60	20	22
B						
a	none	—	none	—	20	25
b	red 7000 erg cm^{-2}	30	none	—	28	31
c	id.	50	none	—	21	21
d	id.	28	blue 2700 erg cm^{-2}	0	24	29
e	id.	48	id.	0	20	24
f	id.	48	id.	30	16	22
g	none	—	id.	30	13	14

light has no effect at all, when the "waiting time" is zero (d and e). It seems strange, however, that in case f (in A as well as in B) red light diminishes the effect of blue light after a lapse of 48 minutes (compare g).

In table 17 and Fig. 9 the results are presented of an experiment in which the plants were irradiated with blue light or red light only. The "waiting time" was varied.

TABLE 17

Irradiation	"waiting time" in min.	Curvature in degrees ("Exposure time" = 60 min)
red 7000 erg cm^{-2}	0	18; 22
id.	30	28
id.	60	16; 19; 19; 18
blue 2700 erg cm^{-2}	0	20
id.	30	5
id.	60	24; 18; 23
none	—	23; 23

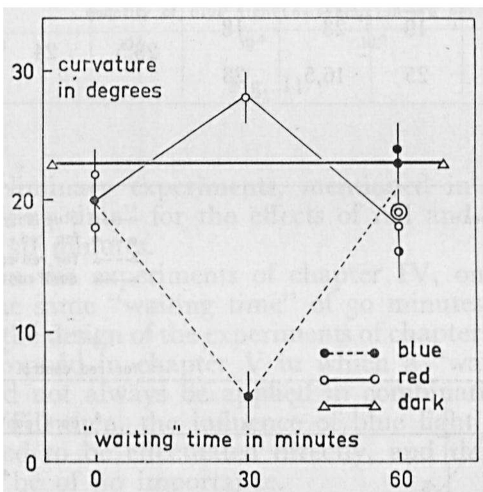


Fig. 9.

It will be noted that only when applying a "waiting time" of about 30 minutes an effect of the irradiation is observed.

Finally the experimental procedure given in *scheme a* was repeated with far red light.

"far red"—lapse of 0 min—"blue"—lapse of 30 min—"curvation time" 1 h.

Table 18, and Fig. 10 and 11 point out, that the results of this design are fundamentally comparable with those of the experiments in which the coleoptiles received a first irradiation with red light as was shown in Fig. 5 and 6.

TABLE 18

Quantity of blue light (λ = 479 mμ) in erg cm^{-2}	Curvature in degrees ("Exposure time" = 60 minutes)							
	after irradiation with blue light only		after irradiation with blue light, immediately preceded by 20,000 erg cm^{-2} of far red light		after irradiation with 20,000 erg cm^{-2} of far red light only		of dark controls	
	exp. 1	exp. 2	exp. 1	exp. 2	exp. 1	exp. 2	exp. 1	exp. 2
none					19	20	15	16
2	15	19	26	23				
9	17	16	21	19				
none							17	17
45	15.5	15	19	17				
225	6	6	9.5	12				
900	5.5	9	8	11				
none					23	20	19.5	17
3,600	6.5	4	8.5	10				
13,500	9	12	6.5	9				
none							20	18
60,000	12	12	11	14				
230,000	—	19	23	18				
none					23.5	24	17	18
460,000	19	25	16,5	23				

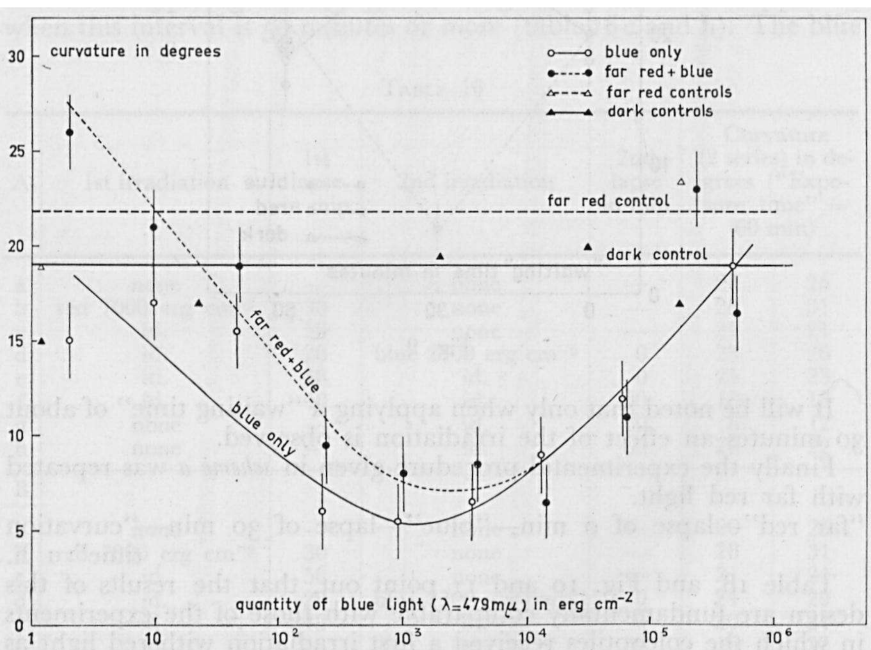


Fig. 10.

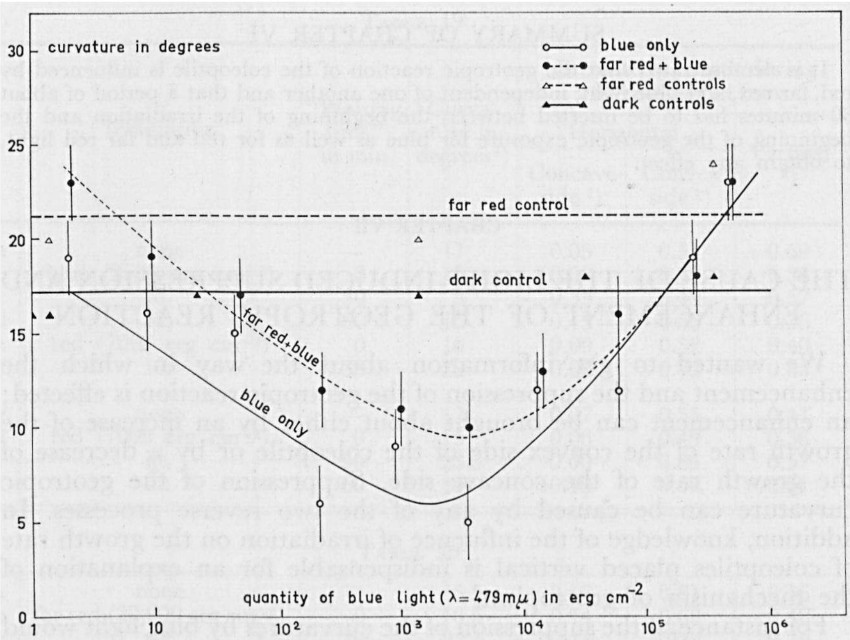


Fig. 11.

DISCUSSION

In the preliminary experiments, mentioned in chapter III, the optimal "waiting time" for the effects of red and far red light was found to be 30 minutes.

Although in the experiments of chapter IV, on the influence of blue light, the same "waiting time" of 30 minutes was maintained, analogous to the design of the experiments of chapter III, this principle had to be dropped in chapter V in which a "waiting time" of 30 minutes could not always be applied in combination with the blue irradiations. Till then, the influence of blue light on the coleoptile was considered to be effectuated directly, and the "waiting time", therefore, to be of no importance.

This led to embarrassing results. It will be noted that, in the discussion of chapter IV, the paper of FRANCK (1951) was mentioned, who considered a "waiting time" of 30 minutes to be optimal for obtaining maximal effect of white light on geotropic reaction. Unfortunately, we came across his paper not before the experiments, described thus far, were finished. Our conclusion that not only red light but also blue light requires a period of about 30 minutes to influence the geotropic reaction of the coleoptile confirms his statement about the influence of white light.

As we have no data yet on which to advance any hypothesis about the mechanism of the effect of light on the gravitational response, a discussion on this mechanism has to be postponed till chapter XII.

SUMMARY OF CHAPTER VI

It is demonstrated that the geotropic reaction of the coleoptile is influenced by red, far red, and blue light, independent of one another and that a period of about 30 minutes has to be inserted between the beginning of the irradiation and the beginning of the geotropic exposure for blue as well as for red and far red light, to obtain any effect.

CHAPTER VII

THE CAUSE OF THE LIGHT-INDUCED SUPPRESSION AND ENHANCEMENT OF THE GEOTROPIC REACTION

We wanted to get information about the way in which the enhancement and the suppression of the geotropic reaction is effected; an enhancement can be brought about either by an increase of the growth rate of the convex side of the coleoptile or by a decrease of the growth rate of the concave side. Suppression of the geotropic curvature can be caused by any of the two reverse processes. In addition, knowledge of the influence of irradiation on the growth rate of coleoptiles placed vertical is indispensable for an explanation of the mechanism of geotropism.

For instance, if the suppression of the curvatures by blue light would appear to be a consequence of an enhancement of the growth rate of the concave side, it would make quite a difference for the interpretation of this phenomenon, whether this would be attended by an acceleration or by a retardation of the growth rate of a coleoptile orientated vertical.

Consequently a series of experiments was set up to investigate the growth rates of vertical and horizontal coleoptiles, after irradiation or in complete darkness. The method of investigation was described in chapter II. Shadowgraphs were made at intervals of 30 minutes during two hours on the usual Gevaert Document Rapid photographic paper by means of monochromatic green light (560 mμ).

In tables 19 and 20 two experiments are represented in which coleoptiles were allowed to curve geotropically with "waiting times" of 0, 30, and 60 minutes. The increases in length of their convex and concave sides are compared with the increase in length of vertical coleoptiles subjected to the same irradiation and in the same period after irradiation. The results are graphically recorded in Fig. 12 and 13.

Table 19 and Fig. 12 show:

a) that the decrease in curvature occurring after irradiation with 2700 erg cm^{-2} of blue light is due to the growth rate of the concave side of the coleoptile being higher than that of the dark control (Fig. 12*c*),

b) that the increase in curvature occurring after irradiation with 7000 erg cm^{-2} of red light is due to the growth rate of the convex side of the coleoptile being higher than that of the dark control (Fig. 12*f* and *i*),

TABLE 19

	Irradiation	"Waiting time" in min	Curvature in degrees [1]	Increase in length during 60 minutes in mm		
				Horizontal		Vertical [2]
				Concave side [1]	Convex side [1]	
a	none	—	17	0.05	0.51	0.60
b	blue (2700 erg cm^{-2})	0	15	0.08	0.49	0.58
c	id.	30	5	0.35	0.49	0.57
d	id.	60	15	0.15	0.56	0.36
e	red (7000 erg cm^{-2})	0	16	0.09	0.52	0.40
f	id.	30	22	0.08	0.72	0.55
g	none	—	17	0.07	0.55	0.41
h	red (7000 erg cm^{-2})	0	15	0.06	0.49	0.36
i	id.	30	23.5	0.00	0.66	0.57
j	id.	60	14	0.19	0.58	0.61

TABLE 20

a	none	—	15.5	0.18	0.61	0.56
b	far red (20,000 erg cm^{-2})	0	16.5	0.14	0.60	0.48
c	id.	30	22.5	0.11	0.73	0.70
d	id.	60	18.5	0.29	0.71	0.75
e	blue (400,000 erg cm^{-2})	0	27	0.26	1.02	0.69
f	id.	30	18	0.48	0.98	0.87
g	id.	60	16	0.23	0.67	0.57

[1]) Each figure giving the mean of 25 plants approximately (3 trays).
[2]) Each figure giving the mean of 16 plants approximately (2 trays).

c) that in two cases, where the curvature is neither increased nor decreased, the straight growth is much lower than that of the dark control (Fig. 12*d*, *e*),

d) that the non-influenced curvature mentioned in table 20, line 5 (7000 erg cm^{-2} of red light; "waiting time" = 60 minutes) may be attended by the growth rate of both the convex and the concave side of the coleoptile being higher than that of the dark control (Fig. 12*j*).

In the same way table 20 and Fig. 13 show:

a) that the increase in curvature occurring after irradiation with 20,000 erg cm^{-2} of far red light or with 400,000 erg cm^{-2} of blue light is due to the growth rate of the convex side of the coleoptile being higher than that of the dark control (Fig. 13*c* and *e*),

b) that the non-influenced curvature mentioned in table 20, line 6 (400,000 erg cm^{-2} of blue light; "waiting time" = 30 minutes) is attended by the high growth rate of both the convex and the concave side of the coleoptile (Fig. 13*f*),

c) that in the same case a straight growth rate much higher than that of the dark control is to be observed (Fig. 13*f*).

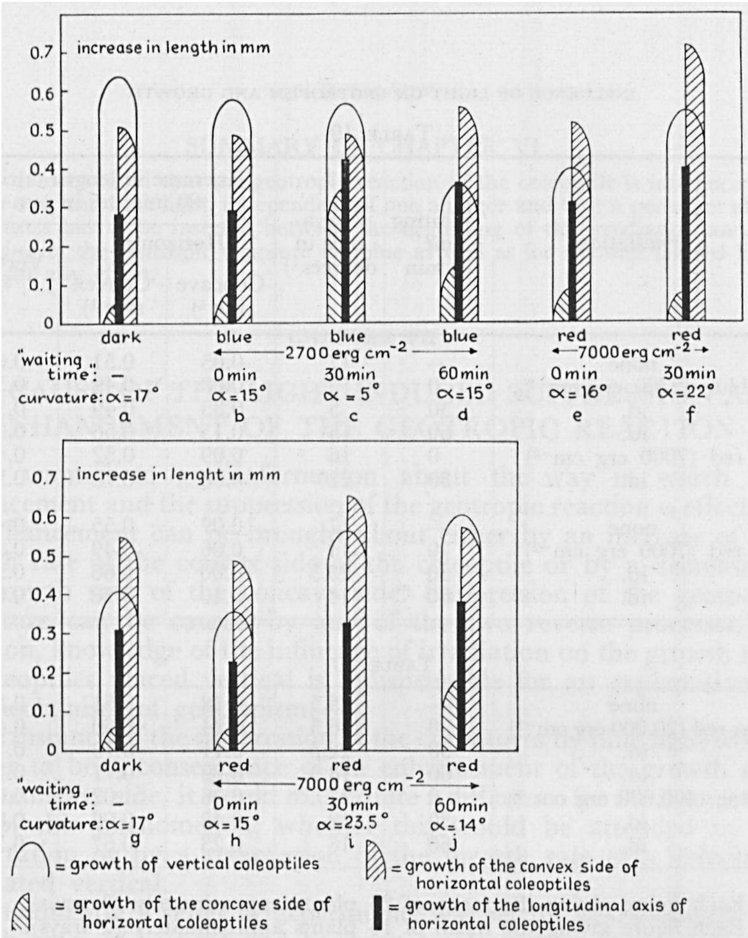

increase in length in mm
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
dark
blue
blue
blue
red
red
2700 erg cm^{-2}
7000 erg cm^{-2}
"waiting" time:
—
0 min
30 min
60 min
0 min
30 min
curvature: α=17°
α = 15°
α = 5°
α=15°
α = 16°
α = 22°
a
b
c
d
e
f
increase in lenght in mm
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
dark
red
red
red
7000 erg cm^{-2}
"waiting" time:
—
0 min
30 min
60 min
curvature: α=17°
α= 15°
α=23.5°
α= 14°
g
h
i
j
= growth of vertical coleoptiles
= growth of the convex side of horizontal coleoptiles
= growth of the concave side of horizontal coleoptiles
= growth of the longitudinal axis of horizontal coleoptiles

Fig. 12.

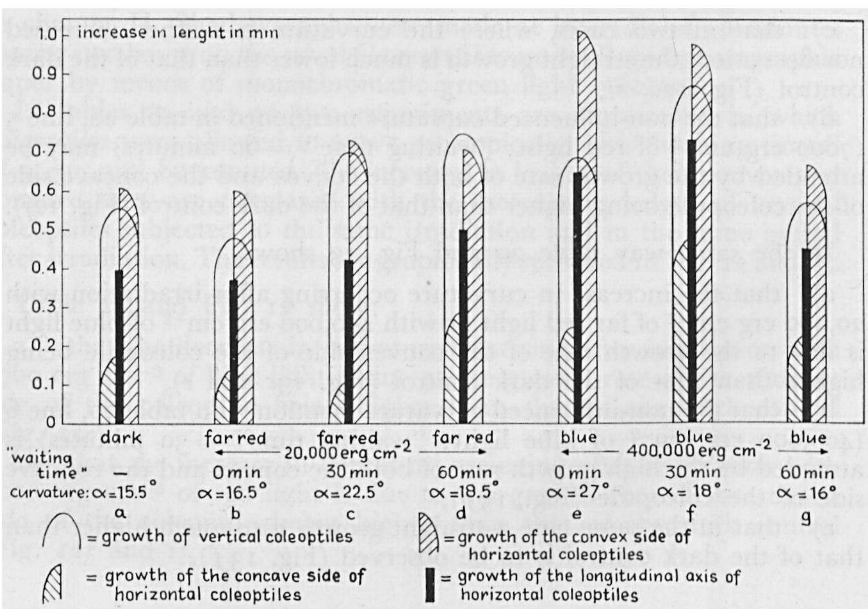

increase in lenght in mm
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
dark
far red
far red
far red
blue
blue
blue
20,000 erg cm^{-2}
400,000 erg cm^{-2}
"waiting time"
—
0 min
30 min
60 min
0 min
30 min
60 min
curvature: α=15.5°
α=16.5°
α=22.5°
α=18.5°
α=27°
α= 18°
α=16°
a
b
c
d
e
f
g
= growth of vertical coleoptiles
= growth of the convex side of horizontal coleoptiles
= growth of the concave side of horizontal coleoptiles
= growth of the longitudinal axis of horizontal coleoptiles

Fig. 13.

Discussion

The following objections can be raised against the experimental design used in the experiments of tables 19 and 20:

a) In view of the following data, it seems plausible to suppose that the green monochromatic light used for shadowgraphing, influenced the growth rate of the coleoptiles (table 21). During the time of observation vertical coleoptiles were shadowgraphed 4 times, horizontal ones 2 times, and for this reason the growth rate of both may not be quite comparable. So as to minimize the influence of green light, the growth phenomena were reinvestigated while using more sensitive photographic material.

Table 21

Total increase in length of vertical plants after two hours (in mm)

Shadowgraphed	Irradiation with		Dark
	Red light	Blue light	
Every 30 min.	1.11	0.93	1.10
At the beginning and at the end of the growth period only.	1.43	0.95	1.38

b) While shadowgraphing every 30 minutes, rapid changes in growth rate escape the notice of the investigator. So in experiments yet to be described shadowgraphs were made every 15 minutes.

c) In chapter IV it was stated that quantities of blue light of about 1800–3600 erg cm^{-2} maximally influence the geotropic reaction of the coleoptile.

As is indicated clearly in table 17, however, this statement is only true when working with a "waiting time" of 30 minutes. Larger quantities of blue light enhance the geotropic curvature, provided that the "waiting time" is reduced to 0 minutes. So, a series of experiments should be set up in which the quantity of blue light, administered to the coleoptiles, as well as the waiting time should be varied (Chapter VIII).

SUMMARY

The experiments described in this chapter were set up to provide data which should enable us to correlate the influence of irradiation of vertical plants with response and reactions to irradiation of horizontal plants. These experiments clearly pointed out that the lowering of the geotropic reaction by blue light is caused by an enhancement of the growth rate of the concave side of the coleoptile, whereas the increase of the geotropic curvatures by red and far red light is due to an enhancement of the growth rate of the convex side.

In those cases where no influence upon the curvature was observable, viz. irradiation with red light, combined with a waiting time of one hour or after irradiation with much blue light, combined with a "waiting time" of half an hour, it can be stated that the growth of both the convex and the concave side of the horizontal coleoptile is enhanced.

CHAPTER VIII

THE INFLUENCE OF THE "WAITING TIME" FOR VARIOUS QUANTITIES OF BLUE, RED AND FAR RED LIGHT ON THE GEOTROPIC REACTION

Blue light

Whereas, with a "waiting time" of 30 minutes, a quantity of blue light ($\lambda = 479$ mμ) of about 50,000 erg cm^{-2} does not influence the geotropic reaction of the coleoptile (cf. chapter IV) the geotropic reaction is markedly increased after irradiation with 400,000 erg cm^{-2}, applying a "waiting time" of 0 minutes (chapter VII).

This suggests that for influencing the geotropic reaction, discrete combinations of quantities of light and "waiting times" are required.

Two series of experiments were set up to settle this point: Firstly a series to study the influence of a number of "waiting times" for a number of fixed quantities of blue light. Secondly a series to study the influence of a number of quantities of blue light for a number of fixed "waiting times". This second series is meant to check the most salient data obtained in the first series.

The results of the first series of experiments are given in Fig. 14 *a-k*, and those of the second series in Fig. 15 *a-d* and Fig. 5, in which the "waiting times" are 0, 60, 75, 75, and 30 minutes resp. Each point represents the mean of two trays of c. 12 plants each.

Fig. 16 shows a spatial graph, schematically combining the Fig. 14 *a-k*, 15 *a-d* and 5. The most striking features in this graph are:

1) the reaction to irradiation with 1800–7200 erg cm^{-2} (a sharp fall at a "waiting time" of 30 minutes; a sharp rise at a "waiting time" of 75 minutes).

2) the reaction to irradiation with 100,000–500,000 erg cm^{-2} (a sharp rise at a "waiting time" of 0 minutes and a less pronounced one at a "waiting time" of 75 minutes).

Red light (660 mμ)

Fig. 17*a* and *b* and table 6 show that the relation between geotropic reaction, quantity of red light and "waiting time" is much less complicated than in the case of blue light.

Irradiations with 2800, 7000 or 90,000 erg cm^{-2} all show the same picture: when choosing a "waiting time" of 30 minutes an increase in curvature is noted.

Far red light (735 mμ)

As can be seen from Fig. 18 and from table 6 giving the results of experiments with 1,000,000 and 12,000 erg cm^{-2} resp. the response evoked by far red is essentially the same as that obtained with red light.

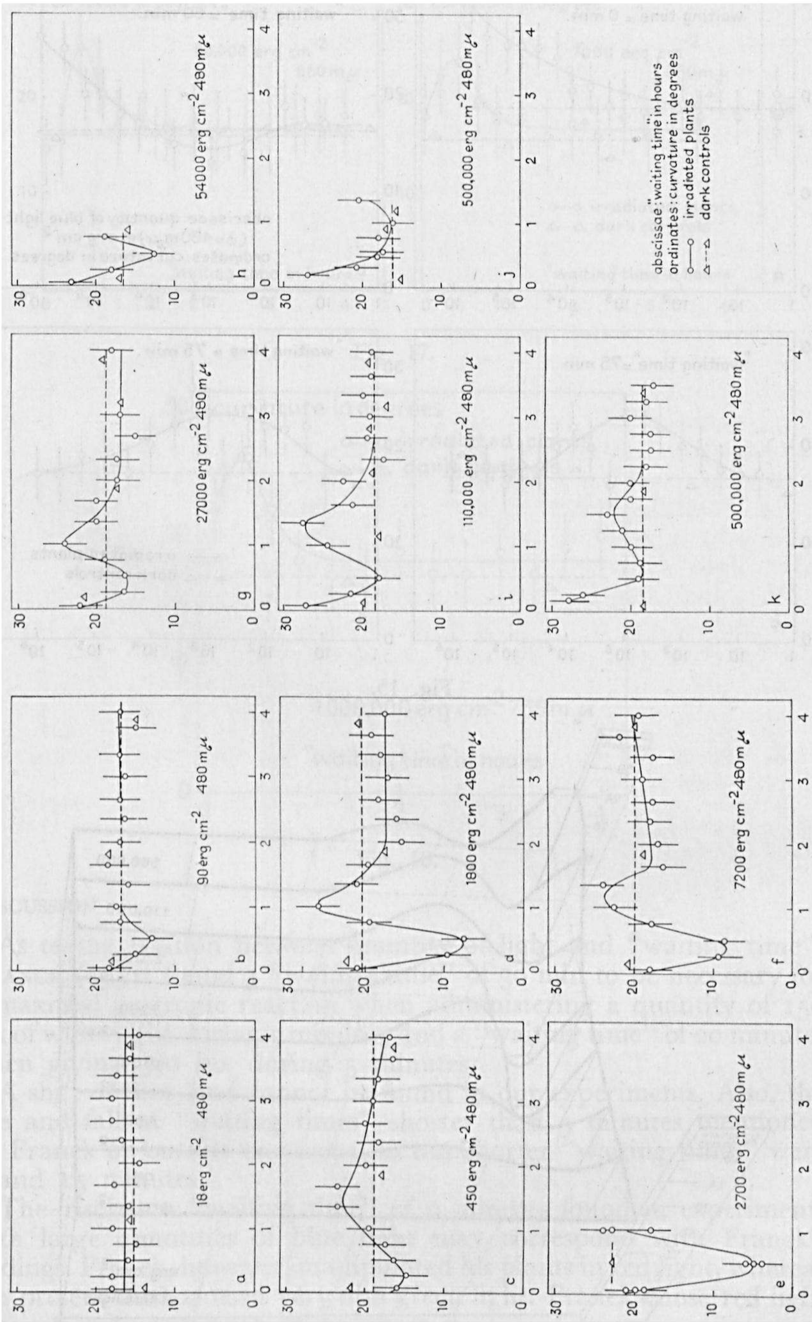

a 18 erg cm^{-2} 480 mμ
b 90 erg cm^{-2} 480 mμ
c 450 erg cm^{-2} 480 mμ
d 1800 erg cm^{-2} 480 mμ
e 2700 erg cm^{-2} 480 mμ
f 7200 erg cm^{-2} 480 mμ
g 27000 erg cm^{-2} 480 mμ
h 54000 erg cm^{-2} 480 mμ
i 110,000 erg cm^{-2} 480 mμ
j 500,000 erg cm^{-2} 480 mμ
k 500,000 erg cm^{-2} 480 mμ
abscissae: waiting time in hours
ordinates: curvature in degrees
irradiated plants
dark controls

Fig. 14.

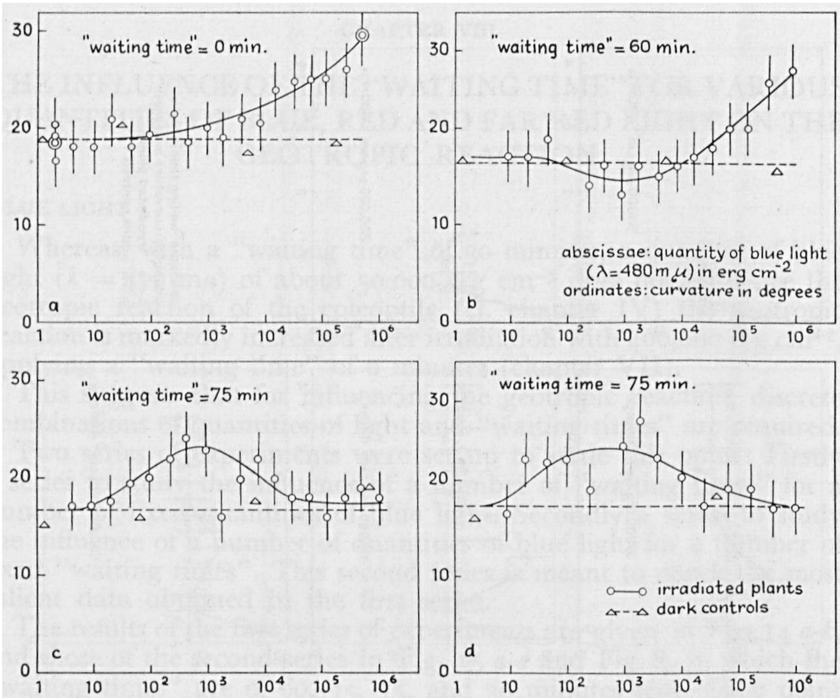

"waiting time" = 0 min.
"waiting time" = 60 min.
abscissae: quantity of blue light
(λ=480 mμ) in erg cm⁻²
ordinates: curvature in degrees
"waiting time" = 75 min
waiting time = 75 min.
irradiated plants
dark controls
a
b
c
d
30
20
10
0
1 10 10² 10³ 10⁴ 10⁵ 10⁶

Fig. 15.

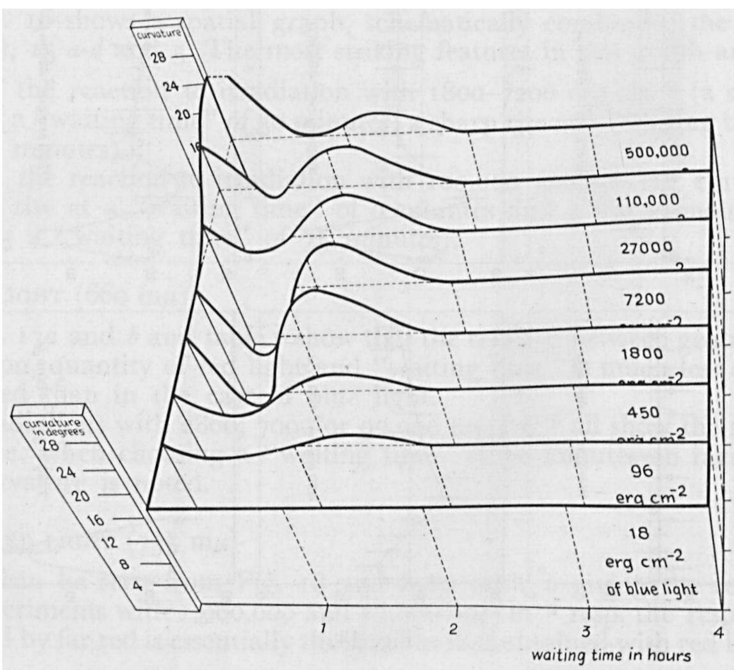

curvature
28
24
20
500,000
110,000
27000
7200
1800
450
erg cm⁻²
96
erg cm⁻²
18
erg cm⁻²
of blue light
curvature in degrees
28
24
20
16
12
8
4
1
2
3
4
waiting time in hours

Fig. 16.

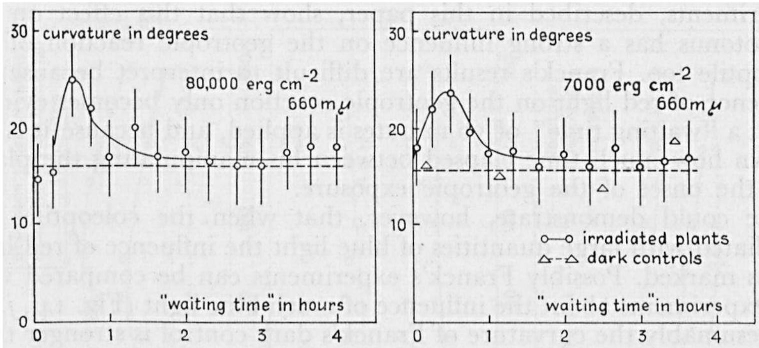


Fig. 17.

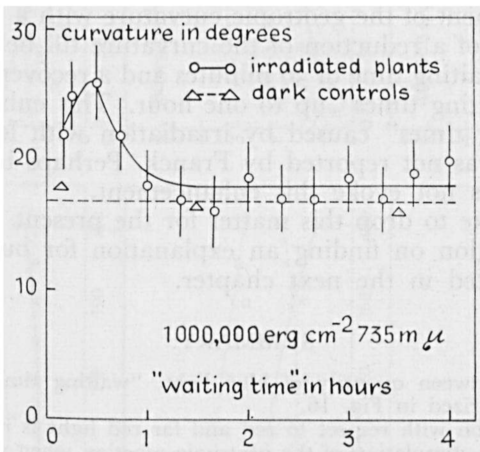


Fig. 18.

Discussion

As to the relation between quantity of light and "waiting time", Franck (1951) found a "waiting time" of 30 min to be necessary for a maximal geotropic reaction when administering a quantity of 150 lux of white light during 5 minutes, and a "waiting time" of 20 minutes when giving 300 lux during 5 minutes.

A shift of that kind cannot be found in our experiments. Also, the rise and fall at "waiting times" shorter than 5 minutes mentioned by Franck lie outside our scope, as our shorter "waiting times" were 0 and 15 minutes.

The rise at a "waiting time" of 0 minutes found in experiments with large quantities of blue light may correspond with Franck's findings. Franck, however, manipulated his plants in red light, whereas the present author used very dim green light. Franck chose red light because it has no phototropic effect.

Blaauw-Jansen (1959) demonstrated that it has a tonic effect resulting in an enhancement of the phototropic reaction. Indeed, experiments, described in this paper, show that this effect on the phototonus has a strong influence on the geotropic reaction of the coleoptile too. Franck's results are difficult to interpret because the influence of red light on the geotropic reaction only becomes evident when a "waiting time" of 30 minutes is applied, and because it is not known how much time elapsed between his manipulating the plants and the onset of the geotropic exposure.

We could demonstrate, however, that when the coleoptiles are irradiated with large quantities of blue light the influence of red light is less marked. Possibly Franck's experiments can be compared with our experiments about the influence of much blue light (Fig. 14*i*, *j*, *k*).

Presumably the curvature of Franck's dark control is stronger than the curvature of the dark control of our Fig. 14, because his plants were exposed to a large dose of red light. This may explain his finding of an enhancement of the geotropic curvature with a waiting time of a few minutes, of a reduction of the curvature till below the control values with a waiting time of 30 minutes and a recovery of the curvatures with "waiting times" up to one hour. The enhancement with longer "waiting times" caused by irradiation with lower quantities of blue light, was not reported by Franck. Perhaps irradiation with white light does not evoke this enhancement.

We should like to drop this matter for the present and to concentrate our attention on finding an explanation for our own data as will be attempted in the next chapter.

SUMMARY

The relation between quantity of blue light, "waiting time" and geotropic reaction is summarized in Fig. 16.

The same relation with respect to red and far red light is much simpler, the only effect being a stimulation of the geotropic reaction when applying "waiting times" of about 30 minutes.

CHAPTER IX

THE INFLUENCE OF PRE-IRRADIATION ON THE GROWTH OF SEEDLINGS IN VERTICAL POSITION

As we wanted to compare the effects of irradiation on the growth of the curving horizontal coleoptile with the effects on the growth of the vertical one, in the next experiment a series of coleoptiles was shadowgraphed every other 15 minutes after being irradiated with different quantities of blue, red or far red light, on Gevaert O 53 graphic ortho film which shows fairly high sensitiviy to light of 560 mμ.

The results of this experiment will be given as graphical records only of the mean increase in length of approximately 25 plants during each period of 15 minutes.

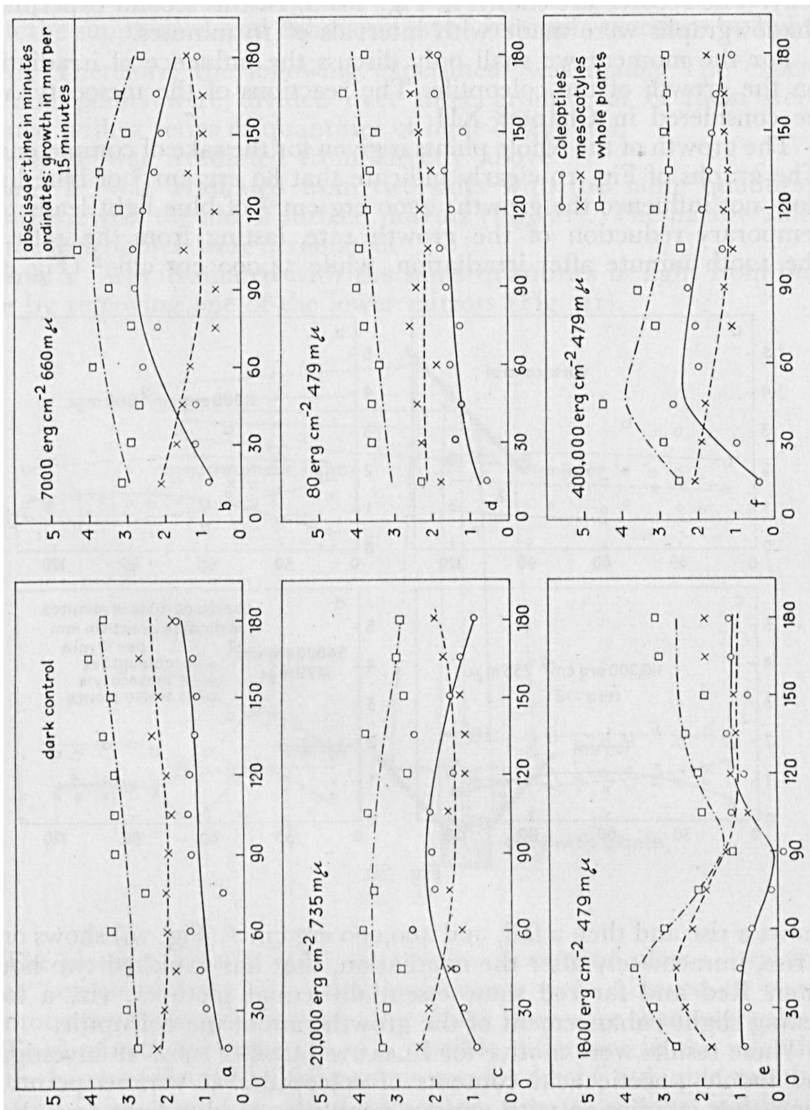


Fig. 19.

In Fig. 19*a-f* the increase in length is shown of "mesocotyls", "coleoptiles" and whole plants.[1])

These results were confirmed by a second experiment in which slightly different light quantities were administered to the plants and in which the growth measurements were continued during two hours

[1]) For definitions see p. 402.

only. The records are shown in Fig. 20*a-d*. In this second experiment shadowgraphs were made with intervals of 10 minutes.

For the moment we shall only discuss the influence of irradiation on the growth of the coleoptile. The reactions of the mesocotyl will be considered in Chapter XII.

The growth of the whole plants is given for the sake of completeness. The graphs of Fig. 19 clearly indicate that 80 erg cm^{-2} of blue light does not influence the growth; 2700 erg cm^{-2} of blue light leads to a temporary reduction of the growth rate, lasting from the 45th till the 120th minute after irradiation, while 54,000 erg cm^{-2} (Fig. 20)

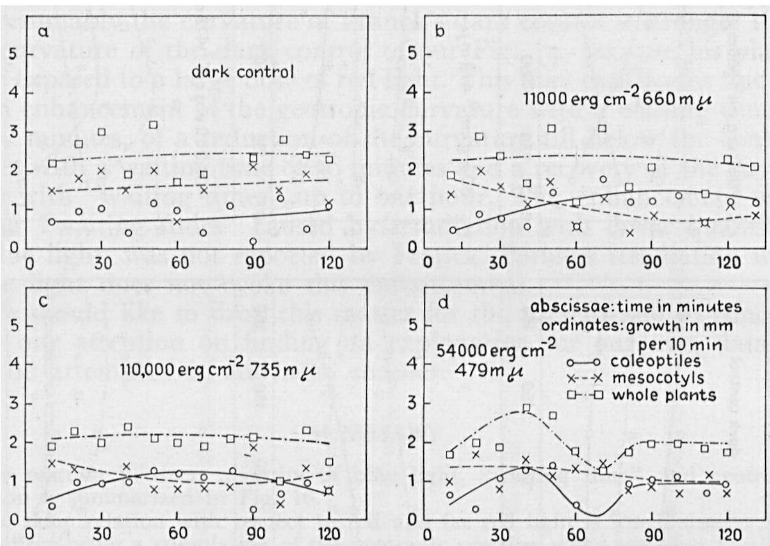


Fig. 20.

shows a rise and then a fall, and 400,000 erg cm^{-2} (Fig. 19) shows only a rise, immediately after the irradiation, that has vanished two hours later. Red and far red show essentially equal pictures: viz. a long lasting slight enhancement of the growth rate of the coleoptile.

These results were motive for BLAAUW-JANSEN (1962) to investigate the indole-3-acetic acid contents of coleoptiles at various points of time after irradiation with various quantities of blue light (see chapter XIV).

CHAPTER X

CORRELATION OF THE PHENOMENA OF GEOTROPISM AND PHOTOTROPISM

When we want to apply the prevailing views on the causes of phototropism to our findings on the influence of light on geotropism, it is necessary to correlate the various phenomena of phototropism (first positive curvature, negative curvature, second positive curvature) with the suppression and enhancement of geotropic reactions by light.

A. Therefore, the following experiment was made: The experimental plants were divided over three groups, all of them were treated with a series of quantities of light of 479 mμ.

series a was irradiated from directly above.

series b was irradiated from two sides with the same quantities as *series a* with the aid of surface mirrors (Fig. 21). The narrow sides of the coleoptiles were irradiated.

series c was irradiated with the same quantities of light from one side by removing one of the lower mirrors (Fig. 21).

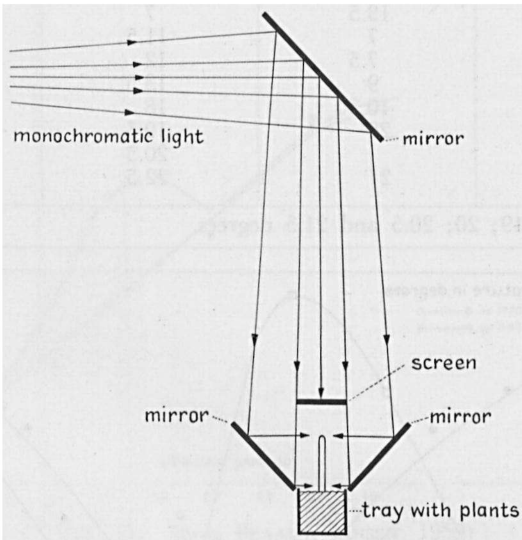


Fig. 21.

The plants of *series a* and *b* were placed horizontal after a "waiting time" of 30 minutes, the plants of *series c* were allowed to curve phototropically for 90 minutes, after which they were shadowgraphed.

To eliminate the influence of a possible difference in the intensity of the beams reflected by the two mirrors for *series b* a double number of trays was taken. In rapid succession two trays were irradiated with equal quantities of light. After a "waiting time" of 30 minutes one tray was turned on its left, the other on its right side.

In this way, two sets of figures were obtained, which were in close agreement with each other. In the 3rd column of table 22 the mean values of these two sets are given.

The results of the experiments mentioned under A are shown in table 22 and in Fig. 22.

It is clear from Fig. 22 that it makes no essential difference for the light quantity required to produce the minimum in the geotropic reaction whether the light is applied from above or from two sides.

TABLE 22

Quantity of light (λ = 479 mμ) in erg cm^{-2}	Geotropic curvature in degrees ("Exposure time" = 60 min)		Phototropic curvature in degrees (Developed within 90 min)
	light from above	light from 2 sides	
4		20	
16		18	
40	14		+20
80		12.5	
200	8	8	+25
400		5.5	
800	1	10	+12
1,600	13.5	7	+ 9
3,200	7	11.5	+ 1
6,500	7.5	12	— 9
13,000	9	13.5	—11.5
25,000	10.5	18	—10
50,000	20	19.5	— 7.5
100,000		20.5	
200,000	21	22.5	+ 5.5

dark controls: 19; 20; 20.5 and 21.5 degrees.

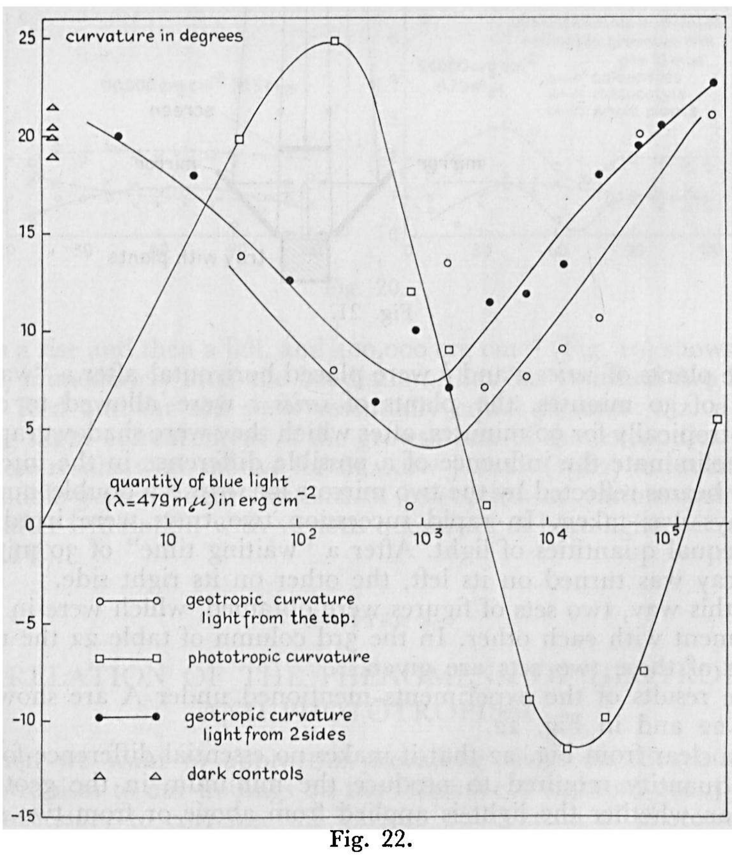


Fig. 22.

The negative phototropic reaction, however, occurs at much larger quantities of blue light than the minimum in the geotropic reaction. This feature may serve us in the general discussion of the phenomena described in this paper.

A further possible point of contact with the hypothesis proposed by BLAAUW-JANSEN (1959), explaining the phototropic behaviour of *Avena* coleoptiles, lies in the realm of the red - far red antagonism, as Blaauw-Jansen proved that the turning point from negative to second positive phototropic curvature shows red - far red antagonism.

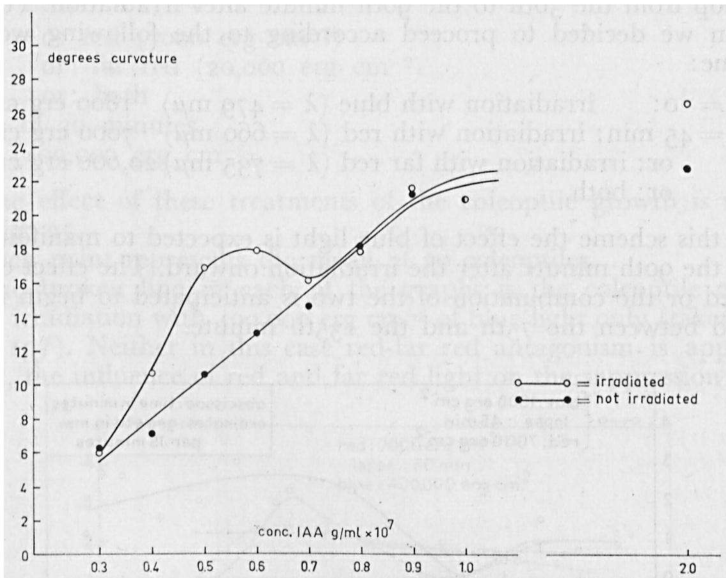


Fig. 23. (from BLAAUW-JANSEN, 1959)

At four instances in the experiments described in Chapters VII and IX we established an increased growth with plants that were pre-illuminated with blue light, viz.:

1. After pre-illumination with 2700 erg cm^{-2} a restoration, after an initial fall, in the growth curve of vertically placed coleoptiles (Fig. 19*e*) corresponding with the second rise in Fig. 20*d*,

2. After pre-illumination with 400,000 erg cm^{-2} a rise in the growth curve of vertically placed coleoptiles (Fig. 19*f*) corresponding with the first rise in Fig. 20*d*,

3. After pre-illumination with 400,000 erg cm^{-2} a rise in the growth of the convex side of the horizontal coleoptiles responsible for the enhancement of the geotropic curvature (Fig. 13*f*) under those conditions,

4. After pre-illumination with 2700 erg cm^{-2} an increase in the growth of the concave side of the horizontal coleoptile responsible for the lowering of the geotropic curvature under those conditions (Fig. 12 *c*).

If a red-far red antagonism could be demonstrated, either on the growth of vertically standing plants in the cases 1 or 2 or on the curvature in the cases 3 or 4, it would be probable that a point of contact with the phototropic behaviour had been hit upon.

Firstly the possibility mentioned under 1 was tested. The restoration of the growth rate appears about 90 minutes after the irradiation with blue light.

From our experiments with red light we know that the effect of red light appears only when the geotropic curvature is allowed to develop from the 30th to the 90th minute after irradiation. For this reason we decided to proceed according to the following working scheme:

time = 0: irradiation with blue ($\lambda = 479$ mμ) 1800 erg cm^{-2}
time = 45 min: irradiation with red ($\lambda = 660$ mμ) 7000 erg cm^{-2}
or: irradiation with far red ($\lambda = 735$ mμ) 20,000 erg cm^{-2}
or: both.

In this scheme the effect of blue light is expected to manifest itself from the 90th minute after the irradiation onward. The effect of red, far red or the combination of the two is anticipated to begin in the period between the 75th and the 135th minute.

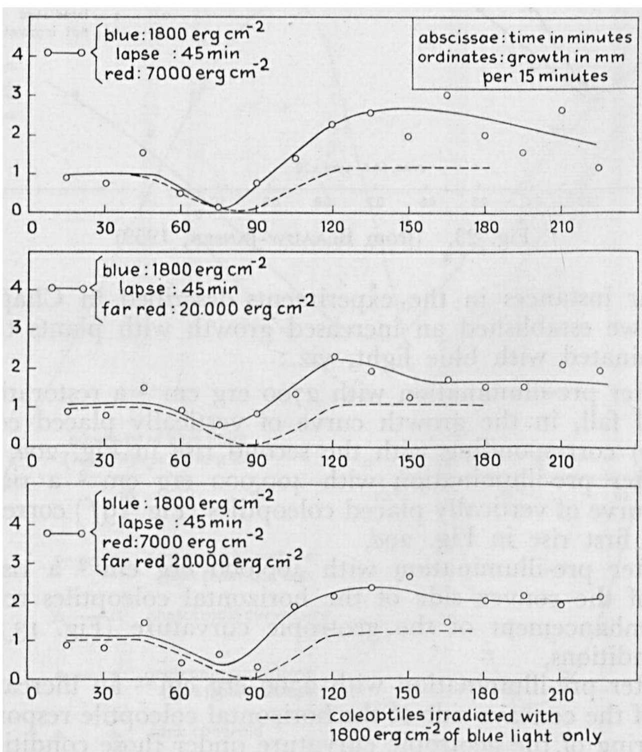


Fig. 24.

In the graphs of Fig. 24 the effect of these treatments on the growth of the coleoptile is given. Each point gives the mean of about 20 plants.

The broken line in each of the graphs is the coleoptile growth after the irradiation with blue light only (taken from Fig. 18*e*). From these data we conclude to the non-existence of a red - far red antagonism in this instance.

As to the second possibility, we see that the increased growth mentioned, appears immediately after the irradiation with blue light.

In the following working scheme the influences of blue and red or far red light are expected to occur at the same time too.

time = 0: red (7000 erg cm^{-2})
or: far red (20,000 erg cm^{-2})
or: both
lapse of 30 minutes
blue (400,000 erg cm^{-2}).

The effect of these treatments of the coleoptile growth is shown in Fig. 25.

Each point represents the mean of 20 coleoptiles.

The broken line in each of the graphs is the coleoptile growth after irradiation with 400,000 erg cm^{-2} of blue light only (taken from Fig. 19*f*). Neither in this case red-far red antagonism is apparent. Next, the influence of red and far red light on the suppression of the

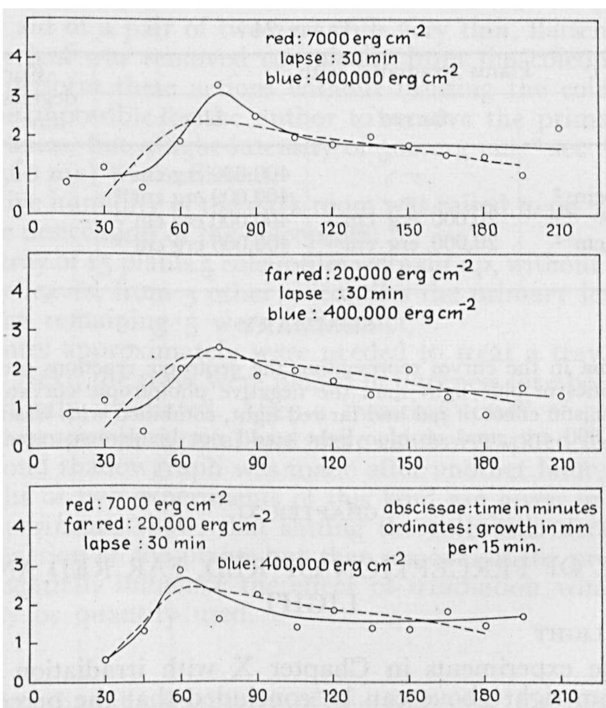


Fig. 25.

TABLE 23

Plants irradiated with			Curvatures in degrees ("Exposure time" =60 min)
blue	red	far red	
2700 erg cm^{-2}	—	—	5.5
2700 erg cm^{-2}	7000 erg cm^{-2}	—	7
2700 erg cm^{-2}	—	20,000 erg cm^{-2}	8.5
2700 erg cm^{-2}	7000 erg cm^{-2}	20,000 erg cm^{-2}	8
—	—	—	17

geotropic curvature by blue light was studied, according to 3. Table 23 shows that no red-far red antagonism was indicated by this experiment.

Likewise, as table 24 shows, the enhancement of the geotropic curvature after 400,000 erg cm^{-2} of blue light was not subject to the red-far red antagonism.

Working scheme:

time = 0: red or far red or both
lapse of 30 minutes
time = 30 min: 400,000 erg cm^{-2} of blue light (administered in the course of 9 minutes)
time = 39 min: plants put horizontal. First shadowgraph.
time = 99 min: second shadowgraph.

TABLE 24

Plants irradiated with			Curvature in degrees ("Exposure time" = 60 min)
red	far red	blue	
—	—	400,000 erg cm^{-2}	27
7000 erg cm^{-2}	—	400,000 erg cm^{-2}	31
—	20,000 erg cm^{-2}	400,000 erg cm^{-2}	31
7000 erg cm^{-2}	20,000 erg cm^{-2}	400,000 erg cm^{-2}	35
—	—	—	17

SUMMARY

The minima in the curves representing the geotropic reactions occur at much lower quantities of blue light than the negative phototropic curvature does.

An antagonistic effect of red and far red light, combined with irradiations with 2700 of 400,000 erg cm^{-2} or blue light could not be demonstrated.

CHAPTER XI

THE SITE OF PERCEPTION OF RED, FAR RED AND BLUE LIGHT

A. BLUE LIGHT

From the experiments in Chapter X with irradiation from two sides or from right above can be concluded that the percepting site (cell or group of cells) is more or less isodiametric.

B. RED, FAR RED AND BLUE LIGHT

For these light qualities the influence of the primary leaf on the perception was investigated. Indirect evidence exists (BLAAUW-JANSEN, 1959) for the possibility that the tip of the primary leaf is the site of perception of red and far red light.

It was thought useful to study the reactions of coleoptiles from which the primary leaf was excised. This was performed in the following way. With the device represented in Fig. 26 (a, b) the coleoptile was slit at two opposite sides, from about 5 mm. below the top to about 3 mm. over the node. The incisions were made as shown in Fig. 26 c.

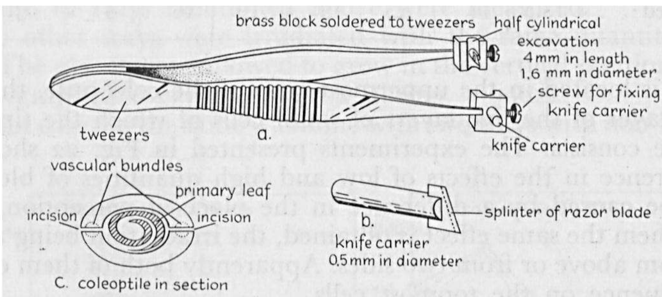


Fig. 26.

With the aid of a pair of tweezers with very thin, flattened points the primary leaf was removed completely from the coleoptile. It is feasible to perform these actions without bruising the coleoptile. It proved to be impossible for the author to remove the primary leaves in total darkness, but a light intensity of 300 erg cm^{-2} sec^{-1} of green light ($\lambda = 560$ mμ) was sufficient.

The relative humidity of the dark room was raised to 96 % in order to avoid the desiccation of the coleoptiles.

In every tray of 15 plants 5 coleoptiles were slit up, without removing the primary leaves, from 5 other coleoptiles the primary leaves were removed, the remaining 5 were left intact.

Ten minutes approximately were needed to treat a tray of plants in this way. Next, the trays were placed in darkness for about 2 hours and subsequently irradiated. After a "waiting time" of 30 minutes the plants were put horizontal and were shadowgraphed for the first time. A second shadowgraph was made after another hour.

The results of two experiments of this kind are given in table 25.

These experiments show, that slitting the coleoptile decreases the geotropic reaction of the plants but that removal of the primary leaf does not essentially influence the effect of irradiation whatever the light quality or quantity used.

DISCUSSION

It is known that the sensitivity of the coleoptile for blue light is maximal in its tip. LANGE (1928) stated that the maximal sensitivity

TABLE 25

	Curvature in degrees ("waiting time" = 30 min) ("exposure time" = 60 min)									
	dark		red 7000 erg cm^{-2}		far red 20,000 erg cm^{-2}		blue 1800 erg cm^{-2}		blue 400,000 erg cm^{-2}	
exp. no.	1	2	1	2	1	2	1	2	1	2
intact plants	14.5	16.5	24	28	22	23	9.5	10	23	22.5
plants slit only	13	11	21	19.5	18	13.5	7	2	17.5	15.5
prim. leaf removed . .	13.5	11	23	19	19	16	4	3	18	17.5

for light is located in the uppermost 50 μ of the coleoptile, that is in the top layer of the five layers of small cells of which the tip of the coleoptile consists. The experiments presented in Fig. 22 show that the difference in the effects of low and high quantities of blue light cannot be caused by a difference in the place of perception, as for both of them the same effect is obtained, the irradiation being applied either from above or from two sides. Apparently both of them exercise their influence on the topmost cells.

We did exclude the primary leaf as the organ which perceives the red and far red light. Perception by the primary leaf and indirect influence of red light via the primary leaf on the coleoptile offered an explanation of the difficulty that red light influences the growth of the coleoptile but induces no phototropic curvature when applied unilaterally (BLAAUW-JANSEN, 1959).

We are not able to suggest any explanation for the influence of red and far red light, neither with respect to our own experiments, nor to Blaauw-Jansen's.

CHAPTER XII

SUPPLEMENTARY EXPERIMENTS

Two further investigations on one of the most salient results of our studies seemed opportune, viz.:

1) The way in which the geotropic curvature and the growth of the coleoptile develop in the course of the "exposure time" after irradiation with 2700 erg cm^{-2} of blue light ($\lambda = 479$ mμ) and applying "waiting times" of 0, 30, 60, 75 and 120 minutes.

2) The tonic condition of the coleoptile in the period beginning with the third hour after the irradiation with blue light, when the geotropic reaction is the same as that of the dark control.

So *firstly* we irradiated 10 trays, each containing c. 15 carefully selected plants, with 2700 erg cm^{-2} of blue light from directly above and put them on their sides in pairs after 0, 30, 60, 75 and 120 minutes resp. Each plant was provided with a grain of vermiculite as a mark about 1 cm below the top. The plants were allowed to curve during a period of 75 minutes and were shadowgraphed on Gevaert O 53 graphic ortho film at the 0th, 15th, 30th, 45th, 60th, and 75th minute of this period.

The same was done with two trays with non irradiated plants. With the aid of a photographic enlarger supplied with a lens of high quality (Leitz Elmar 1 : 3.5) from these shadowgraphs perfectly sharp images, 10 times enlarged, were made and from these images the growth of the concave and the convex side was determined in the way described in chapter II, Fig. 2.

Finally in each of the shadowgraphs the curvatures of the plants from the 12 trays mentioned above were measured.

Two other trays were irradiated with the same quantity of blue light. The plants were allowed to grow in the vertical position and also shadowgraphed every 15 minutes during 3 hours.

In this case too the same was done with two trays with non-irradiated plants.

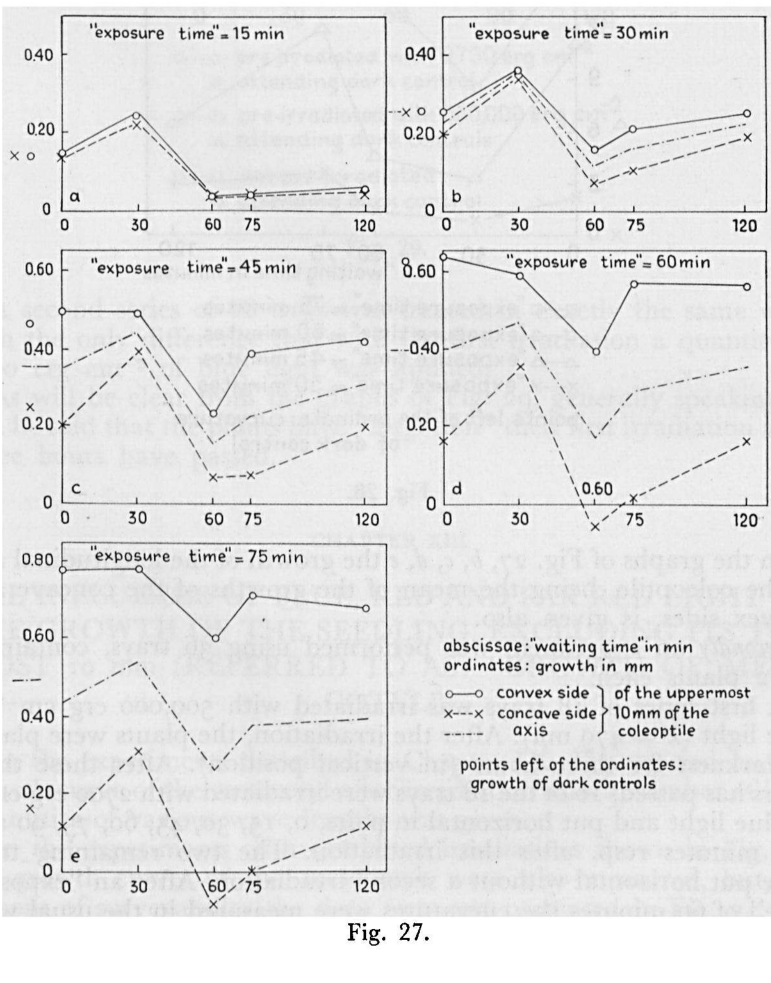


Fig. 27.

The result of this experiment in so far as the first 12 trays are concerned are shown in the graphs of Fig. 27 (growth) and 28 (curvature).

Growth and curvature of the dark controls are given in the points left of the ordinates.

The plants growing vertically showed the behaviour already described in graphs *a* and *e* of Fig. 19.

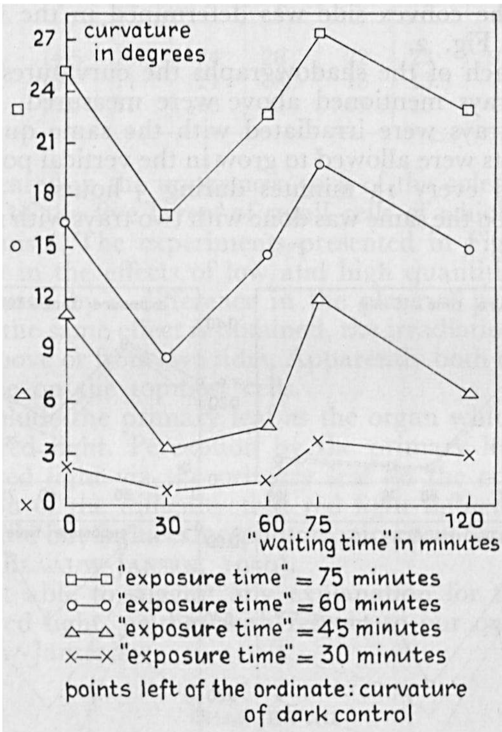


Fig. 28.

In the graphs of Fig. 27, *b*, *c*, *d*, *e* the growth of the longitudinal axis of the coleoptile, being the mean of the growths of the concave and convex sides, is given also.

Secondly an experiment was performed using 36 trays, containing c. 12 plants each.

A first series of 18 trays was irradiated with 500,000 erg cm^{-2} of blue light ($\lambda = 479$ mμ). After the irradiation, the plants were placed in darkness for three hours (in vertical position). After these three hours has passed, 16 of the 18 trays were irradiated with 2700 erg cm^{-2} of blue light and put horizontal in pairs, 0, 15, 30, 45, 60, 75, 90 and 120 minutes resp. after this irradiation. The two remaining trays were put horizontal without a second irradiation. After an "exposure time" of 60 minutes the curvatures were measured in the usual way.

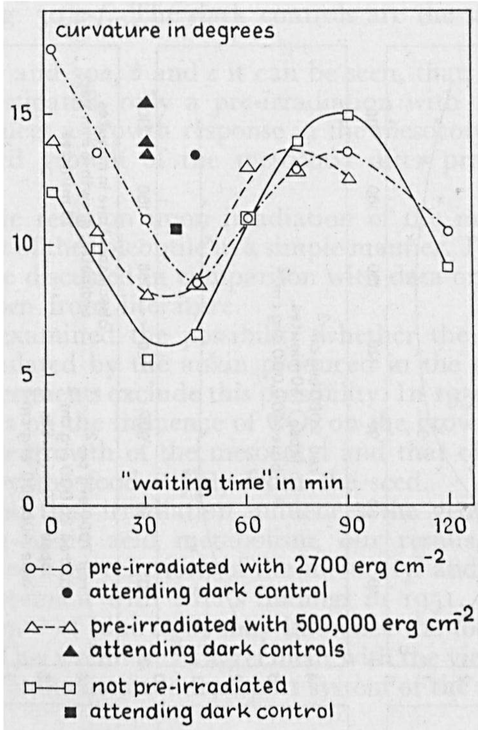


Fig. 29.

A second series of 18 trays was treated in exactly the same way, with the only difference that with the first irradiation a quantity of 2700 erg cm^{-2} of blue light was applied.

As will be clear from the graphs of Fig. 29, generally speaking, it can be said that the plants have "forgotten" their first irradiation after three hours have passed.

CHAPTER XIII

THE INFLUENCE OF BLUE, RED AND FAR RED LIGHT ON THE GROWTH OF THE SEEDLING, EXCLUDING ITS TOP-MOST 10 mm (REFERRED TO AS: "GROWTH OF MESO-COTYL")

In the experiments described in Chapter IX, Fig. 19 and 20, the growth curve of the mesocotyl after irradiation with a number of light quantities of various wave lengths was given.

In the experiments with combined irradiations shown in Fig. 24, the growth curves of the mesocotyls were also measured, but, for reasons of surveyability the data were not presented in Fig. 24, but

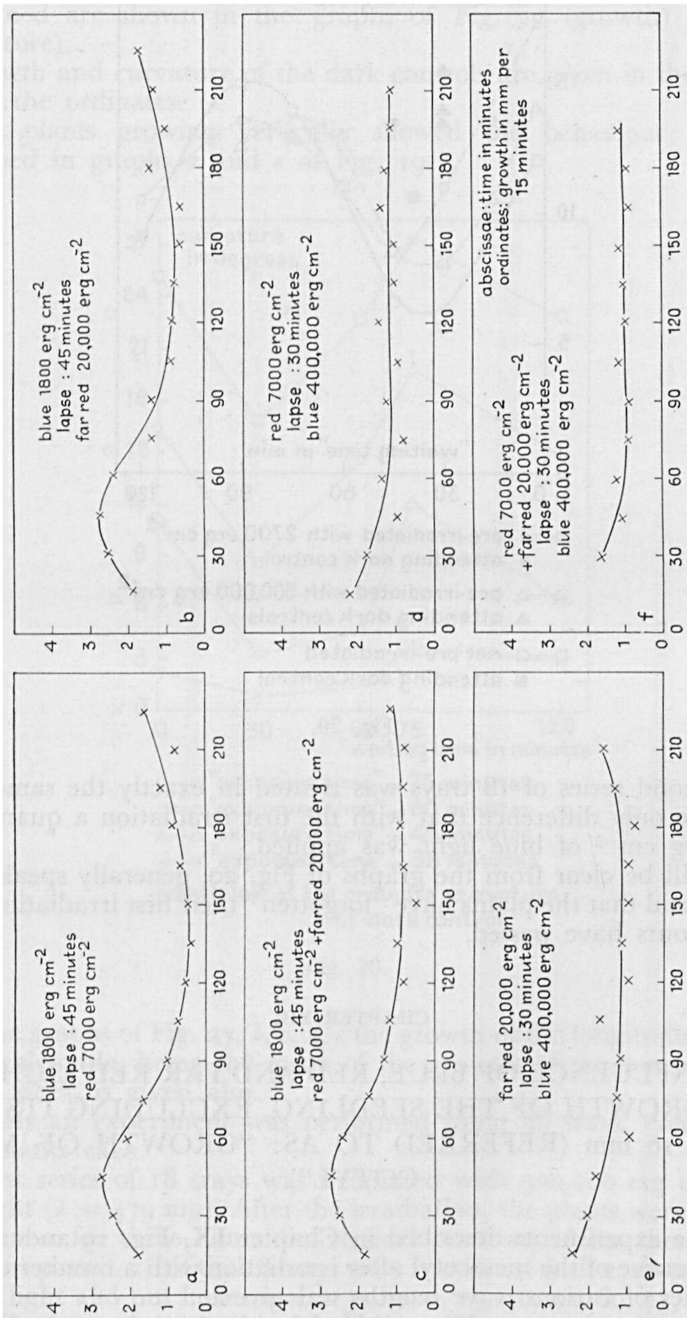

a
blue 1800 erg cm-2
lapse : 45 minutes
red 7000 erg cm-2
b
blue 1800 erg cm-2
lapse : 45 minutes
far red 20,000 erg cm-2
c
blue 1800 erg cm-2
lapse : 45 minutes
red 7000 erg cm-2 +far red 20,000 erg cm-2
d
red 7000 erg cm-2
lapse : 30 minutes
blue 400,000 erg cm-2
e
far red 20,000 erg cm-2
lapse : 30 minutes
blue 400,000 erg cm-2
f
red 7000 erg cm-2
+far red 20.000 erg cm-2
lapse : 30 minutes
blue 400,000 erg cm-2
abscissae: time in minutes
ordinates: growth in mm per 15 minutes
0 30 60 90 120 150 180 210
0 1 2 3 4

Fig. 30.

will follow in Fig. 30 *a-f*. The dark controls are the same as that of Fig. 19.

From Fig. 19*e* and 30*a*, *b* and *c* it can be seen, that, from the pre-irradiations investigated, only a pre-irradiation with 1800 erg cm^{-2} of blue light induces a growth response in the mesocotyl. In all other cases a decreased growth of the mesocotyl after pre-irradiation is observed.

Apparently the reaction upon irradiation of the mesocotyl is not correlated to that of the coleoptile in a simple manner. The phenomena described may be discussed in comparison with data on the growth of mesocotyls, known from literature.

MER (1951) examined the possibility whether the growth of the mesocotyl is regulated by the auxin produced in the coleoptile. The results of his experiments exclude this possibility. In 1959 he concluded from experiments on the influence of CO_2 on the growth of the *Avena* seedling, that the growth of the mesocotyl and that of the coleoptile are correlated by the food supply from the seed.

If one assumes, that irradiation influences the geotropic reaction via the indole-3-acetic acid metabolism, our results, revealing no correlation between the reactions of the mesocotyl and the coleoptile, might be in agreement with Mer's findings in 1951. Pre-irradiation with 1800 erg cm^{-2} of blue light may influence the food distribution in the seedling. This would be in agreement with the view that in some cases irradiation influences the transport system of the seedling.

CHAPTER XIV

GENERAL DISCUSSION

Since in phototropism, tropistic and tonic effects are intermingled and phototropism therefore is difficult to explain, the present author had proceeded in such a way that the tonic effects are caused by light only and the tropistic effects are obtained by gravitational stimulation exclusively. In this way he hoped to deepen the insight in phototropic and geotropic phenomena. The results of his experiments, however, are cause of his loosing every mainstay.

Geotropic phenomena are usually met in terms of indole-3-acetic acid or of a co-factor to IAA (BRAUNER and HAGER, 1958). With respect to phototropic phenomena BLAAUW-JANSEN (1959) discussed the interaction of indole-3-acetic acid and a "red light factor". The existence of the latter she demonstrated in the *Avena* coleoptile.

Now from experiments described in this paper (Fig. 19*f*) it becomes evident that large quantities (400,000 erg cm^{-2}) of blue light increase the growth rate of the coleoptile and that, three hours after irradiation, the coleoptile behaves as if never irradiated, at least with respect to growth (Fig. 19*e*) and geotropic reaction (Fig. 29), in darkness as well as when irradiated once more.

OPPENOORTH (1941) performing estimations of the auxin contents of coleoptiles after irradiation with blue light (26,400 erg cm^{-2}, $\lambda = 436$ mμ) found a gradual increase of the auxin contents. This increase had not yet reached its maximum three hours after the irradiation. Analogous results he found after irradiation with 3000 erg cm^{-2} ($\lambda = 436$ mμ).

According to Oppenoorth irradiation with 330 erg cm^{-2} ($\lambda = 436$ mμ) causes a decrease in the auxin contents, but Fig. 19*d* of this paper shows that the growth of the coleoptile is not influenced after an irradiation with a quantity of blue light of this order of magnitude.

The growth of the coleoptile, therefore, obviously shows no parallel with the auxin contents as estimated by Oppenoorth.

TERPSTRA (1953) has critisized the method of extraction used by Oppenoorth. It seems clear from her investigations, that Oppenoorth not only estimated the "really free auxin" (present in "negligible" quantities), but also part of the "auxin complex" and part of the auxin that can be formed from "auxin precursors" during the extraction process. Oppenoorth performed the extraction by immersing the coleoptiles in ether of 4° C for at least five hours. Terpstra proposed a mode of extraction by which only "free auxin" and "auxin complex" should be determined, viz. grinding, freezing and subsequently extracting with water of 4° C. According to these procedure BLAAUW-JANSEN (1962) estimated the auxin contents of coleoptiles 90 minutes after irradiation with 2700 erg cm^{-2}, 120 minutes after irradiation with 2700 erg cm^{-2} and 60 minutes after irradiation with 400,000 erg cm^{-2}. All irradiations were performed with blue light ($\lambda =$ 479 mμ). In all cases she found a decrease of the auxin contents to $\frac{1}{2}$ or $\frac{1}{4}$ approximately of the contents of the dark controls.

The results of this method of extraction do not show any parallel between the quantity of auxin extracted and the growth response of the coleoptiles reported in this paper. Yet it is Terpstra's opinion that the "auxin complex" is responsible for growth.

If one has no intention to drop the idea that auxin has something to do with growth and geotropic curving, one is forced to assume that the extraction methods described do not lead to the extraction of the auxin-fraction responsible for the phenomena mentioned in this paper. It does not seem to lead any further to discuss the available data on auxin contents in this connection.

The lateral transport of auxin is another topic in literature concerning geotropism and phototropism. At first the present author was inclined to the view, that small as well as large quantities of blue light in some cases hamper the lateral transport. E.g. after an irradiation with 2700 erg cm^{-2} of blue light and applying a "waiting time" of 30 minutes a decreased geotropic reaction, combined with an increased growth of the concave side of the coleoptile, is observable. This would lead to the assumption that under these circumstances the growth of the convex side should be less than normal, presuming that the growth measured along the longitudinal axis is not influenced. From a number of experiments, however, most clearly from the experiment of Fig. 27, it can be seen that under the circumstances described, the growth measured along the longitudinal axis of the horizontal coleoptile is considerably enhanced and so is the growth of the convex side. This leads to a closer contemplation of the data from which it is possible to compare the growth of convex side, concave side and longitudinal axis of the horizontally growing coleoptile, with the growth of the vertical coleoptile. These data are compiled in the graphs of Fig. 12, 13, 19, 24, 25 and 27, which are all in agreement with each other.

Fig. 12, 13 and 27 show the growth of the concave side, the convex side and the longitudinal axis of the coleoptile.

There seems to be no correlation between the growth of the longitudinal axis and the curvature. The facts are that after various pre-irradiations and "waiting times", low curvatures are attended with an increased growth of the concave side, high curvatures with an increased growth of the convex side and medium curvatures with an increased growth of both the concave and convex sides, or with growth rates that do not differ from that of the dark controls. In one case (Fig. 13*e*), the increased curvature is attended with a very strongly increased growth of the convex side and an increased growth of the concave side of the coleoptile.

From Fig. 12*b*, *c* and *d*, but most clearly from Fig. 27 it can be seen that after pre-irradiation with 2700 erg cm^{-2} of blue light ($\lambda = 479$ $m\mu$) a diminished curvature is attended with an increased growth of the longitudinal axis, an increased curvature with a decreased growth of the axis. The most pronounced increase of the curvature ("waiting time" = 75 min), however, is not attended with the most pronounced decrease in growth of the axis; the latter occurring at a "waiting time" of 60 minutes.

In most cases shown in Fig. 12 and 13, perhaps with the exception of those given in Figs. 12*d* and 13*e*, the growth of the longitudinal axis of the horizontal coleoptiles is less than that of the comparable vertical ones. A similar effect of gravity on the tonus of coleoptiles is described by ANKER (1960) who demonstrated that in some instances a coleoptile that is turned every ten minutes from one side to the other, so as to be continually influenced by unilateral gravity, without being allowed to curve, shows a decreased growth compared to a coleoptile growing in the upright position. This geo-growth reaction occurred with non-decapitated coleoptiles as well as with decapitated ones that were immersed in solutions containing 0.05 mg/l of indole-3-acetic acid, but could not be demonstrated with decapitated coleoptiles immersed in solutions containing 0 or 0.2 mg/l. It should be mentioned that Anker's experiments were performed while using orange light (Schott OG2) as a dark room illumination.

As to the explanation of our finding of the two-peaked curves of Figs. 14*c*, *d*, *f*, *g*, *i*, and *k* (schematically reproduced also in Fig. 16), we attempted to correlate them with the phenomena described by Soekarjo and by Blaauw-Jansen. SOEKARJO (1961) demonstrated that at low IAA concentrations a maximum appears in the curvature v. IAA concentration curve of petioles of *Coleus sp.* BLAAUW-JANSEN

(1959) observed the following phenomena with *Avena* coleoptiles: At low IAA concentrations, after an irradiation with red light, she observed an increased growth of coleoptile sections that can be annihilated by a subsequent irradiation with far red light. Also, at low IAA concentrations, she found a maximum in the curvature v. IAA concentration curve, when, after irradiation with red light the curvature of coleoptiles was measured in the standard Avena test (Fig. 23). From coleoptiles that were irradiated with red light, Blaauw-Jansen was able to isolate a substance (the "red light factor"), exerting the same influence as red irradiation does. This substance probably also occurs in non-irradiated coleoptiles, but in a smaller quantity than in irradiated ones. In the light of these findings she was able to propose an attractive elucidation of the phenomena of first positive, negative and second positive phototropic curvature, as a gradual decrease of the IAA contents of the coleoptile is attended with a fall, a rise and another fall of the growth rate (Fig. 31).

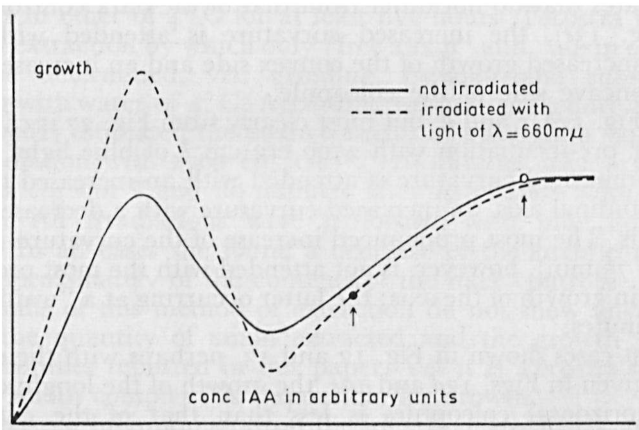


Fig. 31. (from Blaauw-Jansen, 1959)

From her considerations it is clear, however, that in her experiments, 90 minutes after irradiation, the tonic condition of the plant should essentially differ from that of non-irradiated plants.

In the experiment, described in Fig. 29 of this paper, we demonstrated that the tonic state of the plant, three hours after irradiation, is the same as that of non-irradiated plants. Though it seemed possible to correlate Blaauw-Jansen's considerations mentioned above, Oppenoorth's findings concerning the decreasing auxin contents upon irradiation and our finding of the two-peaked curves of Fig. 14, this possibility vanished in the light of the experiment described in Fig. 29 of this paper.

From Figs. 12*e*, *f*, *h*, *i*, and *j* it is obvious, that the increase of the curvation produced after irradiation with red light ("waiting time" = 30 min) is attended with an enhanced growth of the convex side of the coleoptile and with a reduced growth of the concave side. The fact that the influence of pre-irradiation with red light on the geotropic reaction is not observable after a "waiting time" of 60 minutes cannot be ascribed to a restoration of the original situation, as after a "waiting time" of 60 minutes the growth of the concave as well as of the convex side of the coleoptile and concurrently the growth of the longitudinal axis is enhanced, and the same is true for the growth of the vertical coleoptile in those circumstances. Moreover, BLAAUW-JANSEN (1959) demonstrated that in experiments concerning phototropism, the influence of pre-irradiation with red light was observable up to six hours after its administration.

It is remarkable too, that the stimulation of the geotropic reaction by red light ("waiting time" = 30 min) (Fig. 12*f* and *i*) shows the same pattern of growth influences as the stimulation by 400,000 erg cm^{-2} of blue light does ("waiting time" = 10 min) (Fig. 13*e*). Red light, however, in contradistinction to blue light, shows no phototropic activity.

The influence of small quantities of blue light (2700 erg cm^{-2}) seems to be of another nature. From Fig. 6 it can be seen that with low quantities of blue light, the influences of red and blue light are additive; with high quantities of blue light, the influence of red light is absent or, at the least, less pronounced. This leads to the assumption, that much blue light is comparable to little blue light + red light. To check the correctness of this assumption, it would be necessary to try whether the curve of Fig. 14*d* can be transformed in that of Fig. 14*i* by pre-irradiation with 1800 erg cm^{-2} of blue light + 7000 erg cm^{-2} of red light, applying the "waiting times" mentioned in these figures. This, however, does not seem likely.

In all the cases investigated, far red light ($\lambda = 735$ mμ) exerts the same influences as red light ($\lambda = 660$ mμ) does, when administered in combination with various quantities of blue light as well as in combination with red light, or when administered by itself. No red-far red antagonism could be observed.

From our experiments and our attempts to discuss them it becomes clear, that the interpretation of the phenomena described is severely complicated by the fact that unilateral gravity itself has not only a tropistic effect on the coleoptile, but a tonic effect as well, and therefore the present author has not succeeded in his intention to disentangle tonic and tropistic influences.

The only conclusion is, that it seems impossible to interpret the tonic and tropistic phenomena in the terms of the conventional auxin theory.

SUMMARY

Seeds of *Avena sativa* were husked, wetted, germinated for 19 hours on moist filter paper, during which period they were irradiated with orange light (Schott OG2) and subsequently grown in total darkness in moist vermiculite for c. 65 hours. Then they were irradiated with violet, blue, red or far red light (interference filters of λ=415, 479, 660 and 735 mμ resp.) and at once, or some time after the irradiation the growth and the geotropic curving reaction (further referred to as: "geotropic reaction") of the coleoptiles and in some cases the growth of the whole plants, were measured.

For necessary manipulations and for shadowgraphing, very dim green light (interference filter of $\lambda = 560$ mμ) was used.

Irradiations were given from directly above, unless otherwise stated.

As light intensities of c. 1000 erg cm^{-2} sec^{-1} were at our disposal, light quantities up to 500,000 erg cm^{-2} could be administered within a period of ten minutes.

Each of the pre-irradiations mentioned above exerts an influence on the growth of the coleoptile and on the geotropic reaction. This influence is only marked during a relatively short period, occurring a shorter or longer time after the irradiation. In practically all cases, the "exposure time", i.e. the time lapse between the moment of putting the plants horizontal and the moment of registration of the reaction, was one hour. The time lapse between the beginning of the irradiation and the moment of placing the plants horizontal was termed "waiting time".

Red light only

Quantities from 4 up to c. 7000 erg cm^{-2} of red light exert an increasing influence on the geotropic reaction. Larger quantities (up to 800,000 erg cm^{-2}) show the same effect as 7000 erg cm^{-2} does.

As compared to dark controls, the coleoptiles show a number of effects of pre-irradiation with red light, that are compiled in the third part of table 26. The effects mentioned in table 26, are the effects occurring during a period of 60 minutes immediately after the "waiting time".

Table 26

"Waiting time"	Little blue light (1000–10,000 erg cm^{-2})				
	curvature	growth of the vertical coleoptile	growth of the horizontal coleoptile: convex side	growth of the horizontal coleoptile: concave side	growth of the horizontal coleoptile: longitudinal axis
0 min.	0	0	0	0	0
30 min.	--	0	0	++	+
60 min.	0	—	—?	—?	—?
75 min.	+	0	—	--	—
120 min. or more	0	0	0	0	0
	Much blue light (100,000–500,000 erg cm^{-2})				
0 min.[3]	++	+	++	+	++
30 min.	0	+	++	++	++
60 min.	0	0	0	0	0
75 min.	+	0			
120 min. or more	0	0			
	Red or far red light (7000 or 20,000 erg cm^{-2} resp.)				
0 min.	0	—[1]	0	0	0
30 min.	++	+	+	0	+
60 min.	0	+	+	+	+
75 min.		+			
120 min. or more		+ [2]			

[1] A decrease followed by a restoration.
[2] A gradually diminishing increase.
[3] "Waiting time" > 0 min; the time, necessary for illumination, amounting to ten minutes.

0 = no effect
+ = increase
++ = strong increase
— = decrease
-- = strong decrease
? = no clear-cut effect

Far red light only

Essentially the same pattern of influences as that of red light is obtained with far red light, except for the fact that the quantities needed are about three times higher.

Blue light only

The influence of relatively small quantities of blue light (1000—10,000 erg cm^{-2}) seems essentially different from that of large quantities (100,000—500,000 erg cm^{-2}).

The effects of small and large quantities of blue light as compared with dark controls, measured after various "waiting times" are compiled in the first and second part of table 26. Here too, the effects mentioned are the effects that occur during the period of 60 minutes immediately following the "waiting time".

Violet light only

In the few cases that were investigated, small and large quantities of violet light showed the same pattern of influences as was observed with blue light, only somewhat larger quantities of violet light were necessary to obtain effects comparable with that of the pre-irradiation with blue light.

Combinations of irradiations

Generally speaking it can be said that in the investigated cases, the effects of irradiation with blue + red light are additive, except for very large quantities of blue light. Each of the irradiations exerts its own influence, with respect to light quantity as well as to "waiting time". The same seems to be true for blue + far red light.

In several ways it has been attempted to demonstrate red-far red antagonism, which has not been found.

Phototropism and geotropism

Pre-irradiation with a range of quantities of blue light exerts the same influence on the geotropic reaction as is observed after pre-irradiation with the same range, administered from two sides.

When this range is administered unilaterally, first positive, negative and second positive phototropic curvatures are induced.

The curvature v. light quantity curve of the geotropic reaction shows no correlation with that of the phototropic reaction.

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