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THE INFLUENCE OF RED AND FAR RED LIGHT ON GROWTH AND PHOTOTROPISM OF THE AVENA SEEDLING

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CONTENTS

INTRODUCTORY REMARKS

Red light is perceived by the primary leaf of the Avena seedling, and produces or activates an unidentified substance, which we termed the red-light factor (R.L.F.). Red light also reduces the content of indole-3-acetic acid (I.A.A.) in the coleoptile, and increases the first positive phototrophic curvature induced by blue light. At low I.A.A. concentrations the R.L.F. enhances the growth rate: the curve presenting growth as a function of the I.A.A. concentration, which normally shows a single peak, is in this way transformed into a two-peaked curve. The "disposition" phenomena in phototropism are due to the interaction of the R.L.F. and I.A.A. The implications of the use of red light for the commercial growth-substance tests were studied. Far red light too reduces the I.A.A. content of the coleoptile, but it counteracts and eventually annihilates the effect of red light on the formation of the R.L.F.

CHAPTER I

INTRODUCTION AND METHODS

1. Introduction

The dark rooms in which experiments with Avena coleoptiles are made usually are lighted with orange or red light, although it is

known that coleoptiles do respond in some way to these regions of the spectrum.

According to BLAAUW (1909) and to ZOLLIKOFER (1920) red light has a *phototropic* effect on the coleoptiles, but later investigators, who probably used a purer red light, could not confirm this effect.

An effect of red light on the *growth* of coleoptiles was found by VOGT as early as 1915, and this effect was confirmed by KONINGSBERGER (1922), AVERY (1937), SCHNEIDER (1941), LIVERMAN and BONNER (1953) and DE LINT (1957).

It seemed at first sight unlikely that red light would affect the rate of growth, but not the phototropic response of the coleoptiles, as a phototropic curvature is due to a difference in the rate of growth at the opposite sides of unilaterally illuminated coleoptiles. For this reason we decided to see for ourselves whether there was a phototropic response. To this end we placed coleoptiles in a spectrum, and observed that phototropic curvatures were actually formed in the red part of the spectrum too. On closer examination this phenomenon appeared to be due to scattered blue light, but a rough estimate of the intensity of the scattered light showed that the latter could not have caused a curvature of the size that was actually obtained.

This suggested that the red light itself did not induce a curvature, but that it influences the phototropic response to blue light. This possibility was investigated in a series of experiments described in this paper.

2. MATERIAL AND GENERAL METHODS

The experiments were made with seedlings of *Avena sativa* c.v. "*Siegeshafer*". Unhusked grains were thoroughly wetted by shaking them in tap water, and put to germinate on moistened filterpaper in a closed petri dish; the latter was placed under an orange lamp at a temperature of 22° C. After 24 hours the grains were planted in moist vermiculite or in glass holders in a dark room which was kept at the same temperature of 22° C and at a relative humidity of 75 %. When the plants were 88 hours old, they were used for the experiments.

All solutions of indole-3-acetic acid (I.A.A. or heteroauxin) were made with tap water containing 750 mg/l KCl and 0.2 ml/l glacial acetic acid.

The orange light was obtained from incandescent lamps (220 Volt, 20 Watt) by the use of Schott OG 2 filters. Monochromatic light was produced by an incandescent lamp (12 Volt, 100 Watt) in combination with a set of lenses in order to get a parallel beam and with an interference filter made by BALZER (Liechtenstein), of which the tolerance was 0.5 %. Monochromatic light of higher intensity was obtained by the use of an approximatively parallel beam from a more powerful incandescent lamp (60 Volt, 3000 Watt) in combination with a similar interference filter. The light intensity was measured either by means of a thermopile after MOLL (Kipp, Delft) or with a barrier-layer cell from Tungsram. The sensitivity of the thermopile was not

sufficient for the measurement of the lower light intensities. Therefore these measurements were carried out by means of the barrier-layer cell which was calibrated for each wave length by means of the thermopile. As an indicator instrument a ZERNIKE galvanometer (Kipp, Delft) was used. In view of the large variability of the plant material no greater precision than 10 % was aimed at.

Whenever an exact wave length is mentioned, transmission spectra obtained with the interference filters are meant; Fig. 1 is a representative example of such a spectrum. The half-peak width in each case is $\pm$ 8 mμ. A summary of the light sources and filters that were used and of the light intensities that were obtained are given in the table on page 4. The figures given in the tables on experiments are

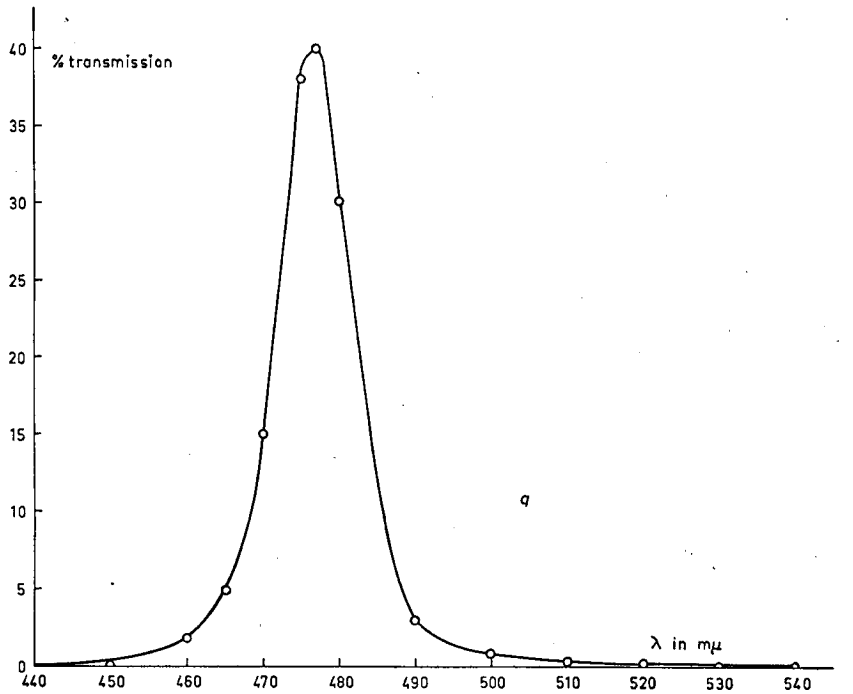

Fig. 1. Example of a transmission spectrum obtained by means of an interference filter.

the mean values of the curvatures, or of the rate of growth, measured in a number of coleoptiles. The variation is expressed as the standard error of the mean:

$$SE_M = \pm\sqrt{\frac{\sum (v-m)^2}{n\,(n-1)}}$$

n = the number of coleoptiles (always mentioned between brackets)
$\sum(v-m)^2$ = the sum of the squared deviations from the mean.

Sometimes the results are given in the form of a graph. The figures

Light	Light source: incandescent bulb		Filter		Light intensity in ergs/cm² sec.
colour, resp. wave length	energy in Watts	tension in Volts	kind	transmission maximum at λ	
Orange	20	220	Schott	> 550 mμ	400
Green	75	220	Schott	520–620 mμ	400
λ in mμ					
480	100	12	int. f.	477 mμ	0.5
	3000	60	int. f.		20
560	100	12	int. f.	562 mμ	0.6
	3000	60	int. f.		20
580	100	12	int. f.	586 mμ	0.5
600	100	12	int. f.	595 mμ	0.9
620	100	12	int. f.	614 mμ	1.4
640	100	12	int. f.	644 mμ	1.9
660	100	12	int. f.	657 mμ	2.1
	3000	60	int. f.		70
680	100	12	int. f.	682 mμ	2.9
700	100	12	int. f.	702 mμ	2.3
740	100	12	int. f.	741 mμ	2.5
	3000	12	int. f.		50

from which the graphs were drawn, are, moreover, always given in a table.

The following general policy was pursued: whenever a conclusion was derived from the results of two or three experiments of the same kind, we did not try to test its validity by repeating the same experiments, but by checking its implications.

Where small differences between experimental series are to be expected, this method presumably should be preferred above an attempt to reduce the error of such a difference by greatly increasing the number of experiments.

CHAPTER II

INFLUENCE OF ORANGE LIGHT

1. Experiments on phototropism

In the first place we investigated whether orange light influences the size of the curvature induced by unilateral illumination with blue light.

Method

Germinated seeds were placed vertically, with the embryo pointing downwards, in vermiculite, 8 in a row. The plants were kept in absolute darkness, except for a period of ten minutes 46 hours after they had been placed in the vermiculite, at which time they were watered.

When the plants were 88 hours old, each series of 8 plants was transferred, also in absolute darkness, to the illumination apparatus. The coleoptiles were exposed with their narrow side to an unilateral

illumination with different amounts of light of $\lambda = 480$ mμ, either at once or after a previous illumination with light of a different wave length.

$1\frac{1}{2}$ hours after the illumination with light of $\lambda = 480$ mμ the coleoptiles were severed from the seeds, placed on photographic paper, and shadowgraphed. The curvatures were measured by means of a protractor.

Experiments

Coleoptiles, whether pre-illuminated from above with 36×10^4 ergs/cm^2 orange light or not pre-illuminated, were illuminated unilaterally with progressively larger amounts of light of $\lambda = 480$ mμ, which all induced a first positive curvature.

In Table I the curvatures measured in two experiments are given. The results of experiment I have been plotted in Fig. 2.

TABLE I

Exp. No.	Light quantity $\lambda = 480$ mμ in ergs/cm^2	Not pre-illuminated curvature in degrees	Pre-illuminated with 36.10^4 ergs/cm^2 orange light curvature in degrees
I	1	6.3 ± 0.5 (6)	4.7 ± 0.8 (8)
	2	10.4 ± 1.6 (8)	9.0 ± 0.8 (9)
	3	12.8 ± 1.1 (8)	9.0 ± 0.9 (7)
	5	13.0 ± 1.2 (6)	16.4 ± 1.4 (8)
	20	12.0 ± 1.1 (10)	21.1 ± 1.0 (7)
	120	8.5 ± 0.3 (12)	22.9 ± 1.0 (8)
II	1	8.0 ± 2.5 (4)	7.7 ± 1.0 (6)
	5	14.0 ± 1.0 (9)	21.2 ± 1.0 (6)
	10	14.0 ± 0.9 (7)	23.2 ± 3.2 (5)
	20	13.7 ± 1.0 (7)	23.5 ± 3.5 (4)
	30	12.5 ± 1.4 (8)	19.4 ± 1.8 (5)

From these experiments it appears that orange light undoubtedly influences the phototropic curvature of the coleoptiles in such a way that the maximum value is enhanced from 14 to 23 degrees, which really seemed surprising.

Now the question arises why no curvatures are observed after unilateral illumination of the coleoptiles with red or orange light. This might be explained by the aid of various suppositions.

a. The coleoptile contains but a very small amount of the pigment that absorbs the orange light. Consequently with unilateral illumination the light-intensity gradient in the coleoptile is not considerable, and light saturation at the light and the shade side is already reached when but relatively small amounts of orange light are administered. In that case curvatures could be expected only after illumination with very low intensities of orange light. It appeared however that under this condition too no curvatures are obtained. This supposition therefore may be discarded.

b. Orange light induces the formation of more blue-absorbing pigment with as a consequence a steepening of the light-intensity

gradient in the coleoptiles when they are illuminated afterwards unilaterally with blue light. The reduction of the first positive phototropic curvature after irradiation with increasing light quantities admittedly may be due to the fact that the growth at the shade side

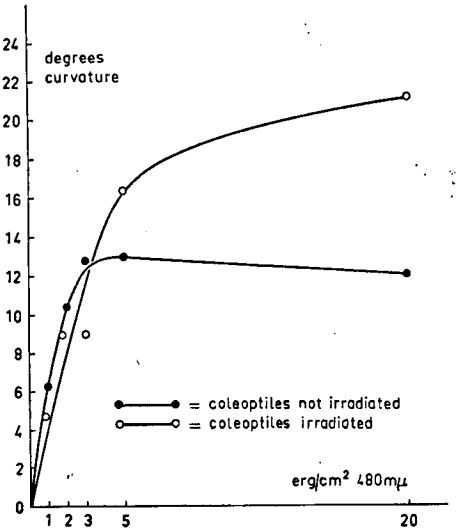

Fig. 2. The phototropic curvature of coleoptiles: • not pre-irradiated, ○ pre-irradiated with orange light.

too begins to react upon the illumination. The presence of more blue-absorbing pigment therefore would entail that a larger amount of blue light would be needed to produce this reaction of the shade side. In this case orange light should have no influence if it was applied after the illumination with blue light. In Table II the curvatures are recorded of coleoptiles which were irradiated with orange light either before or after the illumination with blue light. It appears that there is no difference in the size of the curvatures.

TABLE II

Unilateral illumination	Curvature in degrees
20 ergs/cm² λ = 480 mμ	14.7 ± 1.3 (10)
20 ergs/cm² λ = 480 mμ, followed by 36 × 10^4 ergs/cm² orange light from above	27.9 ± 1.1 (14)
20 ergs/cm² λ = 480 mμ, preceded by 36 × 10^4 ergs/cm² orange light from above	28.5 ± 1.4 (13)

Moreover, it could be shown that pre-illumination with orange light from the future shade side of the coleoptile has the same effect as pre-illumination from the future light side (Table III).

TABLE III

Unilateral illumination with 20 ergs/cm² λ = 480 mμ after

unilateral pre-illumination	curvature in degrees
none	10.7 ± 0.9 (9)
9.6 × 10^4 ergs/cm² orange light from the future light side	22.7 ± 2.0 (7)
9.6 × 10^4 ergs/cm² orange light from the future shade side	22.7 ± 0.8 (9)

For these reasons we consider it unlikely that orange light would induce the formation of a blue-absorbing pigment.

c. A third way to explain the effect of orange light was derived from the results of a number of experiments on the place where the orange light is perceived.

It could be demonstrated that particularly the tip of the seedling is sensitive to orange light.

2. Location of the sensitivity to orange light

Method

In absolute darkness the tips of the coleoptiles were supplied with tin foil caps of 4 mm length, the very same that Arisz had used in 1914 to study the influence of illumination on the base part of the coleoptile.

Then the coleoptiles were supplied with a dose of orange light, whereupon the caps were removed in darkness. Next the coleoptiles were unilaterally illuminated with light of $\lambda = 480$ mμ (20 ergs/cm^2) in order to obtain a first positive curvature as large as possible.

Experiments

TABLE IV

Unilateral illumination with 20 ergs/cm^2 $\lambda = 480$ mμ after

Exp. Nr.	Pre-illumination	Curvature in degrees
I	none	10.7 ± 0.9 (9)
	9.6×10^4 ergs/cm^2 orange light, without caps	22.7 ± 2.0 (7)
	9.6×10^4 ergs/cm^2 orange light, with caps	9.7 ± 1.9 (7)
	28.8×10^5 ergs/cm^2 orange light, with caps	15.5 ± 2.7 (6)
II	none	13.0 ± 1.7 (7)
	24.6×10^4 ergs/cm^2 orange light, without caps	19.0 ± 1.4 (12)
	24.6×10^4 ergs/cm^2 orange light, with caps	11.0 ± 1.4 (11)

From Table IV in which the resulting curvatures are recorded, it is clear that orange light has no influence at all or but a very slight influence if the upper most 4 mm of the seedling are screened from it.

Since the possibilities *a* and *b* had to be rejected, one cannot arrive at another conclusion than that it is not the tip of the coleoptile, but the tip of the primary leaf that responds to the orange light.

It was technically impossible to confirm this conclusion by removing the primary leaf in darkness and by studying the reactions to orange light of the remainder of the plant.

Therefore we passed on to the application of the standard Avena test, because it could be shown that orange light influences the curvatures in the standard Avena test as well.

3. The effect of orange light on the standard Avena test

Method

Avena seedlings were cultivated in glass holders as described in chapter I. They were illuminated with orange light while germinating

on filter paper and also during the first 24 hours after they had been put in the glass holders. From the 48th hour on till the test they were growing in absolute darkness, except at the 72nd hour when a selection was carried out in orange light. When the plants were 88 hours old, the relative humidity of the conditioned room was raised from 75 % to 96 %.

The racks with their 12 plants remained in absolute darkness till the very moment of decapitation. With part of the plants the first decapitation was performed in darkness; the remainder were decapitated in orange light.

The coleoptiles were decapitated for a second time after an sojourn of 2 hours in green light ($\lambda = 560$ mμ). The primary leaf was loosened after the second decapitation because this manipulation could not be performed in darkness.

Two hours after the second decapitation agar blocks of 3.6 mm^3 soaked in a solution of I.A.A., were placed unilaterally upon the stumps. After another two hours shadowgraphs of the test plants were taken. Between these actions the plants were kept in the dark.

Experiments

The curvature of plants which had been decapitated in orange light was compared with the curvature of plants which had been gropingly decapitated in darkness. After the first decapitation all manipulations were performed in green light. In Table V the curvatures produced by agar blocks containing various amounts of I.A.A. are presented.

TABLE V

I.A.A. concentration in g/ml	Curvature in degrees: Decapitation in the dark	Decapitation in orange light
$0.25 \cdot 10^{-7}$	9.0 ± 0.5 (11)	7.8 ± 0.9 (9)
$0.50 \cdot 10^{-7}$	12.5 ± 0.5 (22)	11.4 ± 0.9 (19)
$0.75 \cdot 10^{-7}$	17.6 ± 1.0 (12)	15.2 ± 1.0 (9)
$1.00 \cdot 10^{-7}$	21.2 ± 2.4 (9)	13.5 ± 0.5 (11)
$1.25 \cdot 10^{-7}$	14.1 ± 0.7 (23)	14.7 ± 0.5 (22)
$2.00 \cdot 10^{-7}$	14.9 ± 1.5 (10)	12.1 ± 1.2 (12)

It appears that orange light applied during the first decapitation reduces the size of the maximum curvature (at a concentration of I.A.A. of 10^{-7} g/ml) from about 21° to about 14°. This effect of orange light is unexplained, but it seemed a suitable means to check the supposed perception of the orange light by the primary leaf.

To that purpose the first decapitation was carried out with part of the plants in orange light and with the control plants in darkness. In case the plants were decapitated in orange light, the primary leaf was loosened either immediately after the first decapitation or after the second.

In the first case the primary leaf was left intact only during two or three minutes after it had been exposed to the orange light, in the second case it remained intact for two hours. Further manipulations were carried out in green light of $\lambda = 560$ mμ.

The solution of 10^{-7} g/ml I.A.A. was the only one that was used in

these tests, because in preliminary experiments the effect of orange light proved to be largest at this concentration.

It appears (Table VI) that orange light reduces the curvature only when the primary leaf is left intact during some time after the irradiation. It therefore was concluded that indeed it is the primary leaf which perceives the orange light.

TABLE VI

First decapitation	Primary leaf removed	Curvature in degrees
in the dark	after 2nd decapitation	15.7 ± 0.7 (33)
in orange light	after 1st decapitation	16.6 ± 0.6 (24)
in orange light	after 2nd decapitation	12.9 ± 0.4 (32)

The experiments on the influence exercised by orange light on coleoptiles with screened tip showed that it should be particularly the tip of the primary leaf that responds to the orange light.This was checked as follows.

At the first decapitation, performed either in orange light or in darkness, the tip of the seedling was removed as a whole, whereas in the control series the tip of the coleoptile was taken away but the tip of the primary leaf was left intact. At the second decapitation in all series the primary leaf was loosened. Except the first decapitation all manipulations were carried out in green light of $\lambda = 560$ mμ. A solution of 10^{-7} g/ml I.A.A. was used as a test solution.

From Table VII it is obvious that orange light reduces the curvature only if the tip of the primary leaf is left intact during some time after the irradiation.

TABLE VII

First decapitation	Tip of the primary leaf	Curvature in degrees
in the dark	intact	16.1 ± 0.7 (35)
in the dark	removed	17.1 ± 0.7 (33)
in orange light	intact	12.8 ± 0.6 (35)
in orange light	removed	15.9 ± 0.6 (33)

4. Conclusion and discussion

From the preceding series of experiments it is clear that orange light influences the phototropic reactions and the reaction on I.A.A. of Avena coleoptiles via the tip of the primary leaf. That the tip of a leaf may play a special part, did come to our knowledge from a publication of Sharman (1942), who observed that in *Zea* leaves elongation as well as the differentiation of the tissues start at the leaf tip and from a paper of Roberts (1951), who stated that in wheat the tip of the first leaf has a larger chlorophyll content than the rest of the leaf.

We did not occupy ourselves with the reactions of the primary leaf to orange light, but we confined our investigations to the indirect consequences of such an illumination appearing in the behaviour of the coleoptile. By what means the primary leaf influences the coleoptile, will not be considered. Maybe some substance is formed by the leaf and transported to the coleoptile, maybe the growth of

the primary leaf is influenced by the orange light in such a manner that the substances which are transported upwards from the grain, are distributed differently between coleoptile and primary leaf.

5. SUMMARY

Orange light increases in Avena coleoptiles the maximum size of the first positive curvature induced by unilateral illumination with blue light. The orange light is probably perceived by the tip of the primary leaf. The standard Avena test too is influenced by orange light, which reduces the curvatures caused by the unilateral application of I.A.A. (10^{-7} g/ml). In this case too the tip of the primary leaf proved to be the place of perception of the orange light.

CHAPTER III

DETERMINATION OF THE MOST EFFECTIVE WAVE LENGTH

An action spectrum for the influence which light of the longer wave lenghts exercises on the Avena coleoptile is not known. An action spectrum for the inhibition of growth in the mesocotyl was obtained by WEINTRAUB and PRICE (1947) as well as by GOODWIN and OWENS (1948). However, since it is uncertain whether the same mechanism is responsible for the way in which the coleoptile and the mesocotyl react to wave lengths larger than $\lambda = 600$ mμ, it is not justified to assume that the action spectrum will be the same for both reactions.

We investigated which wave length was most effective in influencing the phototropic curvature of Avena coleoptiles in the way described in chapter II. The variability of the plant material however was so large that it proved impossible to determine an approximatively accurate action spectrum.

Coleoptiles were cultivated as described in chapter II under the heading "phototropism". They were pre-illuminated unilaterally with light of various wave lengths (in chapter II it was shown that the effect of pre-illumination is independent of the direction from which it is applied). Immediately after being pre-illuminated they were irradiated one-sidedly with 20 ergs/cm^2 $\lambda = 480$ mμ, i.e. with the amount of light which gives a maximum positive curvature.

The results of these experiments are summarized in Table VIII. The values given for the curvatures are the mean of six experiments.

TABLE VIII

Pre-illuminated with	Phototropic curvature in degrees
-----	13.1 ± 0.4 (109)
30–70 ergs/cm^2 $\lambda = 560$ mμ	13.4 ± 0.6 (51)
30–70 ergs/cm^2 $\lambda = 580$ mμ	14.9 ± 0.5 (64)
30–70 ergs/cm^2 $\lambda = 600$ mμ	14.1 ± 0.7 (36)
30–70 ergs/cm^2 $\lambda = 620$ mμ	14.3 ± 0.6 (51)
30–70 ergs/cm^2 $\lambda = 640$ mμ	13.6 ± 0.6 (46)
30–70 ergs/cm^2 $\lambda = 660$ mμ	17.8 ± 0.9 (36)
30–70 ergs/cm^2 $\lambda = 680$ mμ	17.1 ± 1.3 (31)
30–70 ergs/cm^2 $\lambda = 700$ mμ	12.6 ± 0.9 (36)

In each experiment the influence of a complete series of wave lengths was investigated in order to avoid difficulties caused by the daily variability of the plants.

It appears that light of the wave lengths $\lambda = 660$ mμ and 680 mμ was most effective in enhancing the maximum size of the first positive curvature. So it was decided to continue our experiments with light of $\lambda = 660$ mμ.

The joint effect of the variability shown by the response to red light and the variability of the phototropic reaction to the blue light, however, makes it impossible to arrive at a complete action spectrum by means of these experiments. To this purpose a process more directly dependent on the red light should be used as indicator, preferably the increase in length of the coleoptiles. A sensitive auxanometer (KONINGSBERGER, 1922) should be used to this end.

SUMMARY

In this chapter it is shown that with the Avena coleoptiles light of the wave lengths $\lambda = 660$ and 680 mμ is most effective in enhancing the maximum size of the first positive phototropic curvature caused by blue light.

CHAPTER IV

THE REACTION OF THE AVENA COLEOPTILE TO LIGHT OF $\lambda = 660$ mμ IN CONNECTION WITH ITS GROWTH-SUBSTANCE METABOLISM

1. DATA FROM THE LITERATURE

Several authors have tried to connect the reactions of plants to red light with their growth-substance metabolism. HILLMAN and GALSTON (1957) summarize the literature on this subject, but in view of the fact that too different materials were treated in too different ways, they consider any attempt to discuss their own work in terms of the reports cited by them as sheer speculation. We subscribe to the view of these authors.

LIVERMAN and BONNER (1953) are the only workers to choose the Avena coleoptile as experimental object. According to their results red and far red light influence the reactions of isolated coleoptile cylinders to heteroauxin. In the hope that the influence of red light on the phototropic behaviour of the coleoptiles might be explained by the findings of these authors, we tried to reproduce their results; however, without success. Probably the discrepancy between their results and ours should be attributed to the use of different light quantities. In our experiments the coleoptile cylinders were illuminated with 700 ergs/cm^2 $\lambda = 660$ mμ, whereas in the experiments of LIVERMAN and BONNER a much larger quantity of light was applied during 30 minutes (filtered light obtained from 2 daylight fluorescent tubes was used). Obviously their experiments cannot be compared to ours. Still the possibility existed that illumination with light of $\lambda = 660$ mμ influences the heteroauxin content of the coleoptile.

To avoid the difficulties and problems with which extraction and quantitative determination of I.A.A. are beset, the I.A.A. content of the coleoptiles was estimated by means of the cylinder test.

Maximal growth of coleoptile cylinders derived from illuminated plants should occur at an I.A.A. concentration of the medium which differs from the I.A.A. concentration inducing maximum growth in cylinders cut from non-illuminated plants.

2. Cylinder test (straight-growth test)

METHOD

The coleoptiles were cultivated in vermiculite as described in chapter I. When the plants were 88 hours old, part of them was illuminated with 700 ergs/cm^2 $\lambda = 660$ mμ, and part was kept in darkness. 1½ hours after the illumination the coleoptiles were placed in a coleoptile microtome after Van der Wey, and 3 mm tips were taken off and rejected. The next 3 mm sections were used in the experiments. Twelve sections were floated on the surface of 10 ml of a solution of I.A.A. in a buffer of 0.0025 m K-maleate adjusted with KOH on a pH of 4.5 and containing 3 % sucrose. All manipulations were carried out in green light of $\lambda = 560$ mμ. After three hours the length of the sections was measured under a microscope.

EXPERIMENTS

A series of experiments was carried out in each of which the influence of a range of I.A.A. concentrations was tested on sections of irradiated and non-irradiated plants. In Table IX the final lengths of the sections are shown in units of the eye-piece micrometer (50.9 units = 3 mm).

Experiments I and II are examples of experiments in which the optimum concentration of I.A.A. was estimated. In experiment I the optimum I.A.A. concentration seemed to be 10^{-8} g/ml for the growth of sections of irradiated plants, against 2.10^{-8} g/ml for the growth of sections of non-irradiated ones. In experiment II however sections of irradiated plants appear to show two growth maxima at different I.A.A. concentrations, whereas sections of non-irradiated plants appear to show only one growth maximum. This phenomenon was confirmed in experiments in which lower I.A.A. concentrations were used. Two examples are presented in Table IX, III and IV and in Fig. 3.

DISCUSSION

Pre-illumination of intact plants with light of $\lambda = 660$ mμ undoubtedly influences the way in which coleoptile sections subsequently react on I.A.A. The essential difference between the concentration-growth curves of coleoptile sections obtained from irradiated and non-irradiated plants may be seen in two fundamentally different ways.

1. The concentration-growth curve of irradiated coleoptile sections shows two maxima, whereas the curve of non-irradiated sections

TABLE IX

Illumination with 700 ergs/cm² $\lambda = 660$ mμ. Final length of sections

Concentr. of I.A.A. in g/ml	I Plants		II Plants	
	illuminated	not illuminated	illuminated	not illuminated
0	52.0 ± 0.7	53.0 ± 0.4	48.5 ± 0.5	51.4 ± 0.4
6.10^{-9}	—	—	54.4 ± 0.3	53.4 ± 0.2
1.10^{-8}	54.1 ± 0.3	51.1 ± 0.2	50.6 ± 0.2	53.2 ± 0.3
2.10^{-8}	53.7 ± 0.4	54.9 ± 0.3	—	—
6.10^{-8}	—	—	50.1 ± 0.4	55.1 ± 0.3
1.10^{-7}	53.3 ± 0.5	53.3 ± 0.3	52.1 ± 0.3	55.1 ± 0.5
1.10^{-6}	52.8 ± 0.5	52.5 ± 0.5	54.3 ± 0.5	55.8 ± 0.6
1.10^{-5}	51.2 ± 0.3	51.0 ± 0.2	—	—
	III		IV	
0	48.8 ± 0.4	51.4 ± 0.6	50.6 ± 0.1	52.4 ± 0.2
1.10^{-10}	52.5 ± 0.3	50.0 ± 0.3	51.2 ± 0.3	51.9 ± 0.3
1.10^{-9}	51.9 ± 0.4	50.2 ± 0.2	52.6 ± 0.1	52.6 ± 0.3
3.10^{-9}	55.8 ± 0.5	50.5 ± 0.2	54.1 ± 0.3	51.9 ± 0.2
1.10^{-8}	50.5 ± 0.3	53.5 ± 0.3	52.3 ± 0.2	53.0 ± 0.2
3.10^{-8}	54.3 ± 0.3	55.4 ± 0.5	55.5 ± 0.3	54.7 ± 0.3
1.10^{-7}	55.8 ± 0.3	55.9 ± 0.2	56.5 ± 0.5	55.8 ± 0.5

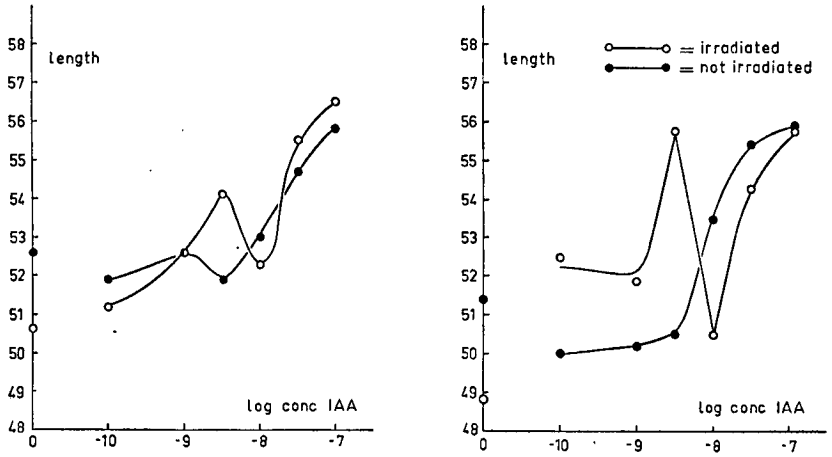

Fig. 3. Final length reached in I.A.A. solutions by coleoptile sections cut from non-irradiated seedlings and from seedlings irradiated with light of $\lambda = 660$ mμ.

shows only one. In this case no attention has been paid to the phenomenon that very low concentrations of I.A.A. inhibit the growth of non-irradiated sections. This phenomenon has also been reported by BARLOW, HANCOCK and LACEY (1957) as appearing in tests that were carried out with sections of wheat coleoptiles. The authors attribute this to the displacement of an endogenous auxin by I.A.A.

2. The two concentration-growth curves are essentially equal. Both show two maxima, but whereas the first maximum of the irradiated sections is to be found at an I.A.A. concentration of about

3.10^{-9} g/ml, the first maximum for the non-irradiated sections appears at an I.A.A. concentration 0. So in this case the curve for the irradiated sections is regarded as compressed and shifted to higher I.A.A.-concentrations as compared to the curve for the non-irradiated sections.

In both cases we pass over the fact that sometimes the sections in the blank buffer solution did not grow, but did shrink on the contrary.

It was investigated whether in the curvature test too the concentration-growth curve would show two maxima; in this way we hoped to exclude the possibility that our conclusion was due to bias.

3. The curvature test

METHOD

The test plants were cultivated as described in chapter II under the heading "Standard Avena-test".

When the plants were 88 hours old, part of them were illuminated with 70 ergs/cm² $\lambda = 660$ mμ. The others were kept in darkness. During 1½ hours after the irradiation the plants remained in darkness, whereafter the first decapitation was carried out in orange light. Immediately after the decapitation the primary leaf was loosened to prevent the orange light from influencing the reactions of the coleoptile (compare chapter II), and an agar block was applied unilaterally. Our intention was to investigate whether irradiation with light of $\lambda = 660$ mμ would induce two maxima of curvature. So in order to prevent the coleoptile from consuming the substance that possibly brings about the first maximum (at a low I.A.A.concentration) I.A.A. was applied immediately after the first decapitation, and the second decapitation was omitted. Each concentration of I.A.A. was tested on six coleoptiles.

EXPERIMENTS

The size of the curvatures as they appeared in three different experiments two hours after the application of I.A.A., are given in Table X. In Fig. 4 the results of experiment II are plotted.

TABLE X

Curvature in the Avena test after illumination with 700 ergs/cm² $\lambda = 660$ mμ

Concent. of I.A.A. in g/ml	I plants		II plants		III plants	
	illumin.	not illumin.	illumin.	not illumin.	illumin.	not illumin.
$0.3\ 10^{-7}$	—	—	6 ± 1	6 ± 2	8 ± 1	10 ± 2
$0.4\ 10^{-7}$	—	—	11 ± 1	7 ± 2	9 ± 1	11 ± 2
$0.5\ 10^{-7}$	13 ± 2	13 ± 2	17 ± 2	11 ± 2	16 ± 2	13 ± 1
$0.6\ 10^{-7}$	15 ± 2	15 ± 2	18 ± 2	13 ± 2	18 ± 2	11 ± 2
$0.7\ 10^{-7}$	24 ± 1	18 ± 1	16 ± 2	15 ± 3	20 ± 2	14 ± 2
$0.8\ 10^{-7}$	23 ± 2	17 ± 2	18 ± 2	18 ± 2	18 ± 3	17 ± 1
$0.9\ 10^{-7}$	19 ± 3	24 ± 2	22 ± 1	21 ± 2	20 ± 1	24 ± 2
$1.0\ 10^{-7}$	25 ± 3	27 ± 2	21 ± 2	21 ± 1	19 ± 3	24 ± 2
$2.0\ 10^{-7}$	26 ± 2	30 ± 2	27 ± 3	23 ± 1	27 ± 3	25 ± 1
$4.0\ 10^{-7}$	30 ± 3	32 ± 1	30 ± 2	23 ± 4	30 ± 5	28 ± 1
$6.0\ 10^{-7}$	33 ± 3	33 ± 1				

DISCUSSION

On condition that each experiment is considered by itself, the curvature-concentration curve shows one maximum for the non-irradiated plants, two for the irradiated ones. The first maximum of the irradiated plants appears in the experiments I and II as a more or less pronounced peak, in experiment III as a platform. In each of

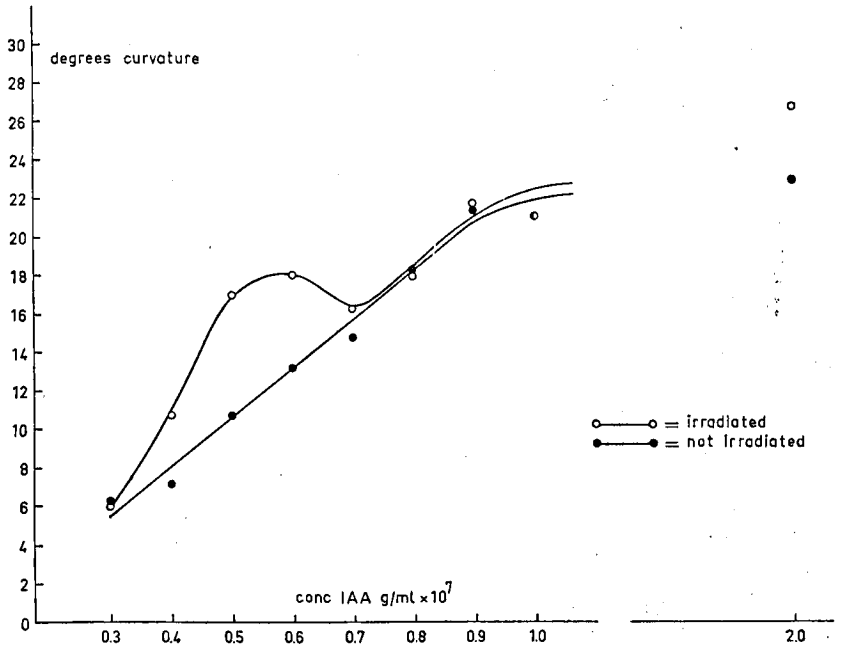

Fig. 4. Curvature reached in the curvature test by non-irradiated coleoptiles and by coleoptiles irradiated with light of $\lambda = 660$ mμ.

the three experiments the curvature of the irradiated plants at an I.A.A. concentration of 0.6 or 0.7 $\times$ 10^{-7} g/ml is significantly larger than the curvature of the non-irradiated ones. Presumably it is not admissible to average the results of the three experiments, the variability shown by the plants from day to day being too large.

To counter the argument that the differences in curvature observed at the same I.A.A. concentration might be due to the variability of the plants and not to the irradiation, the curvatures of three series of non-irradiated plants were studied. They were treated in exactly the same way as the irradiated and non-irradiated series in the above-mentioned experiments. Agar blocks soaked in the same I.A.A. solution were applied to each series. Table XI shows the resulting curvatures. At the same I.A.A. concentration the differences in curvature observed between the plants of series a, b, and c are not nearly so large as those observed between irradiated and non-irradiated plants.

TABLE XI

I.A.A. concentration in g/ml	Curvature in degrees		
	series a	series b	series c
$0.5\ 10^{-7}$	11 ± 2	8 ± 1	9 ± 2
$0.6\ 10^{-7}$	15 ± 1	11 ± 2	12 ± 1
$0.7\ 10^{-7}$	13 ± 1	11 ± 1	12 ± 2
$0.8\ 10^{-7}$	15 ± 0	15 ± 1	16 ± 2
$0.9\ 10^{-7}$	16 ± 2	16 ± 1	15 ± 2
$1.0\ 10^{-7}$	16 ± 3	17 ± 2	16 ± 1
$1.1\ 10^{-7}$	18 ± 3	16 ± 2	18 ± 2
$1.2\ 10^{-7}$	19 ± 2	17 ± 2	17 ± 1
$1.3\ 10^{-7}$	19 ± 2	20 ± 1	20 ± 1
$1.4\ 10^{-7}$	19 ± 1	18 ± 2	17 ± 2
$1.5\ 10^{-7}$	21 ± 2	20 ± 1	19 ± 2

In this case too it is possible that the non-irradiated plants also have a first maximum of curvature, but then this should be situated at a lower I.A.A. concentration than was applied in these experiments. In this case the difference in behaviour observed between irradiated and non-irradiated plants might be accounted for by the hypothesis that the endogenous I.A.A. content of irradiated plants is lower than that of non-irradiated ones.

A second possibility is that irradiation with light of $\lambda = 660$ mμ stimulates the synthesis of a substance which causes the first maximum.

These two possibilities correspond with the possibilities 2 and 1 considered in the discussion on the straight-growth test (par. 2, see p. 12--13).

In order to investigate whether irradiation with light of $\lambda = 660$ mμ lowers the endogenous I.A.A. content or results in the enhanced synthesis of a new compound, extraction and quantitative analysis would be necessary. The results of such an analysis will be given in the next chapter.

4. SUMMARY

It has been shown that irradiation with light of $\lambda = 660$ mμ causes an additional peak in the activity-concentration curve which is obtained if the growth of coleoptile sections or the curvature of coleoptiles is plotted against the I.A.A. concentration. This additional peak is situated at a low I.A.A. concentration.

CHAPTER V

QUANTITATIVE ANALYSIS OF THE I.A.A. CONTENT OF IRRADIATED AND NON-IRRADIATED COLEOPTILES

The purpose of this part of the investigation was to estimate the I.A.A. content of the coleoptiles. Destruction as well as formation of I.A.A. during extraction should be prevented; moreover, complications caused by the formation of auxin complexes should be avoided. The extraction method used in these experiments is based on that described by TERPSTRA (1953).

METHOD

Coleoptile tips of about 6 mm length were ground with some quartz sand; this was performed in orange light. The ground tissue was mixed with 5 ml of tap water at 100° C, frozen at about —5° C, and kept overnight at this temperature. The next day the mass was thawed and filtered. The tissue was rinsed with another 5 ml of tapwater. The 10 ml of extract thus obtained was made alkaline by adding about 10 mg $NaHCO_3$. Next it was shaken three times with 10 ml of ether, freed of peroxides. This ether fraction was discarded.

The aqueous residue was acidified with 0.1 N HCl, methyl orange being used as indicator (final pH ca 4), and shaken three times with 10 ml of ether. These ether fractions were evaporated, and the residue was dissolved once more in a small amount of ether.

This solution was applied to the starting line of a chromatogram. Sheets of Whatman No. 1 were used, and a mixture of isopropyl alcohol, 28 % ammonia and water (8: 1: 1) was employed as solvent. The chromatograms were developed with the solvent ascending for about 18 hours. A marker spot of I.A.A. was run parallel with the chromatogram of the extract.

After developing and drying, the strip of paper with the marker spot was removed and sprayed with a 1 % solution of cinnamic aldehyde in methanol. The strip was dried again and, when brought into a container with HCl-gas, showed the marker I.A.A. as a yellow-red spot. The Rf-value of I.A.A. ranged between 0.4 and 0.6.

The zone corresponding with the position of the I.A.A.-marker spot was cut from the chromatogram, and extracted with 3 × 10 ml of ether for 5 minutes at 37° C. The ether fractions were combined and evaporated, and the residue was transferred into an agar slice of 20 mm^3 in the manner described by TERPSTRA (1953). Next day the agar slices were cut into 6 blocks and tested on six coleoptiles.

EXPERIMENTS

The I.A.A.-content of tips of non-irradiated coleoptiles was compared by means of the curvature test with that of tips of plants, which $1\frac{1}{2}$ hours before the tips were collected had been irradiated with 700 ergs/cm^2 λ = 660 mμ. In Table XII the results of six experiments are given.

TABLE XII

Number of extracted tips	Curvature in degrees	
	extract from non-irradiated tips	extract from tips irradiated with light of λ = 660 mμ
50	15 ± 2	6 ± 1
10	13 ± 3	8 ± 1
30	29 ± 3	15 ± 2
10	34 ± 3	10 ± 1
10	34 ± 3	12 ± 2
40	36 ± 2	17 ± 2

In the course of $1\frac{1}{2}$ hours irradiation with light of $\lambda = 660$ mμ doubtlessly lowers the I.A.A. content of the coleoptile tips to about one half the original content. For the determination of I.A.A. from irradiated tips it appears necessary to extract about 10 tips to each 6 test plants. This matches the results of TERPSTRA (1953). It has not been investigated whether the differences in the size of the curvatures found in the various experiments of Table XII, are due to differences in the I.A.A. content of the extracted tips or to differences in the sensitivity of the testplants.

These results confirm the statement of VAN OVERBEEK (1936), that more auxin can be obtained from tips of non-irradiated coleoptiles than from tips of coleoptiles irradiated with orange light.

SUMMARY

Irradiation of Avena seedlings with light of 700 ergs/cm^2 $\lambda = 660$ mμ lowers the I.A.A. content of the tips of the coleoptiles in the course of $1\frac{1}{2}$ hours to about half the content found in the dark controls.

CHAPTER VI

EXTRACTION OF THE "RED LIGHT FACTOR" (R.L.F.)

Further investigations seemed desirable to make sure whether for the coleoptile the consequence of the irradiation with red light only consists in a decrease of the I.A.A.-content. If this would be so, the absence of the first peak in the growth-concentration curve of non-irradiated coleoptiles should be due to the fact that the hetero-auxin content of these coleoptiles is too high to allow the development of the first maximum, and then it would be impossible to introduce this first peak into the curve of the non-irradiated coleoptiles by adding an extract from irradiated ones, unless I.A.A. would be displaced by the extract from a surface to which it had been adsorbed. In the latter case it would depend on the ratio I.A.A. to extract which of these two would dominate, and then we should expect that the larger the amount of extract supplied to the coleoptiles, the larger the amount of I.A.A. would be that is required to obtain a similar effect. Thus the first growth peak should shift to higher I.A.A. concentrations when larger amounts of the extracted compound, henceforth called "Red Light Factor" or "R.L.F.", are supplied.

METHOD

Extracts from tips of irradiated seedlings were prepared and purified as described in chapter V. However, before the chromatogram was bio-assayed, the zone which, as indicated by an I.A.A.-marker spot, contains the I.A.A., was discarded. The eluates from the rest of the chromatogram were transferred into agar blocks that had been soaked in solutions of I.A.A. of various concentrations as it was supposed, in accordance with the results of the preceding experiments, that the effect of the 'R.L.F.' is observable only when it is applied in

combination with I.A.A. The test plants were cultivated as described in chapter II. They were kept in darkness till the very moment of decapitation, and treated as described in chapter V.

EXPERIMENTS

First a chromatogram obtained with an extract from 300 tips cut from plants that had been irradiated for 1½ hours with 700 ergs/cm² $\lambda = 660$ mμ, was tested on its activity. The chromatogram was divided into two halves by a cut parallel with the starting line, and each half was eluted and bio-assayed. Eluate *a* contained all the substances with Rf-values higher than that of I.A.A., eluate *b* the ones with Rf-values lower than that of I.A.A. Both eluates were tested in combination with I.A.A.-solutions of $\frac{1}{2} \times 10^{-7}$ and $\frac{3}{4} \times 10^{-7}$ g/ml.

TABLE XIII

I.A.A. concentration in g/ml	Control	Curvature in degrees	
		I.A.A. combined with eluate *a*	I.A.A. combined with eluate *b*
0.50×10^{-7}	11 ± 1	10 ± 1	11 ± 1
0.75×10^{-7}	11 ± 1	4 ± 1	11 ± 1

Table XIII, in which the curvatures of the test plants are given, shows that eluate *a* inhibited the curvature. Possibly it contained an active substance in more than optimum concentration.

The experiment was repeated with an extract made from 15 tips. Only the part of the chromatogram above the I.A.A. place was eluted, but it was first divided into 6 zones of 1.4 cm by cuts parallel to the starting line. The Rf-value of I.A.A. in this experiment was 0.56, the flow distance of the solvent 22.5 cm. The result is presented in Table XIV.

TABLE XIV

I.A.A. concentration in g/ml	Curvature in degrees
1×10^{-7}	15 ± 2
1×10^{-7} + zone 1 (front)	10 ± 1
1×10^{-7} + zone 2	10 ± 2
1×10^{-7} + zone 3	10 ± 1
1×10^{-7} + zone 4	7 ± 1
1×10^{-7} + zone 5	22 ± 4
1×10^{-7} + zone 6	8 ± 1

The eluate from zone 5, with slightly higher Rf-value than I.A.A. enhances the curvature induced by I.A.A. (10^{-7} g/ml) from about 15° to 22°.

Now it was tried to imitate the curvature-concentration curve found for irradiated plants by supplying non-irradiated plants with an eluate from a chromatogram in combination with I.A.A.

To this purpose extracts from coleoptile tips cut from plants that had been irradiated 1½ hours previously with 700 ergs/cm² $\lambda = 660$ mμ, were

freed from I.A.A. by chromatography. The zone containing I.A.A. was determined by means of a marker spot. The chromatogram above this zone was extracted and bio-assayed as a whole. Various amounts of the eluate were combined with various amounts of I.A.A., and the mixtures tested.

The results of three typical experiments are given in Table XV. Experiments II and III are plotted in Fig. 5.

TABLE XV

EXP. I

I.A.A. concentration in g/ml	Amount of extract added per test plant expressed in coleoptile tips		
	none	extract from 4/6 tip	extract from 8/6 tip
0.50×10^{-7}	1 ± 1	4 ± 1	6 ± 1
1.00×10^{-7}	9 ± 1	13 ± 1	16 ± 3
1.20×10^{-7}	8 ± 2	10 ± 1	9 ± 1
1.40×10^{-7}	13 ± 2	14 ± 2	9 ± 2
1.60×10^{-7}	13 ± 2	15 ± 2	17 ± 3
1.80×10^{-7}	17 ± 1	16 ± 2	17 ± 2
2.00×10^{-7}	19 ± 1		
4.00×10^{-7}	25 ± 0		

EXP. II

I.A.A. concentration in g/ml	Amount of extract added per test plant expressed in coleoptile tips			
	none	extr. fr. 2/6 tip	extr. fr. 4/6 tip	extr. fr. 8/6 tip
0.50×10^{-7}	7 ± 2	7 ± 4	9 ± 2	9 ± 2
0.62×10^{-7}	10 ± 1	14 ± 1	9 ± 1	10 ± 1
0.75×10^{-7}	10 ± 1	19 ± 4	16 ± 2	11 ± 1
0.87×10^{-7}	13 ± 2	12 ± 2	10 ± 3	5 ± 1
1.00×10^{-7}	13 ± 2	15 ± 3	11 ± 2	12 ± 2
1.25×10^{-7}	17 ± 2	20 ± 4	15 ± 2	13 ± 3

EXP. III

I.A.A. concentration in g/ml	Amount of extract added per test plant expressed in coleoptile tips			
	none	extr. fr. 2/6 tip	extr. fr. 4/6 tip	extr. fr. 8/6 tip
1.00×10^{-7}	5 ± 1	6 ± 1	5 ± 2	6 ± 1
1.25×10^{-7}	6 ± 1	7 ± 1	4 ± 1	5 ± 1
1.50×10^{-7}	9 ± 2	9 ± 2	8 ± 3	8 ± 1
1.75×10^{-7}	15 ± 2	15 ± 1	14 ± 4	15 ± 2
2.00×10^{-7}	19 ± 3	11 ± 2	10 ± 2	9 ± 2
2.25×10^{-7}	17 ± 3	17 ± 3	12 ± 1	12 ± 1
2.50×10^{-7}	18 ± 3	14 ± 1	16 ± 2	15 ± 3

It appears that a purified extract from tips of irradiated coleoptiles transforms the one-peaked curvature-concentration curve of non-irradiated coleoptiles into a two-peaked one. Application of an amount of eluate equivalent with the extract from 1 tip is sufficient to obtain a maximum effect.

These experiments show that the R.L.F. can affect the response of non-irradiated test plants to growth substance. The I.A.A.-content

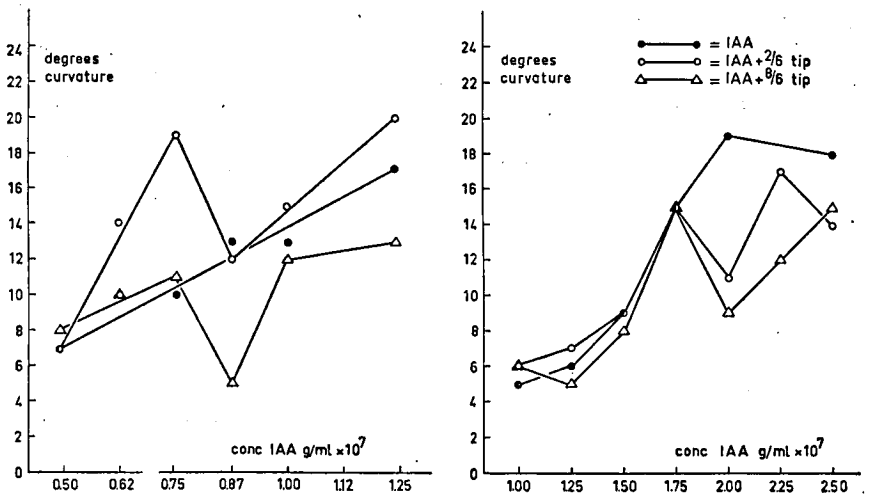

Fig. 5. Influence which a purified extract from coleoptiles irradiated with red light exercises on the curvature of non-irradiated coleoptiles.

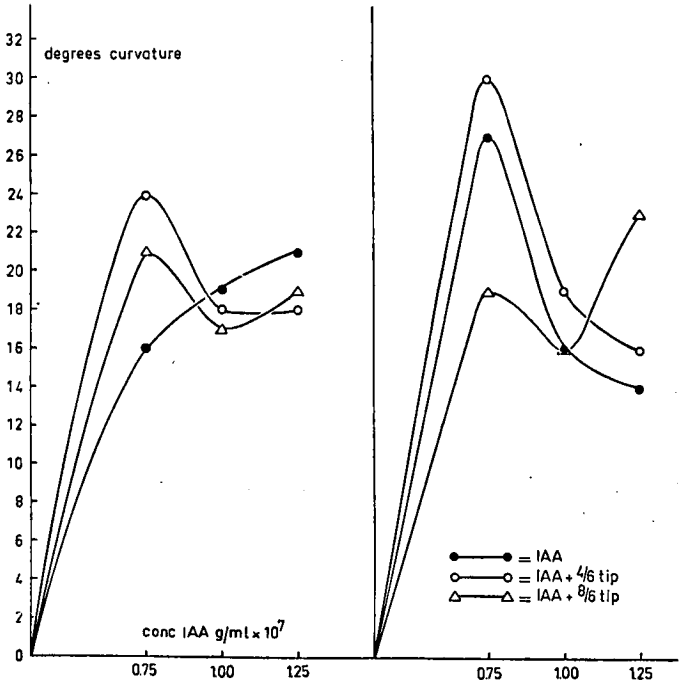

Fig. 6. Influence which a purified extract from coleoptiles irradiated with red light exercises on the curvature of test plants. Left: Test plants not irradiated. Right: Test plants irradiated with light of $\lambda = 660$ mμ.

of these plants therefore is not so high as to make them insensitive to the R.L.F. No evidence is found for the supposition that the first peak shifts to higher I.A.A. concentrations if larger amounts of R.L.F. are supplied. Either this is not so, or the shift is too small to be perceptible.

Finally the R.L.F. was tested in the same experiment on irradiated and non-irradiated plants. Table XVI, the figures of which are plotted in the graphs 6 and 7, shows the results.

TABLE XVI

EXP. I

Amount of extract added per test plant	I.A.A. concentration in g/ml	Not pre-illuminated	Pre-illuminated
none	0.75×10^{-7}	16 ± 3	27 ± 3
none	1.00×10^{-7}	19 ± 1	16 ± 1
none	1.25×10^{-7}	21 ± 2	14 ± 1
4/6 tip	0.75×10^{-7}	24 ± 2	30 ± 3
4/6 tip	1.00×10^{-7}	18 ± 2	19 ± 2
4/6 tip	1.25×10^{-7}	18 ± 3	16 ± 3
8/6 tip	0.75×10^{-7}	21 ± 3	19 ± 2
8/6 tip	1.00×10^{-7}	17 ± 2	16 ± 3
8/6 tip	1.25×10^{-7}	19 ± 2	23 ± 2

EXP. II

Amount of extract added per test plant	I.A.A. concentration in g/ml	Not pre-illuminated	Pre-illuminated
none	0.50×10^{-7}	8 ± 2	8 ± 2
none	0.75×10^{-7}	11 ± 2	17 ± 3
none	1.00×10^{-7}	26 ± 2	26 ± 3
2/6 tip	0.50×10^{-7}	7 ± 2	10 ± 1
2/6 tip	0.75×10^{-7}	15 ± 2	17 ± 3
2/6 tip	1.00×10^{-7}	19 ± 2	19 ± 3
4/6 tip	0.50×10^{-7}	12 ± 1	13 ± 2
4/6 tip	0.75×10^{-7}	17 ± 1	21 ± 2
4/6 tip	1.00×10^{-7}	17 ± 4	17 ± 2
8/6 tip	0.50×10^{-7}	0 ± 0	3 ± 1
8/6 tip	0.75×10^{-7}	18 ± 2	25 ± 2
8/6 tip	1.00×10^{-7}	16 ± 3	20 ± 2

In Fig. 6 (corresponding with experiment I) the graphs of the non-irradiated plants, either supplied with R.L.F. or not (a), are reproduced together with the graphs of the irradiated plants (b). In Fig. 7 (based on experiment II) however, the graphs of irradiated and non-irradiated testplants are plotted together, in Fig. 7a with no R.L.F. added, in Fig. 7b after addition of the R.L.F.-extract from 2/6 tip, in Fig. 7c from 4/6 tip and in Fig. 7d from 8/6 tip.

These experiments indicate that the R.L.F. enhances the curvature of non-irradiated and irradiated plants at the same exogenous I.A.A.-concentration, independent of the amount of R.L.F.

DISCUSSION

It appears impossible to attribute the absence of the first maximum in the curve presenting the size of the curvatures of non-irradiated test plants to their too high endogenous I.A.A.-level.

This appears from the fact that, by applying a purified extract from

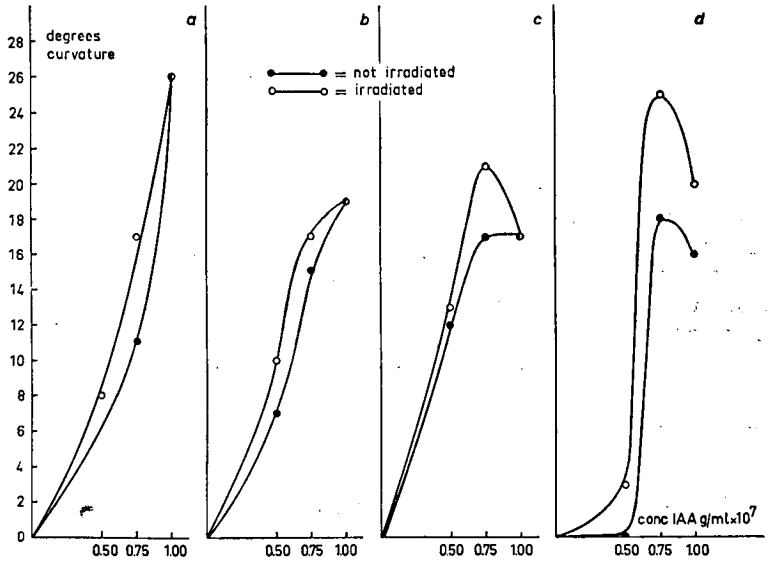

Fig. 7. Influence which a purified extract from coleoptiles irradiated with red light exercises on the curvature of test plants. *a*: No extract added. *b*: Each test plant supplied with extract from 2/6 tip. *c*: Each test plant supplied with extract from 4/6 tip. *d*: Each test plant supplied with extract from 8/6 tip.

irradiated coleoptile tips, the development of such a first maximum can be obtained. Two possibilities for explaining the different behaviour of irradiated and non-irradiated coleoptiles are left:

Firstly, irradiation with light of $\lambda = 660$ mμ may cause or increase the formation of R.L.F.

Secondly, irradiation with light of $\lambda = 660$ mμ may enhance the capacity of the coleoptile to react to R.L.F. already present.

As an argument in support of the first explanation we may adduce the observation that "dark" coleoptiles can react to a supply of R.L.F., which would be difficult to conceive if the second explanation were the right one.

If irradiated coleoptiles contain more R.L.F. than non-irradiated ones, the supply of this substance required by irradiated coleoptiles in order to reach the "optimum" concentration, would be less than that required by the non-irradiated ones. This expectation was not fulfilled in our experiments, but this negative result may possibly be due to the limited range of concentrations that were tested.

SUMMARY

After irradiating seedlings with light of $\lambda = 660$ mμ it appeared possible to isolate from the coleoptile tips a compound (R.L.F.) which causes in the curve presenting the degree of curvature in non-irradiated coleoptiles the appearance of a similar first maximum (at low I.A.A. concentrations) as occurs naturally in that of the irradiated ones. The first peak in the curvature-concentration curve appears in this case in irradiated and non-irradiated coleoptiles at the same exogenous I.A.A. concentration, independent of the amount of R.L.F. supplied or already present (in the irradiated test plants).

CHAPTER VII

IDENTITY OF THE RED LIGHT FACTOR

1. Discussion on the identity of the R.L.F.

With regard to the identity of the R.L.F. only vague indications can be given in this chapter.

1. It is probably an acid. This can be concluded from the fact that it can be transferred by shaking from an acidified aqueous solution into ether.

2. In experiments in which the R.L.F. was heated during two hours either in 1.5 N HCl or in 1.5 N KOH, it appeared that the R.L.F. is acid proof, but that it looses its activity if it is boiled with alkali. The experiment was made according to the method of Terpstra (1953). The curvatures induced by mixtures of I.A.A. and the R.L.F. either untreated or treated with acid or alkali, are given in Table XVII.

TABLE XVII

I.A.A. concentration in g/ml	Control	Curvature in degrees after the addition of extract from one tip per test plant		
		untreated	boiled with acid	boiled with alkali
0.75×10^{-7}	21 ± 2	8 ± 1	8 ± 2	27 ± 3
1.00×10^{-7}	28 ± 3	14 ± 1	16 ± 2	28 ± 2
1.25×10^{-7}	29 ± 2	19 ± 1	20 ± 3	30 ± 3

The amounts of R.L.F. that were applied in this series of experiments, were considerably higher than those used in the experiments of chapter VI.

3. If the R.L.F. is applied to Avena coleoptiles, without addition of I.A.A. it causes no curvatures. For this reason it is improbable that the R.L.F. is identical with Kögl's auxin-a. This would be possible only in the event that Kögl's auxin preparation had been polluted with so large an amount of I.A.A. that the curvatures induced by solutions of this preparation might be ascribed to an additive activity of I.A.A. and auxin-a, but as his preparation was a highly purified one, this is hardly conceivable.

4. Brian and Hemming (1957 and 1958) found that pea epicotyls fail to respond to gibberellic acid if there is no I.A.A. in the culture solution. Gibberellic acid enhanced the growth-promoting activity of

I.A.A. at all the I.A.A. concentrations that were tested. This is not in conformity with the way in which the R.L.F. interacts with I.A.A. Moreover gibberellic acid is not acid proof.

Yet it seemed worth while to determine the Rf-value of gibberellic acid in our chromatograms, and to investigate the activity of gibberellic acid in the straight-growth test and in the curvature test.

2. DETERMINATION OF THE Rf-VALUE OF GIBBERELLIC ACID

4 μg gibberellic acid was mixed with 4 μg I.A.A. and chromatographed as described in chapter V. After being developed, the chromatogram was dried, sprayed with 1 % H_2SO_4 in methanol, and dried again at 60° C during 10 minutes. After this treatment the spot of gibberellic acid can be seen in U.V. light.

The I.A.A. spot was detected on a parallel strip of the chromatogram as described in chapter V.

The results of 5 experiments can be seen in Table XVIII.

TABLE XVIII

The Rf-values for:		
I.A.A.	Gibberellic acid	R.L.F.
0.35	0.52	
0.49	0.59	
0.34	0.54	> 0.45
0.31	0.48	
0.62	0.74	

It appears that the Rf-value of gibberellic acid ranges from 0.5 to 0.7. So this compound is to be found, like the R.L.F., somewhat nearer to the front than I.A.A.

Thus the Rf-value of gibberellic acid does not exclude in advance the possibility that the R.L.F. is identical with gibberellic acid.

3. ACTIVITY OF GIBBERELLIC ACID; CURVATURE TEST

For these experiments agar slices were soaked in mixtures of I.A.A. and gibberellic acid. Preliminary tests showed that 10^{-6} g/ml and 1.5×10^{-6} g/ml gibberellic acid were concentrations suitable for our purpose. The agar blocks were tested on once decapitated Avena coleoptiles. The coleoptiles were cultivated and tested as described in chapter V, and were kept in absolute darkness till the moment of decapitation.

Table XIX shows the curvatures of the test plants two hours after application of the agar blocks; two experiments were made. Experiment II is plotted in Fig. 8 (left).

Obviously gibberellic acid can induce at low I.A.A. concentrations an additional peak in the curvature-concentration curve.

4. STRAIGHT-GROWTH TEST WITH I.A.A. COMBINED WITH GIBBERELLIC ACID

Finally some cylinder tests were carried out with mixed solutions of I.A.A. and of gibberellic acid. The method is described in chapter III.

TABLE XIX

I.A.A. concentration in g/ml	Curvature in degrees concentration of gibberellic acid in g/ml		
	0.	10^{-6}	1.5×10^{-6}
EXP. I			
0.6×10^{-7}	12 ± 2	11 ± 2	11 ± 2
0.7×10^{-7}	11 ± 1	11 ± 3	13 ± 3
0.8×10^{-7}	14 ± 2	20 ± 2	17 ± 1
0.9×10^{-7}	23 ± 1	15 ± 2	15 ± 0
1.0×10^{-7}	26 ± 1	17 ± 2	21 ± 3
1.1×10^{-7}	23 ± 2	23 ± 2	22 ± 3
1.2×10^{-7}	23 ± 1	23 ± 2	26 ± 2
1.3×10^{-7}	24 ± 1	25 ± 2	26 ± 2
EXP. II			
0.62×10^{-7}	5 ± 1	9 ± 2	8 ± 2
0.75×10^{-7}	7 ± 0	4 ± 1	4 ± 2
0.87×10^{-7}	9 ± 2	10 ± 2	7 ± 2
1.00×10^{-7}	12 ± 1	19 ± 2	7 ± 1
1.12×10^{-7}	13 ± 2	13 ± 2	7 ± 2
1.25×10^{-7}	10 ± 2	7 ± 1	6 ± 2
1.37×10^{-7}	12 ± 2	19 ± 2	16 ± 1

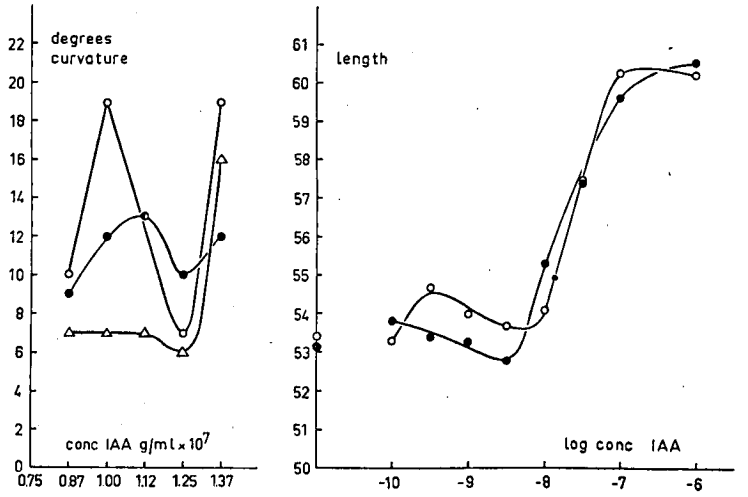

Fig. 8. Influence of gibberellic acid. Left: in the curvature test, right: in the cylinder test; dots: no G.A. added; circle: 10^{-6} g/ml G.A.; triangle: 1.5×10^{-6} g/ml G.A.

The coleoptiles that were to be cut into sections in orange light, were not pre-illuminated with red or orange light.

In Table XX the length reached by the sections during the three hours they floated on the surface of the solutions, is presented in scale units; two experiments were made. Experiment II is plotted in Fig. 8 (right).

TABLE XX

I.A.A. concentration in g/ml	Final length of sections in scale units			
	EXP. I Concentration of gibberellic acid in g/ml		EXP. II Concentration of gibberellic acid in g/ml	
	0.	10^{-6}	0.	10^{-6}
0.	52.5 ± 0.2	52.7 ± 0.1	53.1 ± 0.3	53.4 ± 0.2
1.10^{-10}	53.0 ± 0.2	52.9 ± 0.3	53.8 ± 0.3	53.3 ± 0.2
3.10^{-10}	52.3 ± 0.3	53.0 ± 0.3	53.4 ± 0.2	54.7 ± 0.2
1.10^{-9}	51.3 ± 0.2	52.8 ± 0.2	53.3 ± 0.1	54.0 ± 0.2
3.10^{-9}	53.7 ± 0.3	53.1 ± 0.3	52.8 ± 0.2	53.7 ± 0.3
1.10^{-8}	54.1 ± 0.3	53.5 ± 0.3	55.3 ± 0.3	54.1 ± 0.2
3.10^{-8}	56.3 ± 0.6	55.5 ± 0.4	57.4 ± 0.5	57.5 ± 0.5
1.10^{-7}			59.6 ± 0.7	60.3 ± 0.7
1.10^{-6}			60.5 ± 0.7	60.2 ± 0.8

Gibberellic acid indeed enhances the growth of the sections at low I.A.A. concentrations, and consequently produces an additional peak in the graph.

SUMMARY

The activity of gibberellic acid in the curvature test and in the straight-growth test as well as its place in the chromatogram are comparable to those of the R.L.F. This, however, is not sufficient to prove the identity of the two compounds.

CHAPTER VIII

THE R.L.F. IN CONNECTION WITH THE PHOTOTROPIC REACTIONS OF AVENA COLEOPTILES

As shown by ARISZ (1915) the phototropic curvature of Avena coleoptiles is a periodic function of the light energy provided that the latter is supplied within a short time. With increasing amounts of light under these circumstances a first positive, a negative and a second positive curvature are produced. These different responses may be attributed to

a. a redistribution of auxin due to a lateral shift of the auxin transport to the shade side,
b. an inactivation of auxin by the light,
c. an increase in the auxin synthesis at higher light intensities,
d. a reduced response of cells to auxin under the influence of the light.

Arguments for and against each of these possibilities were reviewed by SCHRANK (1950) and WENT (1956). By none of the investigators whose experiments had a bearing on the question of the cause of these differences in phototropic behaviour, the influence of red light was taken into account.

Now it was shown in the preceding chapters that the growth of a coleoptile which is irradiated with light of $\lambda = 660$ mμ decreases with decreasing I.A.A. concentrations, but increases again at low I.A.A. concentrations before it is reduced to zero. This relation

between growth and I.A.A. concentration curve is represented tentatively by one of the curves shown in Fig. 9. (broken line).

The curve for the plants that were not pre-illuminated (solid line), has been based on the data obtained by the experiments discussed in this chapter, as will be shown on p. 31.

Fig. 9 seems to offer a new possibility for the explanation of the negative and the second positive curvature.

Let it be assumed, with OPPENOORTH (1941), that the auxin content of the coleoptile decreases with increasing quantities of blue light. As the irradiated side of the coleoptile receives larger amounts of blue light than the shade side, the illuminated side will contain less

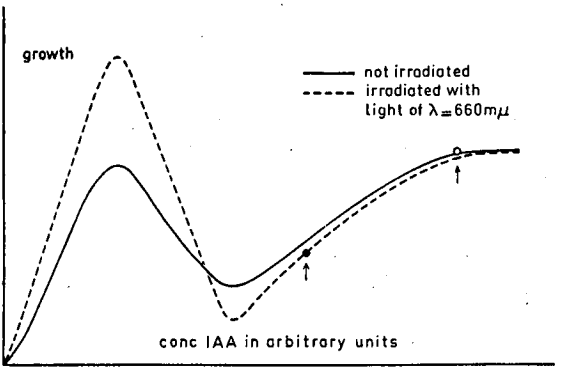

Fig. 9. Tentative course of the growth rate curve of intact coleoptiles at different I.A.A. concentrations. circle: I.A.A. level of non-irradiated plants; dot: I.A.A. level of plants irradiated with red light.

auxin than the shade side. Now with increasing amounts of unilateral light energy the I.A.A. content of the light side as well as that of the shade side can be imagined to move along the graph of Fig. 9 to the left, that of the light side preceding that of the shade side.

At the outset (at low light energy, i.e. at a high but decreasing auxin content of the coleoptile) the growth of the light side may be less than the growth of the shade side (first positive curvature). When mounting the slope towards the first peak from the right side of the graph, however, the growth rate of the light side may surpass the growth rate of the shade side (negative curvature) whereas, when the first peak has been passed, the tables are once more turned (second positive curvature).

This supposition could be checked by investigating the influence which a pre-illumination with light of $\lambda = 660$ mμ exercises on the growth rate of a coleoptile whose phototropic reaction lies at the turning point from the negative to the second positive curvature. If this turning point really corresponds with the first peak of the curve which presents the relation between growth and I.A.A. concentration, the growth rate of coleoptiles should at this point be enhanced by an irradiation with light of $\lambda = 660$ mμ.

In the same series of experiments the influence which irradiation

with light of $\lambda = 660$ mμ and of $\lambda = 480$ mμ and with combinations of these wave lengths exercises on the straight growth of coleoptiles, was studied also in the regions of the first positive and negative curvature.

METHOD

The coleoptiles were cultivated as described in chapter II for the experiments on phototropism. When the seedlings were 88 hours old, a particle of vermiculite was fixed by means of lanoline on the coleoptile at a distance of about 1 cm from the tip; this operation was performed in green light of $\lambda = 560$ mμ; the particle was to serve as mark. Next the coleoptiles were photographed by use of light of $\lambda = 560$ mμ, and irradiated unilaterally with light of $\lambda = 660$ mμ, $\lambda = 480$ mμ or both.

Two hours after the irradiation the coleoptiles were photographed again. The increase in length of the coleoptiles from tip to mark was measured in mm.

EXPERIMENTS

The results of these experiments are recorded in Table XXI. The result of one of them is plotted in Fig. 10.

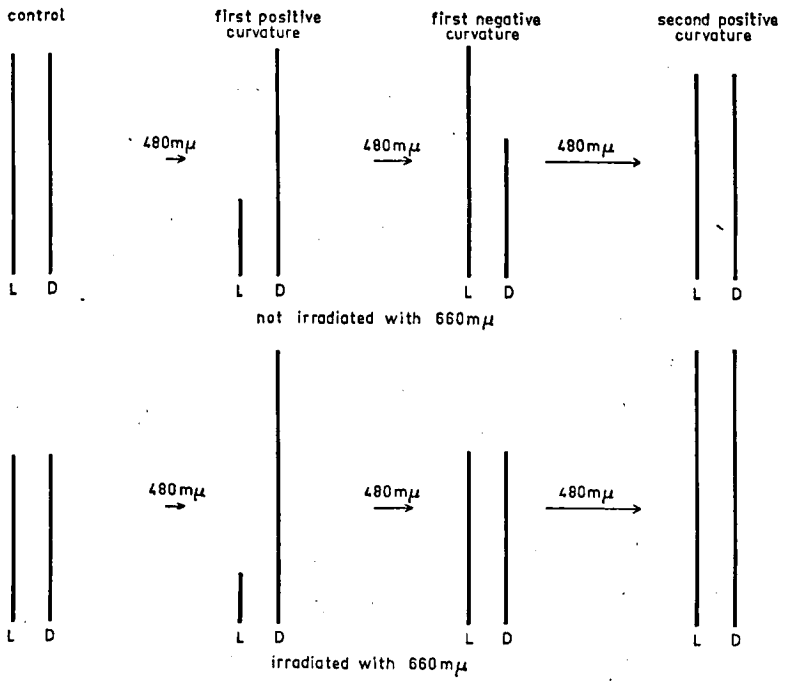

Fig. 10. Increase in length in 2 hours of the light side (L) and of the shade side (D) of coleoptiles that were one-sidedly illuminated with blue light of various intensities; the coleoptiles of the lower row previously irradiated with light of 660 mμ, those of the upper row not previously irradiated.

TABLE XXI

Growth in mm during 2 hours after different irradiations

	Region of the *first positive* phototropic curvature		
	No light of $\lambda = 480$ mμ	20 ergs/cm^2 $\lambda = 480$ mμ	
		light side	shade side
not pre-illuminated	0.55 ± 0.06 (8)	0.12 ± 0.04 (16)	0.49 ± 0.06 (16)
pre-illuminated with 700 ergs/cm^2 $\lambda = 660$ mμ	0.40 ± 0.04 (23)	0.21 ± 0.04 (17)	0.66 ± 0.06 (17)
not pre-illuminated	0.57 ± 0.05 (20)	0.20 ± 0.04 (7)	0.59 ± 0.08 (7)
pre-illuminated with 700 ergs/cm^2 $\lambda = 660$ mμ	0.43 ± 0.07 (12)	0.13 ± 0.05 (9)	0.71 ± 0.09 (9)
	Region of the *negative* phototropic curvature		
	No light of $\lambda = 480$ mμ	4800 ergs/cm^2 $\lambda = 480$ mμ	
		light side	shade side
not pre-illuminated	0.57 ± 0.05 (20)	0.60 ± 0.16 (8)	0.36 ± 0.08 (8)
pre-illuminated with 700 ergs/cm^2 $\lambda = 660$ mμ	0.43 ± 0.07 (12)	0.45 ± 0.08 (8)	0.45 ± 0.08 (8)
not pre-illuminated	0.55 ± 0.06 (18)	0.50 ± 0.17 (3)	0.30 ± 0.17 (3)
	Transition region from *negative* to *second* positive phototropic curvature		
	No light of $\lambda = 480$ mμ	24000 ergs/cm^2 $\lambda = 480$ mμ	
		light side	shade side
not pre-illuminated	0.50 ± 0.06 (12)	0.56 ± 0.05 (14)	0.56 ± 0.05 (14)
pre-illuminated with 700 ergs/cm^2 $\lambda = 660$ mμ		0.72 ± 0.06 (15)	0.72 ± 0.06 (15)
not pre-illuminated	0.58 ± 0.05 (15)	0.53 ± 0.12 (06)	0.53 ± 0.12 (06)
pre-illuminated with 700 ergs/cm^2 $\lambda = 660$ mμ		0.77 ± 0.04 (17)	0.77 ± 0.04 (17)
not pre-illuminated		0.78 ± 0.04 (11)	0.78 ± 0.04 (11)
pre-illuminated with 700 ergs/cm^2 $\lambda = 660$ mμ		0.97 ± 0.08 (12)	0.97 ± 0.08 (12)

It appears that:

a. irradiation with 700 ergs/cm^2 $\lambda = 660$ mμ alone inhibits the growth of the coleoptiles;

b. unilateral light of $\lambda = 480$ mμ (20 ergs/cm^2) if given to coleoptiles not irradiated with $\lambda = 660$ mμ, inhibits growth at the light side only, but in coleoptiles pre-irradiated with $\lambda = 660$ mμ, it enhances in addition the growth rate at the shade side;

c. in the region of the negative curvature the growth of the shade side is inhibited;

d. at the turning point from negative to second positive curvature a previous illumination with light of $\lambda = 660$ mμ enhances the

growth rate of the coleoptiles; the growth rate of these pre-illuminated coleoptiles is also larger than that of controls kept in complete darkness.

DISCUSSION

Table XXI shows that with increasing quantities of unilateral blue light the growth at the light side first passes a minimum (first positive curvature) and that then the growth at the shade side too passes a minimum (negative curvature). This holds true for coleoptiles, no matter whether they are pretreated or not pretreated with red light. This fits in with the hypothesis that was expounded in the introduction to this chapter and according to which the growth of light and shade side can be schematically represented by the graph of Fig. 9. Moreover irradiation with light of $\lambda = 660$ mμ enhances the growth of the coleoptiles in the transition region between the negative and the second positive curvature. So the growth of light and shade side of coleoptiles not irradiated with light of $\lambda = 660$ mμ runs along the solid graph of Fig. 9, whereas the growth of coleoptiles pre-irradiated with light of $\lambda = 660$ mμ is indicated by the broken line. Indeed, there is a striking resemblance between the growth pattern shown by the coleoptiles under influence of increasing quantities of blue light and that shown by them under the influence of decreasing quantities of I.A.A. However, in the introduction to this chapter we assumed that by increasing the quantities of blue light the I.A.A. content of the coleoptile decreases. If this is right, the negative and second positive curvature must be due to the activity of the R.L.F. Unfortunately, the results of the experiments by the aid of which OPPENOORTH (1941) thought that he could prove the inactivation of auxin by blue light, have lost their convincing character since TERPSTRA (1953) demonstrated that Oppenoorth's method of auxin extraction is not reliable. Secondly, OPPENOORTH estimated the overall auxin content of his extracts in the manner that was usual at that time, and not the I.A.A. content itself (i.e. after chromatographic separation). So the evidence, advanced thus far, should be completed by a determination of the I.A.A. content of coleoptiles irradiated with 24 000 ergs/cm^2 $\lambda = 480$ mμ, that is at the transition of the negative to the second positive curvature.

The experiments of Table XXI show that at the turning point of positive and negative curvature coleoptiles which are not irradiated with red light, nevertheless show a first growth peak, though a less pronounced one than is shown by coleoptiles that have been irradiated with red light. This is indicated by the solid line of Fig. 9. Presumably either decapitation and sectioning of the coleoptiles obliterate this less pronounced peak, or large quantities of blue light have the same effect as red light in producing a first growth peak.

From these experiments information is obtained also on the cause of the first positive curvature. They confirm the observation of BEYER (1927) that with the first positive curvature the growth at the irradiated side of the coleoptile is inhibited, the growth at the

dark side on the other hand enhanced. It appears, however, that this enhancement of the shade side occurs only in pre-irradiated coleoptiles. The most plausible explanation is the classic one, viz. that the unilateral blue light causes an accumulation of the available auxin at the shade side of the coleoptile. That this accumulation would not occur with coleoptiles that have not been irradiated with red light, is unlikely. Maybe the difference in the reaction of irradiated and non-irradiated coleoptiles is connected with the reduction of the I.A.A. content of the coleoptiles by red light. Possibly the I.A.A. level of the non-irradiated coleoptiles is above the optimum so that an increase of the I.A.A. content at the shade side cannot result in an enhancement of the growth rate. So in our opinion the first positive curvature is due to a shift of I.A.A. towards the shade side; the negative and the second positive curvature to a reduction of the I.A.A. content at both light and shade side of the coleoptile, which brings the coleoptile into the action region of the R.L.F., possibly combined with an accumulation of I.A.A. This is in line with the views of WENT (1956).

This chapter may end with a short contemplation of the changes in "disposition" ("Stimmung" or "tonus"), i.e. the changes which the power to react to a stimulus undergoes under the influence of a preceding stimulus. ARISZ (1914) stated that the duration and the intensity of a preceding all-round illumination determine the way in which the coleoptile reacts on a following one-sided light stimulus. By pre-illumination with white light the plant can reach a stage in which it reacts on a unilateral illumination by a negative curvature. With a longer duration of the pre-illumination the faculty to produce a positive curvature returns. In our opinion pre-illumination reduces the I.A.A. content of the coleoptile. If it is true that the I.A.A. level determines whether a positive or a negative curvature will result, the so called phototonus of a coleoptile must entirely depend upon its I.A.A. level.

SUMMARY

In this chapter evidence is adduced for the hypothesis that the first positive curvature is due to a shift of heteroauxin to the dark side of the coleoptile; the negative and the second positive curvature on the other hand would be due to a reduction of the I.A.A. level in the coleoptile as a result of which the red light factor gets the opportunity to influence growth. Consequently the red light factor can influence not only the growth rate but the phototropic response as well.

CHAPTER IX

THE INFLUENCE OF FAR RED LIGHT (of $\lambda = 740$ mμ) ON THE AVENA COLEOPTILE

In several instances an antagonistic effect of red and far red light has been found.

In the preceding chapters it was shown that light of $\lambda = 660$ mμ (red light) has two different and probably independent effects on the Avena coleoptile,

firstly: the I.A.A. content of the coleoptile is decreased; the higher maximum reached by the first positive phototropic curvature is presumably connected with this decrease;
secondly: at low I.A.A. concentrations a second growth maximum is induced, which causes an increase in the rate of growth of the coleoptiles at the turning point between the negative and the second positive phototropic curvature.

It seemed worth while to investigate whether irradiation with light of $\lambda = 740$ mμ (far red light) antagonizes one of these effects or perhaps even both.

The methods used to this end were the same as those described in the preceding chapters far the corresponding experiments on the influence of red light.

EXPERIMENTS

First effect

a. Avena coleoptiles were unilaterally irradiated with 20 ergs/cm^2 $\lambda = 480$ mμ in order to produce a maximum positive curvature. They were pre-illuminated either with 700 ergs/cm^2 $\lambda = 660$ mμ or with 6000 ergs/cm^2 $\lambda = 740$ mμ or with both. In Table XXII the resulting curvatures are shown.

TABLE XXII

Phototropic curvature (in degrees) induced by a unilateral illumination with 20 ergs/cm^2 $\lambda = 480$ mμ without or after a pre-illumination with

	EXP. I	EXP. II	EXP. III
—	9.0 ± 0.8 (14)	12.6 ± 0.9 (10)	9.9 ± 0.9 (7)
700 ergs/cm^2 $\lambda = 660$ mμ	13.3 ± 1.4 (09)	17.1 ± 1.8 (08)	14.0 ± 1.8 (5)
700 ergs/cm^2 $\lambda = 660$ mμ + 6000 ergs/cm^2 $\lambda = 740$ mμ	12.2 ± 1.3 (10)	20.7 ± 0.8 (11)	13.4 ± 0.8 (5)
	EXP. IV	EXP. V	EXP. VI
—	12.3 ± 1.4 (7)	9.9 ± 0.9 (7)	8.6 ± 1.1 (8)
6000 ergs/cm^2 $\lambda = 740$ mμ	16.2 ± 1.3 (6)	15.4 ± 1.4 (5)	14.0 ± 0.8 (8)

It appears that illumination with light of $\lambda = 740$ mμ does not reverse the effect of illumination with light of $\lambda = 660$ mμ. On the contrary, it has the same effect, viz. a larger maximum curvature.

b. The same conclusion can be drawn from experiments on the influence which light of $\lambda = 740$ mμ exercises on the absolute growth of the coleoptiles in the region of the first positive curvature. Like light of $\lambda = 660$ mμ, it inhibits the growth of coleoptiles that were not subjected to unilateral blue light (Table XXIII), and it increases the growth at the shade side of coleoptiles that are developing a phototropic curvature after an unilateral irradiation with 20 ergs/cm^2 $\lambda = 480$ mμ.

TABLE XXIII

Growth of the light and shade side (in scale units) after unilateral irradiation

	No light of $\lambda = 480$ mμ	Light side	Shade side
no pre-illumination	0.58 ± 0.08 (20)	0.13 ± 0.04 (14)	0.62 ± 0.05 (14)
pre-illuminated with 6000 ergs/cm² $\lambda = 740$ mμ	0.46 ± 0.08 (12)	0.07 ± 0.03 (11)	0.66 ± 0.07 (11)
no pre-illumination	0.57 ± 0.05 (20)	0.20 ± 0.04 (7)	0.59 ± 0.08 (7)
pre-illuminated with 6000 ergs/cm² $\lambda = 740$ mμ	0.46 ± 0.09 (10)	0.22 ± 0.06 (12)	0.93 ± 0.09 (12)

c. Finally it could be proved that light of $\lambda = 740$ mμ reduces the I.A.A. content of Avena coleoptiles in the same way as light of $\lambda = 660$ mμ does; this is shown in Table XXIV.

TABLE XXIV

Curvature test of extracts

Extract from	Non-irradiated coleoptiles	Coleoptiles irradiated with 6000 ergs/cm² $\lambda = 740$ mμ
30 tips	10 ± 3	4 ± 0.5
20 tips	8 ± 2	3 ± 1
20 tips	7 ± 2	1 ± 1

Second effect

a. By application of the straight growth test (compare chapter IV) we studied the influence which light of $\lambda = 740$ mμ exercises on the first peak of the curve which presents growth as a function of the I.A.A. concentration in coleoptiles that have been irradiated with light of $\lambda = 660$ mμ. In Table XXV the final lengths of coleoptile sections at the critical I.A.A. concentrations are given in scale units. It appears that the growth-enhancing effect of light of $\lambda = 660$ mμ is completely or partly neutralized by illumination of the seedlings with light of $\lambda = 740$ mμ.

TABLE XXV

Growth of coleoptile cylinders in solutions of I.A.A.

	in a concentration of			
	10^{-9} g/ml		3.10^{-9} g/ml	
	I	II	I	II
not pre-illuminated	52.6 ± 0.2	51.2 ± 0.2	51.4 ± 0.3	51.3 ± 0.3
pre-illuminated with 700 ergs/cm² $\lambda = 660$ mμ	53.3 ± 0.2	52.7 ± 0.3	52.6 ± 0.1	53.5 ± 0.3
pre-illuminated with 6000 ergs/cm² $\lambda = 740$ mμ + 700 ergs/cm² $\lambda = 660$ mμ	52.8 ± 0.3	51.9 ± 0.2	52.0 ± 0.3	52.3 ± 0.1

b. Finally it was investigated whether irradiation with light of $\lambda = 740$ mμ reverses the effect which light of $\lambda = 660$ mμ exercises

on the growth of coleoptiles in the transition region between the negative and the second positive phototropic curvature. To this end coleoptiles were pre-illuminated either with 700 ergs/cm² $\lambda = 660$ mμ or with 700 ergs/cm² $\lambda = 660$ mμ immediately followed by 6000 ergs/cm² $\lambda = 740$ mμ and then irradiated one-sidedly with 24000 ergs/cm² $\lambda = 480$ mμ (see chapter VIII). Table XXVI gives the increase in length of the coleoptiles two hours after this treatment. It can not be doubted that the effect of light of $\lambda = 660$ mμ is annihilated by light of 740 mμ.

TABLE XXVI

Growth of coleoptiles after an irradiation with

Pre-illumination	No light of $\lambda = 480$ mμ	24000 ergs/cm² $\lambda = 480$ mμ
none	0.45 ± 0.06 (11)	0.40 ± 0.05 (15)
700 ergs/cm² $\lambda = 660$ mμ		0.64 ± 0.07 (15)
700 ergs/cm² $\lambda = 660$ mμ + 6000 ergs/cm² $\lambda = 740$ mμ		0.47 ± 0.07 (12)

DISCUSSION

Irradiation with light of $\lambda = 740$ mμ antagonizes the effect of light of $\lambda = 660$ mμ on the growth peak at low I.A.A. concentrations that appears in the curve which presents growth as a function of the I.A.A. concentration. The influence which light of $\lambda = 660$ mμ exercises on coleoptiles in the transition region between the negative and the second positive phototropic curvature too is antagonized by light of $\lambda = 740$ mμ. This finding is another argument in favour of our attempt to link up the phenomena of the negative and second positive curvature with the occurrence of a first growth peak, i.e. the one found at low I.A.A. concentrations. Illumination with light of $\lambda = 740$ mμ and with light of $\lambda = 660$ mμ, however, have the same effect on the growth of coleoptiles, that were not exposed to unilateral blue light, as they have on the first positive curvature and on the I.A.A. content. It therefore may be concluded that the hypothesis that a relation exists between some of the effects of red light, viz. the enhancement of the first positive curvature, the decrease of the growth rate, and the decrease of the I.A.A. content, gains in probability.

A twofold effect of far red light was found also by De Lint (1957). In his experiments on the effect which irradiation excercises on the final length of Avena coleoptiles, far red irradiation decreased the length as compared to that of non-irradiated coleoptiles, but it partly reversed the inhibition induced by red or yellow light.

Now our Fig. 3 shows that the introduction, by irradiation with red light, of a first peak in the curve which presents growth as a function of the I.A.A. concentration, is accompanied by the introduction of a depression at somewhat higher I.A.A. concentrations. Fig. 5 shows that a similar depression is caused by the R.L.F. extract and that the depression is the more pronounced the higher the amount of R.L.F. As irradiation with far red light annihilates the first growth

peak caused by the R.L.F., it will, in all probability, also annihilate the growth depression due to the R.L.F. Presumably DE LINT has reduced the I.A.A. content of his coleoptiles by irradiation with red or yellow light to the level where the R.L.F. causes the growth depression discussed above, and he has abolished the depression with far red light. Since far red light, as well as red light, reduces the I.A.A. content of the coleoptile, it must be impossible that the final length of the dark controls is attained by coleoptiles that have been exposed to a far red irradiation. DE LINT found that they do not do this.

SUMMARY

One of the two effects of red light on the Avena coleoptile is neutralized by far red light, namely the transformation of the one-peaked curve which presents growth as a function of the I.A.A. into a two-peaked one.

On the other hand far red light has the same depressing effect as red light on the growth rate of non-illuminated coleoptiles and on the maximum of the first positive phototropic curvature.

CHAPTER X

CONSEQUENCES WHICH THE DISCOVERY OF THE INFLUENCE EXERCISED BY RED AND ORANGE LIGHT ON AVENA COLEOPTILES HAS FOR THE APPLICATION OF AUXIN TESTS

In the preceding chapters it was shown that orange and red light influence the reactions of the coleoptile on I.A.A., and that this influence is exercised via the primary leaf. Without intending a further comprehensive analysis we performed some experiments in order to learn the effect of orange light on the results of the curvature test.

I

Usually one switches on the orange light when entering the dark-room with the intention to carry out a curvature test. In consequence the last seedlings to be decapitated have much more time to react on the orange light than the first ones because, as has been shown above, the seedling reacts only so long as the primary leaf remains intact. If it can be shown that this commercial practice influences the curvatures of the test plants, one should operate carefully when using orange light. We investigated this in the next experiments.

At the beginning of the experiment the racks with coleoptiles that were to be tested, were all illuminated at the same time with orange light. The curvature test was carried out as usual, i.e. the plants were decapitated twice with an interval of two hours. The primary leaf was removed after the first decapitation. The subsequent treatments were applied first to series *a*, next to series *b*. There was an interval of 30 minutes between the treatment of the first rack of series *a* and that of the first rack of series *b*.

TABLE XXVII

Effect of orange light on the standard Avena (curvature) test

I.A.A. concentration in g/ml	Curvature in degrees	
	series *a*	series *b*
0.	—1 ± 0	—2 ± 1
0.25 10^{-7}	9 ± 1	7 ± 1
0.50 10^{-7}	11 ± 2	12 ± 1
0.75 10^{-7}	16 ± 1	13 ± 1
1.00 10^{-7}	20 ± 1	16 ± 1
1.25 10^{-7}	15 ± 2	14 ± 1

Table XXVII gives the curvatures of the plants two hours after the application of the agar blocks with I.A.A. In series *b* the maximum curvature is decreased as compared to series *a*. If this test method is used to compare quantities of auxin, the use of orange light therefore is apt to pervert the results. This source of error can be eliminated by keeping the test plants in the dark till the very moment of decapitation.

II

With the standard curvature test it is usual to decapitate the coleoptiles twice; this practice is based on the finding of VAN DER WEY (1931) that the sensitivity to auxin is enhanced by a second decapitation. After the first decapitation the primary leaf is usually loosened, though VAN DER WEY did not indicate whether he did this or not.

Now we made it our point to find out whether the "increase in sensitivity" (by which VAN DER WEY denoted the fact that at low auxin concentrations larger curvatures were obtained) is due to the second decapitation or to the circumstance that the removal of the primary leaf was postponed.

Four series of plants were tested with the same series of dilutions of a I.A.A. solution. Each rack of plants was kept in darkness till its turn for decapitation had come.

Series a was decapitated twice with an interval of $1\frac{1}{2}$ hours. The primary leaf was pulled out in the usual way after the first decapitation.

Series b was treatened like series *a*, but the primary leaf was not loosened until after the second decapitation.

Series c was decapitated once. The primary leaf was pulled out immediately after decapitation, but the agar blocks were applied after $1\frac{1}{2}$ hours to make this series comparable to series *a*.

Series d like *c*, but the I.A.A. was applied immediately after decapitation.

The results are recorded in Table XXVIII.

The largest curvatures at low I.A.A. concentrations are not to be found in series *a*, but this series shows the best proportionality between curvature and I.A.A. concentration. Obviously a second decapitation does not enhance the reaction to low concentrations of I.A.A. Probably VAN DER WEY came to this conclusion by comparing a series *d* with

TABLE XXVIII

I.A.A. concentration in g/ml	Curvature in degrees			
	a	*b*	*c*	*d*
0.50×10^{-7}	7 ± 2	11 ± 2	9 ± 2	7 ± 1
0.75×10^{-7}	10 ± 2	13 ± 2	15 ± 2	10 ± 2
1.00×10^{-7}	12 ± 2	15 ± 1	15 ± 2	14 ± 2
1.25×10^{-7}	16 ± 2	15 ± 1	17 ± 2	12 ± 2
1.50×10^{-7}	17 ± 2	13 ± 1	18 ± 1	14 ± 1
2.00×10^{-7}	19 ± 2	20 ± 2	18 ± 1	18 ± 1
2.50×10^{-7}	17 ± 2	18 ± 2	17 ± 1	23 ± 5

a series *b*. Indeed series *b* responds more vigorously than series *d* to small amounts of I.A.A., but this is caused by the fact that in series *b* the primary leaf was left intact during $1\frac{1}{2}$ hours after the beginning of illumination, and not by the second decapitation. This appears from a comparison between series *a* and series *c*, which shows that the second decapitation decreases the "sensivity" instead of enhancing it.

So if a good proportionality between curvature and I.A.A. concentration is wanted, one should decapitate twice, but if very sensitive plants are needed, one should decapitate once and apply I.A.A. $1\frac{1}{2}$ hours after decapitation.

III

It apparently needs no special experiments to ascertain that illumination of the test plants with orange or red light will influence likewise the results of the straight-growth (cylinder) test.

IV

It seemed worth while to know how long after the illumination the influence of orange light persists. In chapter II we showed that irradiation with orange light enhances the maximum of the first positive curvature. To solve the question indicated above, we irradiated coleoptiles unilaterally with 20 ergs $\lambda = 480$ mμ 0, 1, 2, 4 or 6 hours after a pre-illumination with orange light. From table XXIX it appears that the influence of orange light is still recognizable six hours after its application.

TABLE XXIX

Interval between pre-illumination with orange light and irradiation with blue light	Phototropic curvature in degrees after 20 ergs/cm² $\lambda = 480$ mμ	
	pre-illuminated coleoptiles	not pre-illuminated coleoptiles
0 hour	32 ± 1 (7)	15 ± 1 (5)
1 hour	21 ± 3 (5)	12 ± 2 (7)
2 hours	19 ± 2 (7)	14 ± 1 (7)
4 hours	19 ± 2 (5)	13 ± 2 (4)
6 hours	16 ± 0 (5)	12 ± 1 (5)

SUMMARY

1. Orange light, as used in the air-conditioned dark rooms, influences the reactions of Avena coleoptiles to indole-3-acetic acid (I.A.A.).

2. The orange light does not act upon the coleoptile directly, but through the intermediary of the tip of the primary leaf.

3. Light of $\lambda = 660$ mμ as well as light of $\lambda = 740$ mμ appeared to be active.

4. Irradiation of the Avena seedling as a whole, with 700 ergs/cm^2 $\lambda = 660$ mμ has two effects:

   a. it reduces the I.A.A. content of the coleoptiles.

   b. it enhances either the amount or the effect of an unidentified substance, here called the red light factor (R.L.F.).

5. The R.L.F. regulates the growth of the coleoptile in a narrow range of I.A.A. concentrations. It enhances the growth rate at low I.A.A. concentrations. In this way the one-peaked curve which presents growth as a function of the I.A.A. concentration is transformed into a two-peaked one.

6. Irradiation with 6000 ergs/cm^2 $\lambda = 740$ mμ annihilates effect 4b of light of $\lambda = 660$ mμ, but reduces, like the latter, the I.A.A. content of the coleoptile.

7. An explanation of the negative and of the second positive curvature could be based on a further study of the interaction of the R.L.F. with I.A.A.

8. The results of our experiments are a warning that the influence of orange and red light on the results of growth-substances tests should not be underestimated.

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