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DOUBLE ACTION OF NEAR INFRARED IN LENGTH GROWTH OF THE *AVENA* COLEOPTILE

by

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1. INTRODUCTION

For a large number of higher plants it has been shown that for several reactions an antagonism between red and near infrared irradiations exists. This has been demonstrated in earlier work from this laboratory (10, 11) and also by BORTHWICK, HENDRICKS *et al.* (1).

In our laboratory we have especially studied internode and petiole elongation as influenced by light. Notwithstanding the fact that our equipment is rather extensive, we still were limited in space, so that we have looked for a smaller plant. We found *Avena* seedlings quite acceptable, with the elongating coleoptile as the object. This allows a more rapid procedure, and at the same time yields more accurate results, owing to the much larger number of plants one can use, as compared with, *e.g.*, tomato.

Some work has already been done in *Avena* coleoptile overall growth under white light conditions (7, 12), and in coloured light (6). THOMSON (7), for white light, concluded that light speeds up all growth reactions. According to this author, irradiated *Avena* coleoptiles have a higher growth rate, but growth sooner stops than in dark. SCHNEIDER (6) has shown the same for the action of red light. WENT (12) has demonstrated that the inhibition is linear with log energy. Different spectral regions give parallel lines, the positions of which determine the absorption spectrum (4).

LIVERMAN and BONNER (3) have demonstrated that *Avena* coleoptile sections show the antagonism between red and near infrared irradiations. Growth was measured after 6 or 16 hrs. It was possible to annihilate the red light growth promotion by subsequent near infrared irradiation.

2. MATERIAL AND METHODS

Equipment for irradiation. For the near infrared irradiation our laboratory has two pieces of equipment available. The first is described by WASSINK and VAN DER SCHEER (9) for supplementary illumination of plants. The light source for this cabinet consists of 6 incandescent lamps of 60 W each. This source is combined with a water layer of 3 cm and a blue and a red glass. The radiation in this cabinet is between 700 and 1,000 mμ and the intensity is about 800 ergs/cm²sec. The second unit is designed to obtain much higher intensities; 18 bulbs of 100 W each are mounted over about the same surface as in the first apparatus. It has the same filtering as the previous one, however with a water layer of 5 cm, and allows to apply an intensity range from 40,000 down to 500 ergs/cm²sec.

A set-up for yellow irradiation yields an intensity range from 14,000 ergs/cm²sec. down to

about 100 ergs/cm²sec. The light source consists of 6 sodium vapour tubes, mounted closely together, the total installed energy is 840 W. The filter combination is 5 cm water and 1 cm half-saturated copper sulphate solution. A sheet of yellow cellophane is intercalated to keep the blue lines out. The light obtained consists almost exclusively of the yellow Na-lines.

The red light cabinet used in this investigation is of the same construction as the one used for low intensity irradiation in near infrared. The light is produced by 4 red fluorescent 40 W tubes, combined with a red glass to remove the short wave length emission (further details in [9]).

Plant material. We have used the common experimental oat variety "Zege" (in German "Siegeshafer"). The grains are scaled, and soaked in tap water for 2 hrs. Hereafter they are put side by side along the border of a square glass plate which is covered with a piece of filter paper and is lying in a petridish. The germules have to hang over the glass margin, and the grains must be turned with their grooves downward. Germination is allowed in darkness for 2 days. After this time equally germinated seeds are selected and stuck into a 1½% agar layer in a petridish. This is done in a very weak "daylight" fluorescent room illumination. The agar acts as a water reservoir and at the same time as a support for the growing plants.

Each glass plate takes about 70 seeds from which we select 40–50 seedlings for 2 agar plates. In each experimental treatment, mostly 2 agar plates with seedlings are used originating from different glass plates. Only the lower halfs of the petridishes were used, and a set of them was covered with one large board sheet with a 2 cm high wooden rim. The second day this cover is lifted by intercalating a wooden frame of 6 cm height. The cover is important to assure the appropriate degree of humidity. Especially during the first day on agar the plants require a very high humidity. Only under these conditions the young roots reach the agar. As soon as the roots have settled on the agar, the air humidity is less important for a normal further growth.

The dishes with the plants are in darkness for 3 days. Then the plumule is about 5 cm high, of which the coleoptile occupies only between 1 and 2 cm. Hereafter, so on the 5th day, the plants are ready for irradiation. This normally takes not more than a few hours. Afterwards, the plants are brought into darkness again to complete the growth of the coleoptiles. This takes up to 5 more days, so that the total duration of an experiment is not more than 10 days.

Ultimately, we measure the lengths of the full-grown coleoptiles. The figures are averages of around 40 single measurements.

3. EXPERIMENTAL RESULTS

Normal light effects. The results of three equal experiments in yellow light are represented in Table 1. In these experiments the plants were continuously irradiated from the 5th day onward until all first leaves had grown through the coleoptiles. Four light intensities were given and a dark control was included. The lengths of the coleoptiles are given in mm (Table 1, a), and as percentage of the dark control (Table 1, b). The dark controls differ only slightly which enables us to use the more convenient percentage-way of presentation. Subsequent tables will give the results in percentages of the dark control only. Table 1 shows that the coleoptiles are more inhibited at higher light intensities.

TABLE 1. Inhibition of *Avena* coleoptile length by irradiations of different intensities. Three identical experiments. From the 5th day on continuously irradiated with yellow light at intensities of 4,400; 900; 200, and 80 ergs/cm²sec.
a. Lengths of coleoptiles (means out of ± 40) in mm.
b. Coleoptile lengths in percentages of dark controls.

Yellow light intensity (ergs/cm²sec.)	a			b		
	1	2	3	1	2	3
4,400	36.0	34.8	36.1	53	53	55
900	36.1	36.9	35.6	53	56	55
200	39.3	40.1	37.8	57	61	58
80	42.9	40.6	43.4	63	62	67
Dark	68.4	65.5	64.9	100	100	100

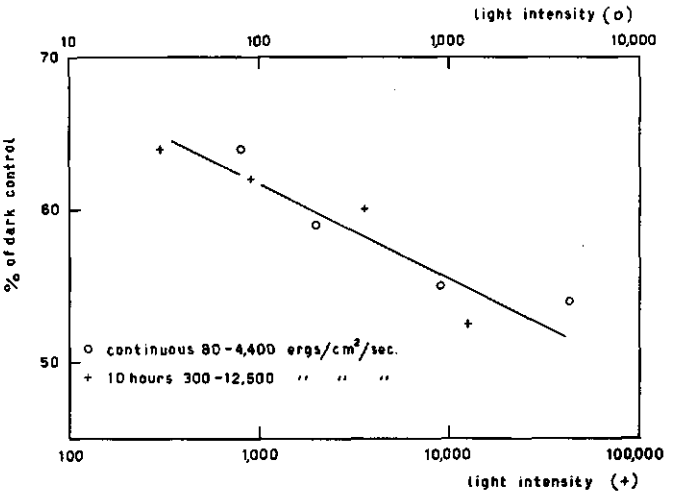

FIG. 1. Inhibition of *Avena* coleoptile length as influenced by intensity of irradiation. Yellow light during 10 hours (+) or continuously (0) in the intensity ranges from 300 to 12,500 and from 80 to 4,400 ergs/cm²sec. respectively.

Figure 1 demonstrates that the inhibition is linear with log i. The points in the graph result from two experiments in four different light intensities. Both series are on the same slope; it appears incidental, however, that they are on the same line, since the irradiation pattern is quite different.

Table 2 presents the results of some experiments in 800 ergs/cm²sec. red radiation of different duration. Irradiations of longer duration have stronger inhibiting effects than those of shorter duration. With high light intensities it is of no use to irradiate longer than for about 10 hrs. After this time, the first leaves from the treated plants break through the coleoptiles, and the coleoptile growth stops completely. This moment is reached sooner with more light energy which is in accordance with the figures of THOMSON (7).

TABLE 2. Inhibition of *Avena* coleoptile length by irradiations of different durations. Irradiation for durations of 1000, 100, 10, 1, and 0 minutes with red light at an intensity of 800 ergs/cm²sec. Means in percentages of dark controls from four different experiments.

Exposure time (minutes) \ Experiment number	1	2	3	4	Mean
0	100	100	100	100	100
1	86	85	84	–	85
10	82	75	79	85	80
100	73	73	76	75	74
1000	–	–	54[1]	59	57

[1] Only 600 minutes.

Similar to the intensity function of light, inhibition is linear with log time of irradiation. An example of several experiments showing this feature is presented

in figure 2. In regions of lower inhibition there is a deviation from linearity. During transporting the plants from the light position into darkness, we perhaps were not working in complete darkness which will be checked soon.

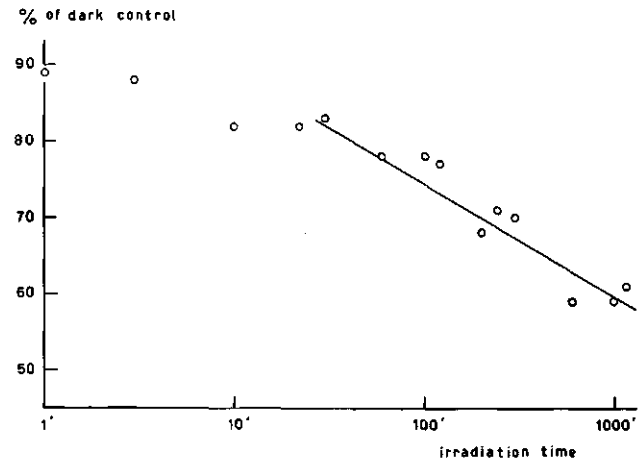

FIG. 2. Inhibition of *Avena* coleoptile length as influenced by duration of irradiation. Red irradiation of 800 ergs/cm²-sec. during exposure periods from 1 to 1,000 minutes.

So far we have seen that the effect of light on inhibition of the length of *Avena* coleoptiles is linear with log time in time series and with log intensity in light intensity series. This makes it reasonable to think of a log-linearity of inhibition against $i \times t$, and the results shown in figure 3 support this idea.

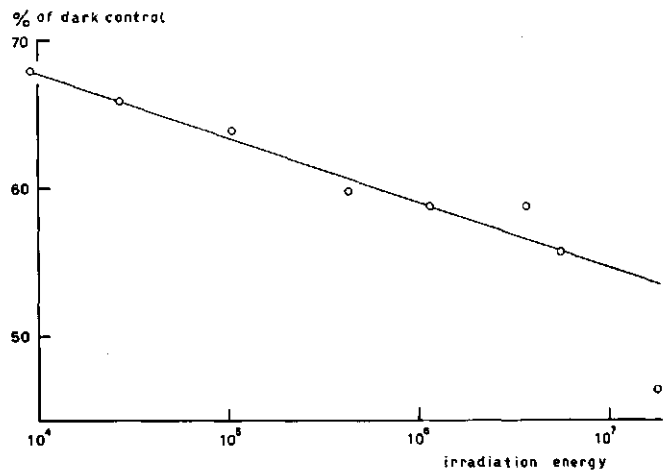

FIG. 3. Inhibition of *Avena* coleoptile length as influenced by irradiation energy. Yellow light during 5 or 24 hours at intensities from 300 to 12,500 ergs/cm²sec.

The effect of successive irradiations is additive as is obvious from Table 3. A weak irradiation following a strong one has only a small effect owing to the log-position of total energy given. For the same reason a strong subsequent irradiation has a noticeably smaller length-decreasing effect than a preceding one of the same energy.

TABLE 3. Inhibition of *Avena* coleoptile length by one or two successive irradiations with yellow light. Intensity in ergs/cm²sec. Duration in minutes.

Intensity (ergs/cm²sec.)	8,000	8,000	180	Relative coleoptile length
Time of irradiation (minutes)	0	0	0	100
	60	0	0	88
	100	0	0	85
	100	0	100	80
	60	60	0	79
	100	100	0	77

Special effects of near infrared. An inhibition owing to a rather strong irradiation with yellow light is reinforced by a similar subsequent irradiation, while it can be partly annihilated by subsequent near infrared (Table 4).

TABLE 4. Inhibition of *Avena* coleoptile length by two successive irradiations. First irradiation in the spectral region indicated on top, intensity in ergs/cm²sec., duration in minutes. Second treatment as indicated at the left side.

1st Treatment / 2nd Treatment	Yellow			Near infrared	Dark
	8,000 60 min.	8,000 100 min.	12,500 120 min.	40,000 120 min.	
Dark	88	85	69	89	100
Yellow [1]	79	77	55	—	—
Near infrared . .	90	92	82	85	—

[1]) Each foregoing treatment duplicated.

It appears impossible, however, to recover the 100% value with a subsequent near infrared irradiation (the third horizontal line reaches about 90% only). This is to be expected considering that 120 minutes of near infrared have an inhibiting effect of 11%, yielding 89%, while a double quantum even goes down to 85%. That there really is a partial reversal of the yellow inhibition is shown by the differences between the 1st and the 3rd horizontal lines: 88 to 90, 85 to 92, and 69 to 82.

The first mentioned difference is only small, the inhibition resulting from the yellow irradiation in this combination was hardly stronger than that of the near infrared irradiation alone. In such a case it is impossible to decide in how far the inhibition is an effect of the yellow light (irreversible part) or of the near infrared (newly induced).

Table 5 contains another example of the irreversible inhibition. In these experiments the number of exposure to yellow and near infrared has been increased. The table shows that, aside of the well-known reversal (column 1 versus 2), the final inhibition becomes stronger with every new I–Y combination (column 1), while at the same time the irreversible part of the inhibition

TABLE 5. Reversibility between yellow and near infrared in *Avena* coleoptile length. I = 2 hours of 40,000 ergs/cm²sec. near infrared, and Y = 2 hours of 8,000 ergs/cm²sec. yellow light.

Irradiation pattern	Relative coleoptile length
Dark	100
Y	80
I	94
I Y	70
Y I	90
Y I Y	68
Y I Y I	89
Y I Y I Y	64

increases (column 2). A comparison of Table 5 with the results of near infrared treatment from Table 4 suggests that the irreversible part is fairly completely determined by the near infrared inhibition alone.

Table 6 shows some more results in accordance with this suggestion, the reversal is complete with an infrared treatment of less than 2 hrs. Again, it is impossible to recover a length above that of the near infrared alone. The length reached after a sufficiently strong near infrared dose seems to be independent of any pretreatment with shorter wavelength radiation.

TABLE 6. Inhibition of *Avena* coleoptile length by two successive irradiations. First treatment: 0, 1, 2 or 4 hours red light (800 ergs/cm²sec.). Second treatment: 0, 1, 2, or 4 hours near infrared (800 ergs/cm²sec.).

2nd Treatment \ 1st Treatment		Red light (hrs)			
		0	1	2	4
Near infrared (hrs)	0	100	78	82	71
	1	103	91	89	91
	2	93	93	92	92
	4	96	91	92	90

The normal light function of near infrared. Continuous irradiation with near infrared for one day with 4 intensities yields the data of Table 7. As can be expected from what has been demonstrated so far, higher intensities of near infrared bring about increasing inhibition, in the same way as holds *e.g.*, for yellow irradiation.

TABLE 7. Inhibition of *Avena* coleoptile length. Irradiation for 24 hours with near infrared at different intensities (ergs/cm²sec.).

Intensity (ergs/cm²sec.)	Relative coleoptile length
8,000	81
1,200	85
750	87
300	89
Dark	100

The same is true for a time series at one intensity of near infrared (Table 8): a longer period in near infrared produces a stronger inhibition of the coleoptile length.

TABLE 8. Inhibition of *Avena* coleoptile length by near infrared irradiations of different duration (minutes). Light intensity 800 ergs/cm²sec. Two representative experiments.

Irradiation time (minutes)	Near infrared (800 ergs/cm²sec.)	
0	100	100
18	101	100
60	98	96
180	89	89
600	85	84
1800	84	84

Apparently, near infrared irradiation has two effects on the *Avena* coleoptile:
1) a similar effect as *e.g.* red and yellow, be it much weaker, *viz.* decreasing the length obtained in complete darkness,
2) a reversal of an inhibition induced by red and yellow.

It is unlikely that our infrared source is less pure than that of others. Moreover, DOWNS has published similar effects (2).

The normal light inhibition reaction also takes place in combinations of near infrared followed by yellow or red, the two effects in inhibition are additive. The total effect is the same as if two light periods of the same wavelength region were given, taking into account the specific activity of each wavelength region.

Near infrared irradiation of 800 ergs/cm²sec. given during 0, 1, or 4 hrs, and followed by red light of the same intensity during 0, 1, 10, or 100 minutes yields the inhibition data compiled in Table 9. It should be remarked that in Table

TABLE 9. Inhibition of *Avena* coleoptile length by two successive irradiations. First treatment: near infrared (0, 1, or 4 hours; 800 ergs/cm²sec.). Second treatment: red (0, 1, 10, and 100 minutes; 800 ergs/cm²sec.).

2nd Irradiation \ 1st Irradiation		Near infrared (hrs)		
		0	1	4
Red light (minutes)	0	100	88	92
	1	85	81	80
	10	75	74	70
	100	73	69	64

9 the figure 92 in the last column probably is too high as compared with 88 in the middle row. Nonetheless for all red irradiation periods a longer pretreatment with near infrared results in a stronger inhibition of the ultimate length of the coleoptiles. This again must be due to the inhibiting effect of the near infrared irradiation as such.

Neither exceptional is the effect of a weak near infrared dose following a high intensity inhibition with near infrared. In this case no reversal of inhibition appears. This is presented in Table 10. This table suggests that it is im-

TABLE 10. The inhibition of *Avena* coleoptile by two successive irradiations with near infrared. First treatment darkness, or 4 hours with 8,000 ergs/cm²sec. near infrared. Second treatment: 0, 15, 30, 60, 120, or 240 minutes near infrared (800 ergs/cm²sec.).

First treatment / Second treatment		Near infrared (4 hrs)	Dark
Near infrared (minutes)	0	87	100
	15	87	97
	30	89	92
	60	89	91
	120	87	90
	240	86	90

possible to annihilate the inhibition of a strong near infrared treatment by a following irradiation with near infrared of lower intensity.

4. DISCUSSION

It is evident from the presented data that red, yellow and also near infrared irradiations inhibit the length growth of *Avena* coleoptiles as compared with darkness. The inhibition appears to be linear with log i, log t and log i × t. This has been found earlier by other workers on growth of leaves of pea seedlings (PARKER *c.s.* [4]). We found inhibition also in blue (8) and violet light, as will be discussed later in a more extensive paper. The dark control plants are the longest possible.

In general the effects of two successive irradiations are additive, *e.g.* in case yellow or red follows yellow, red or near infrared. Near infrared as the second irradiation, however, only behaves this way when it follows near infrared. Following red or yellow (and we found the same with blue and violet) it reverses the inhibiting effect. The inhibiting effect can only be reversed onto the inhibition level of the near infrared irradiation alone.

It may be suggested that the de-inhibiting effect of near infrared deals only with that part of the inhibiting effect which has not yet been fixed *i.e.*, proceeded beyond a certain degree of realization. When light brings about an increase in overall growth rate (6, 7) for a time longer than the irradiation period, then near infrared might restore the original growth rate immediately, while this occurs only slowly in darkness. This capacity of near infrared to annihilate an inhibiting effect resulting from a preceding irradiation does not hold for such effect produced by near infrared itself nor for the loss of length produced during the first irradiation and thereafter until the near infrared came into action.

It may be remarked, that the completely opposite results of LIVERMAN and BONNER (3) are due to the different methods. The results of SCHNEIDER (6) and THOMSON (7) explain this divergence. Our figures are obtained from full-grown coleoptiles, whereas LIVERMAN and BONNER have measured their sections after 6 or 16 hours of growth. They measure the growth rate, while we measure total growth.

We may conclude that an inhibiting effect by near infrared is fully fixed during the irradiation itself; while the inhibition in other spectral regions is only partly fixed during irradiation, for the larger part afterwards in darkness. If

this is correct we must expect different action spectra for continuous irradiation and for irradiation during short periods only.

The results DOWNS ([2], figure 2) published agree with our findings. The reversal by near infrared of the daylength determining function of red light is decreased when near infrared is given over long periods (see also [5]). Thus, also here long periods of near infrared have an effect into the red direction.

One may assume that the antagonism between shorter wavelength regions and near infrared operates by way of a single length growth inhibiting factor, which accelerates overall growth rate. The concentration of this factor then should be raised (normal light function), or lowered (special near infrared and dark function).

SUMMARY

Avena coleoptile growth is affected by light; all wavelength regions appear to be inhibitive, including near infrared.

Besides this, near infrared has a de-inhibition capacity on inhibiting effects induced by a preceding irradiation in the blue, yellow or red wave length regions. This reaction obtains only when a certain inhibition has been induced, but not yet fixed, *i.e.*, has not yet proceeded beyond a certain degree of realization.

The functional system may be an inhibitor of length growth produced by visible light and destroyed by near infrared radiation.

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