

THE INFLUENCE OF RED LIGHT ON GEOTROPISM OF DECAPITATED AVENA COLEOPTILES

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SUMMARY

The influence of red light on the geotropic curvature of decapitated *Avena* coleoptiles provided with agar blocks soaked in a solution of IAA is similar to the influence of red light on the geotropic curvature of intact coleoptiles. A small quantity of red light ($0.6\text{--}2\text{ J m}^{-2}$) enhances the geotropic curvature, a larger quantity of red light ($150\text{--}200\text{ J m}^{-2}$) causes a shift of the curvature to the apical part of the coleoptile stump.

1. INTRODUCTION

Since the discovery of the influence of red light on the phototropic curvature of *Avena* coleoptiles by CURRY (1957) and BLAAUW-JANSEN (1959) independently of each other, several authors have reported on the influence of red light on the geotropic curvature of *Avena* and *Zea* coleoptiles. It was found that the time between the irradiation with red light and the onset of geotropic stimulation influences the nature of the response (WILKINS 1965). In this paper only the influence of red light given shortly before the onset of the geotropic stimulation is reported.

BLAAUW (1961, 1963), HUISINGA (1964), and WILKINS (1965) all observed that red light given shortly before the onset of the geotropic stimulation enhances the geotropic curvature of *Avena* coleoptiles and restricts the curvature to the apical part of the coleoptile. BLAAUW (1963) and HUISINGA, moreover, found that the influence of red light on the distribution of the curvature is more energy-dependent than the influence of red light on the enhancement of the curvature.

Though the perception of gravity (DOLK 1930 and 1936; ANKER 1956) and the gravity mediated lateral transport of auxin (Dolk; GILLESPIE & THIMANN 1963; HAGER & SCHMIDT 1963) are not limited to the tip of the coleoptile, decapitated coleoptiles are not able to perform geotropic curvatures unless auxin is given to them (BRAUNER 1923; SEUBERT 1925; WENT 1926; ANKER 1954). So the coleoptile tip seems necessary as producer of auxin. Red light, however, lowers the production of auxin by the tips of *Avena* coleoptiles (VAN OVERBEEK 1936; BRIGGS 1963) as well as the content of ether extractable auxin in the apical part of the coleoptiles (BLAAUW-JANSEN 1959).

To obtain more information on the influence of red light on the geotropic curvature of *Avena* coleoptiles experiments were made with decapitated coleoptiles that had been artificially provided with auxin. A possible influence of red light on the geotropic curvature by way of a decrease in auxin production is thus excluded.

The influence of red light on the geotropic curvature of coleoptiles treated in this way proved to be similar to the influence of red light on geotropic curvature of intact coleoptiles.

2. MATERIAL AND METHODS

Avena seedlings (var. Victory C.I. 2020) were raised as described by BLAAUW & BLAAUW-JANSEN (1964). After an initial irradiation with red light for 21 hours after soaking of the seeds the seedlings were put in darkness. When the seedlings were about 94 hours old the coleoptiles were excised in spectrally pure green light (560 nm) of very low intensity (about 2.10^{-5} W m⁻² at the level of the plants). The source has been described by BLAAUW-JANSEN & BLAAUW (1966). The coleoptiles were placed on pins in holders with twelve pins after removal of their primary leaves. After a subsequent period of several hours in darkness part of the coleoptiles were decapitated. On the stumps agar blocks (2.5%) of $1 \times 2 \times 2$ mm soaked in 0.1 ppm IAA solution were put. The length of the coleoptile tips removed was 0.4 or 1.4 mm. This was done with the instrument depicted in *fig. 1*. The distance between the plate against which the coleoptile

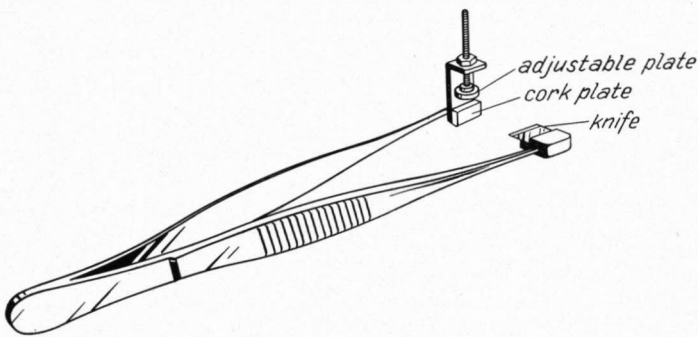


Fig. 1. Decapitation knife

tip was rested and the knife was adjustable. The length of the tips obtained with this simple device was sufficiently reproducible. These manipulations, which took 15 minutes for one holder, were done in the same green working light used during the excision of the coleoptiles. One holder at a time was dealt with and received about 2.10^{-2} J m⁻² green light during the treatment. Non-decapitated coleoptiles used as controls were irradiated with the same amount of green light. Immediately after this treatment the coleoptiles were irradiated from above with a dose of red light of 653 nm. The irradiation time varied from 1 second to 4 minutes. Some coleoptiles were not irradiated. The source of the red light is described in BLAAUW-JANSEN & BLAAUW (1966). Half an hour after the end of the manipulations in green light and the onset of the irradiation with red light the coleoptiles were put in a horizontal position and continuously stimulated geotropically. At the beginning and at the end of the curvature time

of 2.5 or 3 hours the coleoptiles were shadowgraphed on Kodak Royal X Pan sheet film with light of 560 nm obtained from an incandescent lamp with a 560 nm "Depal" double band filter. About 10^{-5} J m^{-2} was sufficient to produce satisfactory blackening.

In another experiment part of the coleoptiles was irradiated with red light with the tips shielded from the light with 5 mm plastic caps. In this experiment the coleoptiles were irradiated from two sides with the aid of surface mirrors. Placing and removing of the capas, which stayed on the coleoptiles for a few minutes only, was done in darkness. In this experiment no green light was used shortly before the irradiation with red light. Temperature during cultivation and experiment was 23°C , relative humidity about 95%. The development of the geotropic curvature took place in a closed box kept extra moist with wet filter paper.

The curvatures were measured in degrees. The shape of the curvatures was approximated by using the ratio between the curvature of the basal part of the coleoptile i.e. the coleoptile minus the apical centimeter and the curvature of the whole coleoptile. This ratio was determined for each coleoptile. It is low when the curvature is limited to the apical part of the coleoptile and high when the curvature is distributed evenly over the coleoptile.

The values are given with 95% confidence intervals calculated by multiplying the standard deviation of the mean by $t_{0.05}$ for the appropriate number of degrees of freedom.

3. RESULTS

The influence of a large (200 or 150 J m^{-2}) and a small dosage (2 or 0.6 J m^{-2}) of red light of 653 nm on geotropic curvature of coleoptiles from which tips had been removed of 1.4 mm or 0.4 mm was compared with the influence of red light on the geotropic curvature of intact coleoptiles. The decapitated coleoptiles were always provided with agar blocks soaked in a 0.1 ppm IAA solution.

We may assume that through removal of tips of 1.4 mm no auxin is produced in the stump (NIELSEN 1924; WENT 1928). However, the curvature of these stumps is small (*cf.* DOLK 1930, 1936; ANKER 1956). Therefore also coleoptiles were used from which a tip of 0.4 mm had been removed. These stumps are still able to produce auxin, but through the artificially applied auxin the auxin content of the coleoptile is less dependent on the auxin production of the tip than with intact coleoptiles.

Both with coleoptiles from which a tip of 1.4 mm had been removed and with coleoptiles from which a tip of 0.4 mm had been removed red light had the same influence as with intact coleoptiles. A small dosage of red light clearly enhances the geotropic curvature; in addition to this effect a large dosage causes the curvature to be limited to the apical part of the coleoptile (*table 1*). With coleoptiles from which a tip of 0.4 mm has been removed the curvature is already limited to the apical part of the coleoptile through 2 J m^{-2} of red light.

INFLUENCE OF RED LIGHT ON GEOTROPISM

Table 1. Influence of red light on magnitude and shape of the geotropic curvature of decapitated coleoptiles with agar blocks soaked in IAA 0.1 ppm and of intact coleoptiles. Time between onset of irradiation and onset of geotropic stimulation was 0.5 hour. Curvature time was 2.5 hours (exp. 1) or 3 hours (exp. 2). Curvatures in degrees and ratios between the curvature of the basal part of the coleoptile and of the whole coleoptile are given with 95% confidence intervals.

Exp.	Quantity of red light (J m ⁻²)	Curvature (degrees)	Ratio	Length of removed tip (mm)	Number of coleoptiles
1	200	14 ± 4	0.09 ± 0.05	1.4	10
	2	12 ± 3	0.21 ± 0.05	1.4	11
	-	10 ± 3	0.21 ± 0.08	1.4	10
	200	36 ± 10	0.08 ± 0.02	0.4	11
	2	32 ± 7	0.08 ± 0.02	0.4	11
	-	21 ± 5	0.23 ± 0.09	0.4	10
	200	39 ± 6	0.06 ± 0.03	-	12
	2	33 ± 6	0.15 ± 0.04	-	12
	-	22 ± 3	0.24 ± 0.04	-	11
	2	150	18 ± 4	0.09 ± 0.05	1.4
0.6		12 ± 3	0.33 ± 0.07	1.4	8
-		10 ± 3	0.29 ± 0.09	1.4	8
150		44 ± 7	0.10 ± 0.03	0.4	6
0.6		31 ± 4	0.18 ± 0.05	0.4	9
-		28 ± 6	0.22 ± 0.05	0.4	9
150		37 ± 9	0.07 ± 0.02	-	7
0.6		23 ± 4	0.24 ± 0.02	-	8
-		16 ± 2	0.28 ± 0.05	-	9

Table 2. Influence of red light on magnitude and shape of the geotropic curvature of intact coleoptiles. Of part of the coleoptiles the tips were shielded with caps (+) during irradiation with red light. Time between onset of irradiation and onset of geotropic stimulation was 0.5 hour. Curvature time was 3 hours. Curvatures in degrees and ratios between the curvature of the basal part of the coleoptile and of the whole coleoptile are given with 95% confidence intervals.

Quantity of red light (J m ⁻²)	Curvature (degrees)	Ratio	Caps	Number of coleoptiles
160	34 ± 4	0.07 ± 0.02	+	10
160	36 ± 5	0.09 ± 0.03	-	11
3	28 ± 5	0.19 ± 0.03	+	10
3	39 ± 3	0.14 ± 0.02	-	11
-	17 ± 4	0.31 ± 0.04		11

When the tips of intact coleoptiles are shielded with caps during the irradiation with red light the same phenomena occur, though the enhancement of the curvature by a small dosage of red light is decreased by the shielding of the tip (table 2).

4. DISCUSSION

From our results it may be concluded that the enhancement of the geotropic curvature by red light, and the influence red light exerts on the shape of the curvature, is not mediated by way of a process that is entirely or predominantly localized in the tip. This is in accordance with other data on the influence of red light. Red light lowers the auxin content in *Avena* coleoptiles (BLAAUW-JANSEN 1959) and decreases the production of auxin which is localized in the tip (VAN OVERBEEK 1936; BRIGGS 1963), but from the work of Briggs it appears that with maize coleoptiles at least the decrease in the production of auxin only occurs when the tip is irradiated with red light before its separation from the coleoptile. The decrease in the production of auxin by red light is apparently a secondary effect (*cf.* HUISINGA 1964). Also the production of the red light factor found by BLAAUW-JANSEN (1959) is not localized in the tip.

Though intact coleoptiles curve more, decapitated coleoptiles provided with auxin are able to curve geotropically. The curvature is less and localized more basally in the stump according as the length of the removed tip is greater (ANKER 1956). However, on this lower level the effect of an irradiation with red light on the magnitude and the shape of the curvature is the same as in intact coleoptiles.

From the influence of red light on coleoptiles of which the tip was shielded during the irradiation with red light it appears that it is not necessary that the part of the coleoptile that is most sensitive to geotropical stimulation is irradiated with red light. However, the shielded tips will have received some light through scatter in the coleoptile.

The influence of red light on the geotropic curvature of coleoptiles has been explained in varying ways. WILKINS (1965) proposed several possibilities: change in the sensitivity of the geoperception mechanism, in the capacity of the tissue to transport auxin laterally, or in the growth rate of the coleoptile caused either by a marked increase in the rate of release of a growth substance from a bound form or the release of a substance that increases the sensitivity of the tissue for auxin e.g. the red light factor found by BLAAUW-JANSEN (1959). From the findings of CURRY, THIMANN & RAY (1956) that red light increases the growth rate of the apical 10 mm of *Avena* coleoptiles shortly after the irradiation and those of BRIGGS (1963) that red light decreases the production of auxin in the *Avena* coleoptile tip Wilkins concluded that red light enhances the sensitivity of the tissue to auxin shortly after the irradiation.

BLAAUW (1963) and HUISINGA (1964) account for the influence of red light on the shape of the geotropic curvature only. They suppose that red light influences the shape of the curvature by changing the basipetal transport of auxin. According to Blaauw red light would accelerate this transport, thus lowering the auxin content in the tip to such an extent that the red light factor found by Blaauw-Jansen would cause an enhancement of the growth. Huisinga supposed that red light decreases the basipetal transport of auxin. In this way the coleoptile tip would contain more auxin proportionately than the more basal

parts of the coleoptile. On account of experiments on the influence of red light on the plastic stretching of *Avena* coleoptile sections, however, BLAAUW-JANSEN & BLAAUW (1966) assumed that red light does not influence the transport rate of auxin and does not reduce the auxin content in the coleoptiles zones examined. In their opinion red light probably does influence some system of transport or supply.

Our results do not enable us to make a choice between the proposed mechanisms through which red light might influence geotropic curvature of *Avena* coleoptiles, but some remarks may be made.

The possibilities proposed by Wilkins that red light should increase the rate of release of a growth substance from a bound form or should enhance the sensitivity of the tissue to auxin do not explain the influence of red light on the shape of the geotropic curvature. Moreover, when with greater sensitivity of the tissue to auxin one means that a certain quantity of auxin causes the tissue to show a growth that is enhanced after an irradiation with red light more than it is without such an irradiation, then a greater sensitivity of the tissue to auxin needs not result in an enhanced geotropic curvature, because the curvature is mediated by a difference in growth of the opposed flanks of the coleoptile. The same may be said of an enhanced auxin content of the tissue by the release of bound auxin. Whether indeed a difference in growth will occur depends on the auxin content of the tissue (*cf.* ANKER 1956). As the auxin content of decapitated coleoptiles will differ from the auxin content in non-decapitated coleoptiles our results render these suppositions of Wilkins less attractive. The same may be said of the supposition of BLAAUW (1963) abandoned by this author for that matter.

The supposition of Huisinga that red light decreases the basipetal transport of auxin does not seem to be contradicted by our results that the action of red light is not mediated through the tip. Indeed, NAQVI & GORDON (1966) found that red light decreases the transport velocity of auxin in maize coleoptile sections.

If the influence of red light is not mediated through a change in the transport of auxin but through a change in some other system of transport or supply as is supposed by BLAAUW-JANSEN & BLAAUW (1966) according to our results this system is not much influenced by the tip.

No explanation yet can be given for the phenomenon that with coleoptiles from which a tip of 0.4 mm has been removed a small quantity of red light already causes the curvature to be limited to the apical part of the coleoptile. It will be the subject of future investigations.

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