581.184.5:582.998

MEDEDELINGEN LANDBOUWHOGESCHOOL
WAGENINGEN • NEDERLAND • 80-11 (1980)

PHOTOTROPISM IN SEEDLINGS OF SUNFLOWER, *HELIANTHUS ANNUUS* L.

(with a summary in Dutch)

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(Received 7-VII-80)

H. VEENMAN & ZONEN B.V. – WAGENINGEN – 1980

Mededelingen Landbouwhogeschool
Wageningen 80-11 (1980)
(Communications Agricultural University)
is also published as a thesis

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ABBREVIATIONS

ABA	abscisic acid
°C	degrees Celsius
CCC	chlormequat = (2-chloroethyl) trimethylammoniumchloride
cm	centimeter
dpm	desintegrations per minute
Fig.	figure
FR	far red
g	gram
GA_3	gibberellin A_3
GLC	gas-liquid chromatography
h	hour
H.E.R.	high-energy reaction
I	intensity
IAA	indolyl-3-acetic acid
L.E.R.	low-energy reaction
L.S.C.	liquid scintillation computer
M	molar
m	meter
mCi	millicurie
min	minute
ml	milliliter
mm	millimeter
mMol	millimolar
μW	microwatt
nm	nanometer
p	probability
pvc	polyvinyl chloride
P.V.P.	polyvinylpyrrolidone
R	red
R_f	relative to the front
spec. act.	specific activity
T	time
T.L.C.	thin-layer chromatography
var.	variety
vol.	volume
w	weight
Xa	xanthoxin

1. GENERAL INTRODUCTION

Phototropism, the movement of plant organs to orientate themselves in a direction determined by the incidence of light, has been known for a very long time. According to MÖBIUS (1937), ± 100 years B. C. DE VARRO noticed heliotropism, the first known form of phototropism. He wrote: 'It is very remarkable that some flowers are directed towards the sun in the morning, and follow the sun until sunset'.

In the 17th and 18th century, simultaneously with the rise of physical science, phototropism was explained by the then known physical laws. RAY (1686) explained phototropism as a phenomenon of temperature, while HALES (1727) ascribed phototropism to evaporation (see MÖBIUS, 1937). HALES' theory was contradicted by BONNET (1754), who placed his plants under water during unilateral illumination and showed that they still bent (see DU BUY and NUERNBERGK, 1935). Light was not yet recognised as the cause of bending. The theory of water displacement, or the lack of water at the illuminated side, however, was and is still of current interest: PRIESTLEY (1926) explained phototropism as a phenomenon of guttation, and MCINTYRE (in press) measured water quantities at the illuminated and the shaded side of unilaterally illuminated plants.

SACHS (1887) supposed that the direction of the light through the plant is responsible for curvature, independent of the intensity. He did not realise, or did not include in his hypothesis, that light must be absorbed by the plant to be physiologically active.

In 1880 DARWIN published his well-known book, 'The Power of Movements in Plants', in which he described phototropism. In this book he wrote: 'some influence is transmitted from the top to the lower part, causing the latter to bend'. He came to this conclusion by covering the top of seedlings so that no light was shown to the top during illumination. He found that almost no curvature occurred. DARWIN experimented with coleoptiles, but also with dicotyledons. In the latter group of plants he only found a reduction in curvature. It is remarkable that, when using dicotyledons, he did not cover the top, but part of the hypocotyl just underneath the petioles.

It should be noted that curvature only seems to start at the top, but in reality it occurs along the whole coleoptile or hypocotyl. DIGBY and FIRN (1979) showed this phenomenon for geotropism and FRANSSEN et al. (in press) for phototropism.

Although SACHS and DARWIN did their research on phototropism in the same period, there was little mutual appreciation for each others work. DARWIN, in an attempt to compare the transmission of stimuli in plants, as discovered by him, with that in animals, pointed at the similarity with nervous conduction. He even compared the tip of the plants, from which the influence for curvature was transmitted, with the brain of a lower classified animal. SACHS and others did not agree with this opinion.

BLAAUW (1909) developed another theory. His first discovery was that both intensity and duration of illumination are important for curvature. As long as I (intensity) × T (time) is constant, the bending will be the same. With this discovery it became clear that phototropic curvature occurs as a result of a photochemical effect in the plant. Later it was found that this rule is not generally valid, but is only true for the first positive and the first negative response (ARISZ, 1915), or only for the first positive response (BRIGGS, 1960).

According to MÖBIUS (1937), DE CANDOLLE (1832) tried to make phototropism understandable in terms of light and growth rate. BLAAUW (1915) used these items in developing his theory for the explanation of phototropism. For his experiments BLAAUW used, e.g., etiolated seedlings of *Helianthus globosus*. He illuminated the hypocotyls with several intensities of light and found a reduction in growth rate. He also examined the decline in light intensity in unilaterally illuminated hypocotyls by bisecting them diagonally, placing them on a photographic plate, and determining the blackening. In this way he was able to calculate the curvature of seedlings; a comparison between the calculated and actually measured curvatures showed that they agreed. In his last publication on phototropism 'Licht und Wachstum III', subtitled 'Die Erklärung des Phototropismus' (1918), he concluded: 'light is the growth affecting factor and phototropism is dependent of growth rate'. This theory does not apply to all phototropic curvatures: root curvature, for instance, cannot be explained this way. Also it fails to account for the supposed influence from the tip: BLAAUW alleged that with illumination of the tip only the light-growth reaction would extend itself to the lower parts, but did not experimentally verify this.

BLAAUW (1915) compared the averages of curvatures and of growth reductions. The tables, however, show a large variation and BLAAUW did not apply statistical methods. Moreover, he used white light including red and far-red irradiation. Although these regions can affect elongation growth, they are ineffective in causing phototropic bending. Later it became known that the growth rate can increase or decrease during de-etiolation, dependent on the age of the seedlings (THOMSON, 1954; SMITH, 1975).

The theory of BLAAUW fell into the background as a result of the spectacular discovery of the growth-regulating hormones. Simultaneously with BLAAUW other plant physiologists, inspired by DARWIN's theory, were developing a new hypothesis which eventually led to the discovery of auxin by WENT (1928).

BOYSEN-JENSEN (1910) cut the tip of *Avena* coleoptiles, put it back on the decapitated coleoptiles, illuminated the tip unilaterally while the base was kept in darkness, and showed that the base responded. PAÁL (1919) extended these experiments: he cut off the tip of *Avena* coleoptiles and put them back on other decapitated *Avena* coleoptiles. The tip was unilaterally illuminated and again the base responded. These experiments were reproducable when a thin slice of agar separated the top from the decapitated coleoptiles, but not when they were disconnected by a thin metal plate. STARK and DRECHSEL (1922) found that tips of *Avena* coleoptiles, put back on decapitated seedlings of different species, also induced curvature.

WENT (1928) extended these experiments. He did not put the tips back on to the coleoptiles, but let them diffuse into agar blocks. When he placed the agar blocks asymmetrically on decapitated *Avena* coleoptiles, he could mimic phototropic curvature. WENT was able to explain phototropic curvature in terms of auxin. He placed the tip of etiolated *Avena* coleoptiles on two well-separated agar blocks, illuminated the tip unilaterally, and tested the activity of the agar blocks in his *Avena* curvature bioassay (see below). The agar blocks which had been underneath the illuminated side showed less curvature than the ones underneath the shaded side, with a ratio of approximately 1:2. His hypothesis, lateral auxin transport in the tip of the coleoptile, has been the main theory of phototropism for the last fifty years (CHOLODNY-WENT theory). During the unilateral illumination he also noticed a small decrease in the total auxin content. WENT did not consider in his theory the possible interference of a growth inhibitor. If a growth inhibitor was formed during (unilateral) illumination, the illuminated part of the tip might diffuse more growth inhibitor into the agar block than the shaded part and, therefore, that block would show less curvature in the bioassay. Concerning BLAAUW's theory, WENT concluded that the light-growth reaction alone cannot account for the phototropic curvature; his calculations for curvature from growth rate changes gave much lower figures than the actually measured ones. It is difficult to judge whether the calculations were done correctly; WENT calculated the growth rate within 2 hours, using results from his bioassay, for the whole length of the coleoptile. However, it is not clear from his results that curvature occurred along the whole coleoptile.

In 1925, VAN DILLEWIJN concluded that the first positive, first negative and second positive response of *Avena* coleoptiles can be explained by growth-rate changes, according to the hypothesis of BLAAUW.

WENT was able to quantify the amount of growth hormone diffusing from the tip: by placing more tips on the agar blocks and replacing the agar blocks asymmetrically on decapitated coleoptiles, he induced more curvature in these coleoptiles. In this way he developed the *Avena* curvature bioassay. He found that his standard curve was linear from 1 to 5 tips; when more than 5 tips diffused into agar, the curvature reached a plateau, the maximal curvature being 16°. Later, when other plant hormones were discovered, it was found that most of them showed a logarithmic dose-response relationship instead of a linear one. This led to the reinvestment of the bioassay by BENTLEY and BICKLE (1952) and SCOTT and JACOBS (see JACOBS, 1979). They found a logarithmic relationship between auxin concentration and curvature. In WENT's time the first positive, first negative, second positive and indifferent responses of *Avena* coleoptiles were known (ARISZ, 1915). In his thesis (1928) all the unilateral illuminations used by WENT were in the range of the first positive response. He used white light for his illuminations, and dim red light as a safe light. Afterwards it was discovered that red light alters the phototropic sensitivity in relation to the first positive, first negative and second positive responses (BRIGGS, 1963a).

In the literature, experiments are described which are difficult to explain in terms of auxin transport. In 1911, VAN DER WOLK described that the base was

less sensitive to light than the tip. In illuminating only the basal half of *Avena* coleoptiles he never found curvature of the upper half, but this upper half responded more rapidly to unilateral light than control coleoptiles. In illuminating only the upper half and removing this half within 3 min after unilateral illumination, he found that also the lower half bent. Another phenomenon, the straightening reaction or autotropism, which was known to the earlier workers (e.g. ARISZ, 1915), was not discussed by WENT and is difficult to explain in terms of basipetal and lateral auxin transport.

In the thirties, the active substances were considered to be auxin a and b, chemically different from indoleacetic acid, the 'heteroauxin' (KÖGL, 1937). Afterwards it became evident that these compounds have no auxin activity at all (see JACOBS, 1979). This renders the results of KONINGSBERGER and VERKAAIK (1938) difficult to explain. They applied auxin a or IAA to decapitated *Avena* seedlings and found that only the seedlings with auxin a responded phototropically, not the seedlings to which IAA was applied. DU BUY and NUERNBERGK (1930), measuring growth-rate changes of the illuminated and the shaded sides of curving coleoptiles, found different kinetics for the phototropic curvature of intact plants and for the curvature of decapitated plants in which the curvature was induced by unilateral application of agar blocks, in which diffusate from coleoptile tips. This indicates that the curvature induced by exogenously applied hormones differs from that produced by unilateral illumination.

VAN OVERBEEK (1933) did his experiments with dicotyledons, viz. with dark-adapted *Raphanus* seedlings. He concluded that both the asymmetrical distribution of growth substance due to unilateral illumination and the sensitivity of the cells to the growth substance play a role. He established an asymmetrical distribution in the hypocotyl, without the involvement of the tip or the cotyledons, and also found that the inhibition of growth due to light was not the result of inhibition of growth-substance synthesis. He stated: 'Die Blaauwsche Theorie und die Theorie von Went sind also keine Antithesen, sondern die Grundgedanken beider Theorien ergänzen einander bei der Erklärung des Phototropismus der Raphanus-Hypokotyle'. Furthermore, VAN OVERBEEK (1933) showed that dark-adapted radish seedlings grow more rapidly than light-grown ones, but contain less growth substance, which contradicts the idea of a direct relationship between the amount of growth and the quantity of growth hormone.

Originally, WENT (1926) investigated the growth response of *Avena* coleoptiles illuminated by light from 3 sides using different intensities and concluded that the tip response (first positive response) might well be a BLAAUW-effect. He also wrote: 'Now we understand why the tip response requires 60 or 78 min to reach its minimum (minimum in growth rate of the coleoptile by illumination of the tip only) because the response is stimulated in the tip and then has to diffuse into the lower parts where it finally manifests itself'. A growth retardation of the coleoptile, induced by light from 3 sides, does not agree with his theory published afterwards on growth-substance distribution. In 1956 WENT explained the tip response as a growth-substance effect and the base response as a BLAAUW-effect,

in agreement with VAN OVERBEEK (1933).

GALSTON (1959) argued that the light-growth reactions are too small, by a factor 4, to account for the measured curvatures. He also remarked that the very strong growth reaction, as observed by VAN OVERBEEK (1933), cannot be explained in terms of auxin content, auxin destruction, or auxin transport, and therefore light must somehow alter the response of the tissue to auxin.

WILDEN (1939) investigated the asymmetrical distribution of growth substance(s) in the first positive, first negative, and second positive response of *Avena* coleoptiles. She found more growth activity at the illuminated side during the first negative response.

In the 19-fifties and sixties, research on phototropism was directed towards the following 3 main questions:

- The asymmetrical distribution of IAA during the response. This was mainly done by exogenous application of radioactive IAA to the tip of coleoptiles.
- The destruction or inactivation of IAA by light.
- The sensitivity of the tissue to IAA, under several conditions.

PICKARD and THIMANN (1964) investigated the distribution of exogenously applied, radioactive IAA in *Zea mays* seedlings. Their results were in agreement with the CHOLODNY-WENT theory for the first positive, first negative, and second positive response. The results of BRIGGS et al. (1957) and BRIGGS (1963b) pointed in the same direction. REISENER (1958), however, could not find an asymmetrical distribution of IAA in *Avena* coleoptiles after phototropic stimulation. SHEN-MILLER and GORDON (1966) found an asymmetrical distribution of exogenously applied IAA, but not of endogenous IAA.

GALSTON and BAKER (1949) showed that IAA is destroyed in the presence of riboflavin. However, photo-oxidation is too small to account for the extent of phototropic curvature (BRIGGS et al. 1957; PICKARD and THIMANN, 1964; ZENK, 1968).

The sensitivity of the tissue to IAA was studied by several workers, e.g. NAQVI and GORDON (1967). They concluded that light had an inhibiting effect on IAA transport in *Zea mays*. The same phenomenon was described earlier by VAN OVERBEEK (1932), for *Raphanus* seedlings.

Already in 1887 SACHS, using solutions with a different colour, showed that blue light, but not red light or green light, was phototropically active. A more detailed action spectrum is described by CURRY (1969) for the first positive response of *Avena*. No activity at all was found at wavelengths higher than 500 nm. Research done to elucidate the photoreceptor pigment has not been conclusive so far; the attention is mainly directed towards carotenoids and flavins.

Although it was known in the beginning of this century that blue light was the phototropically active wavelength range, most experiments have been done with unilateral incandescent white light. DU BUY (1933) was the first to use blue unilateral light.

DU BUY and NUERNBERGK (1934) first published the relation between the total intensity of actinic light and the curvature of *Avena* seedlings, using white light. These results were converted into graphical form by WENT and THIMANN (1937),

and show a first positive, first negative, second positive, indifferent and third positive response. Parts of this curvature have also been obtained with monochromatic blue or ultra-violet irradiation (see BRIGGS, 1963c). The first negative response does not always occur as actually negative, depending on the total intensity of light given (BRIGGS, 1960). Also red light, given before the phototropic stimulus, can change this response considerably (BLAAUW and BLAAUW-JANSEN, 1964; BRIGGS, 1963a).

Most experiments to elucidate the phototropic mechanism have been done with monocotyledonous seedlings, on the basis of the theory of WENT. As shown above, even in the case of phototropic curvature of etiolated *Avena* coleoptiles, much contradictory evidence occurs in the literature and it is difficult to gain a clear picture of the response. This was also pointed out by MER (1969), who discussed the absence of a correlation between the amount of auxin and the growth rate, and the transfer of conclusions obtained with decapitated plants to intact ones. DENNIS (1977) emphasized that the pool size, diffusion and metabolism of hormones are more important for a physiological response than the amount of a hormone at a specific time.

The phototropic response of dicotyledonous seedlings is even less understood. BLAAUW (1915) developed his theory for *Helianthus tuberosus* and VAN OVERBEEK (1932, 1933) investigated the response of *Raphanus* seedlings. The latter concluded that the main synthesis of growth substance was in the cotyledons, and in the tip after the removal of the cotyledons, on the analogy of the tip of coleoptiles.

STEYER (1967) compared the phototropic response of monocotyledonous and etiolated dicotyledonous seedlings and demonstrated that both showed the first positive and second positive response, except that in *Helianthus* the first positive response was only 4°.

BARA (1957) and DIEMER (1961), both testing the CHOLODNY-WENT theory in dicotyledonous seedlings, investigated the phototropic response of *Helianthus annuus*, mainly in decapitated green seedlings. Decapitation decreased the curvature and this decrease could not be explained simply as a reduction in growth rate (DIEMER, 1961). Application of IAA to the decapitated seedling could restore the curvature, either completely (DIEMER, 1961) or partly (BARA, 1957). Both BARA and DIEMER used green seedlings which received a dark treatment (17 or 10 h, respectively) before the onset of the experiments. SHIBAOKA and YAMAKI (1959) also found an influence of the cotyledons in dark-adapted sunflower seedlings.

LAM and LEOPOLD (1966) concluded that the cotyledons of sunflower seedlings play an important role in phototropism, in supplying auxin; however, they also detected considerable growth-inhibiting activity in their extracts. Their results are contradicted by those of SHUTTLEWORTH and BLACK (1977), who found no influence of the cotyledons and pointed out that LAM and LEOPOLD (1966) did not measure proper phototropic curvature, but a curvature in the hypocotyl caused by illumination of the cotyledons with red light. The hypocotyl itself did not receive unilateral illumination.

BRENNAN et al. (1976) stated an influence of the tip on the phototropic response in mung bean and found no influence of the first leaves.

BRUINSMA et al. (1975), although finding an influence of the cotyledons, concluded that IAA could not be the cause of phototropism in *Helianthus annuus*, IAA being equally distributed in both halves of the curving hypocotyl. They proposed a theory in which the illuminated side of the hypocotyl is inhibited in growth by an inhibitor, a possibility first proposed by BRAUNER (1922). This idea was also substantiated by the results of BAYER (1961), SHIBAOKA (1961), and THOMPSON and BRUINSMA (1977), and later supported by DÖRFFLING (1978). THOMPSON and BRUINSMA (1977) found evidence that the inhibitor concerned is xanthoxin (see also Chapter 7).

This introduction is not meant as a complete survey of the literature about phototropism. Many reviews have been written about this subject, e.g. by DU BUY and NUERNBERGK (1935), MÖBIUS (1937), WENT and THIMANN (1937), VAN OVERBEEK (1939), GALSTON (1959), REINERT (1959), THIMANN and CURRY (1960), THIMANN and CURRY (1961), BRIGGS (1963c), THIMANN (1967), CURRY (1969), GALSTON (1974), LEOPOLD and KRIEDEMANN (1975), THIMANN (1977), REINHOLD (1978), DENNISON (1979) and GRESSEL and HOROWITZ (in press), and they all deal with the subject more extensively. The aim of this introduction is to point out that the knowledge of the mechanism of phototropism is far from clear.

In this thesis phototropism is studied in *Helianthus annuus* L. seedlings.

The plant material and the methods are described in Chapter 2.

In Chapter 3 factors which may influence the phototropic response are investigated, particularly geotropism, circumnutation, the variability of the phototropic response in the course of the year, and the age and length of the hypocotyl in relation to its bending properties.

Chapter 4 deals with the influence of the different organs of the seedling and the epidermis of the hypocotyl on the phototropic curvature.

In Chapter 5 the influence of different wavelengths on hypocotyl phototropism, and in Chapter 6 the effect of pretreatment with red or far-red irradiation on the phototropic response are investigated.

Chapter 7 deals with the involvement of hormones in sunflower phototropism, in particular with the possible role of the growth inhibitor, xanthoxin.

The final Chapter, 8, presents a synthesis of the conclusions derived from the experiments of the investigation.

2. MATERIAL AND METHODS

2.1. Experimental material

All experiments were carried out with *Helianthus annuus* L., var. Giganteus seedlings. Sunflower plants, originally grown from seeds obtained from Van Tubergen, B.V., Haarlem, The Netherlands, were grown in the garden of the Department of Plant Physiology. The seeds of the harvest of each year were used for experiments in subsequent's years.

2.2. Culture conditions

Seeds were sterilised in 3 % sodium hypochlorite for 45 minutes, rinsed in tap water for an hour, and placed between wet filter paper for approximately 20 hours in darkness. Small and ungerminated seeds were disregarded and the remaining germinated seeds were potted in a mixture of humus soil : sand : clay = 1 :1 :1 in a piece of pvc pipe, 4.0 cm high with a diameter of 3.1 cm, closed with a rubber plug at the base. Plants were cultured in darkness or in a rhythmic period of 14 hours light/10 hours dark, until used for experiments. When light-grown seedlings were used, they had 6 hours light after their last dark period.

The temperature during culturing and the experiments was 25 ± 2 °C, and the relative humidity was 60 : 10 %, unless mentioned otherwise.

2.3. Age and length of the seedlings

The age of the seedlings was calculated from the time of potting; the length of the seedlings was measured with a ruler to the nearest mm, from the point they emerged from the soil to the attachment of the cotyledons.

2.4. Phototropic experiments

Phototropic experiments were carried out in an experimental box (Fig. II.1. and II.2.). The dimensions of this box were 134 cm, 76.5 cm and 37 cm. Inside the box was another box (dimensions 126 cm, 16 cm and 15 cm) containing a fluorescent tube (see under light sources). Slits were cut in the sides of this box (5 cm wide), and in this way hypocotyls could be subjected to unilateral illumination. All internal surfaces were painted black or lined with black velvet to avoid reflection.

The seedlings were placed at 25 cm distance from the fluorescent tube when illuminated with white light, and at 6 cm distance when illuminated with blue

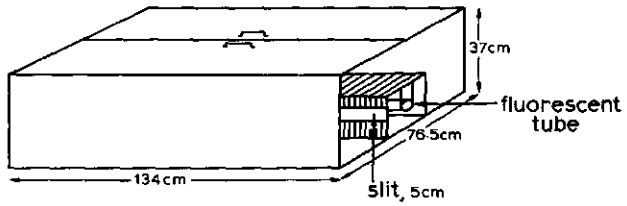


FIG. II.1. A schematic presentation of the experimental box, one side wall removed.

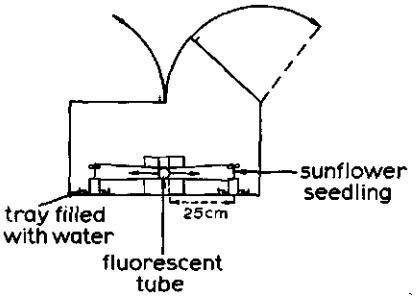


FIG. II.2. A cross-section of the experimental box.

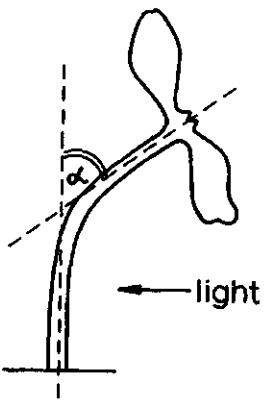


FIG. II.3. The angle of curvature measured in sunflower hypocotyls.

light, in a tray filled with tap water to maintain the humidity. After the onset of the light treatment the experimental seedlings were placed in this tray, one by one, at the same speed as needed to make the subsequent measurements.

The curvature was measured with a protractor. The angle between the straight part of the hypocotyl emerging from the soil and the straight part below the cotyledons was measured (Fig. II.3.). At least 10 plants were used in each experimental group.

2.5. GEOTROPIC AND PHOTO-GEOTROPIC EXPERIMENTS

For geotropic experiments seedlings were placed horizontally with the cotyledons in vertical or horizontal position. Sometimes, depending on the size and

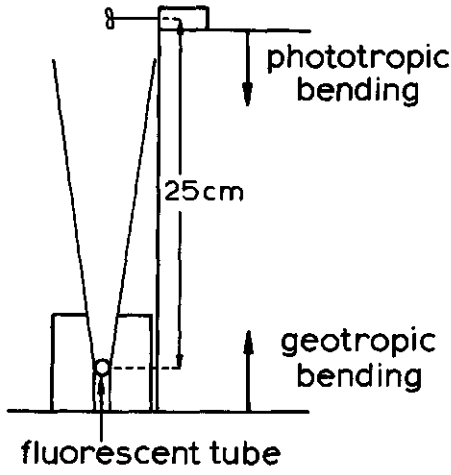


FIG. II.4. The photo-geotropic experimental design.

direction of the cotyledons, it was necessary to place them on a small platform, in order to avoid disturbance of the cotyledons.

Photo-geotropic experiments were carried out as indicated in Fig. II.4. The pots were placed horizontally on a platform with the hypocotyls protruding. Underneath the hypocotyls was a fluorescent tube, at 25 cm distance, so that for phototropic bending curvature had to occur against the direction of gravity and vice versa.

2.6. COVERING AND REMOVAL OF PARTS OF THE SEEDLING

2.6.1. *Cotyledons*

Cotyledons were covered with aluminium foil, or black or transparent plastic. The aluminium foil was neatly folded around the cotyledons, and the plastic was taped together at the lower side of these organs. The actions, both covering and removal, were performed carefully to avoid stress to the seedlings. In order to minimise light conduction through the opening of the foil or plastic, these materials were folded carefully around the petioles.

Removal of the cotyledons took place just above the attachment with the hypocotyl.

2.6.2. *Apex and cotyledons*

Removal of the apex, together with the cotyledons, took place just underneath the attachment of the cotyledons with the hypocotyl.

2.6.3. *Roots*

The seedlings were removed from their pots and the roots were cut off at the root-hypocotyl junction. The derooted seedlings were transferred to new pots with a very humid soil mixture, and planted again to a depth of 0.5 cm.

2.6.4. *Hypocotyl*

Covering of the hypocotyl took place in the same way as covering of the cotyledons. Especially the removal of foil or plastic was carried out very carefully to avoid stress. Because of the growth of the hypocotyls during this treatment, the covering was extended every 4 to 5 hours.

2.6.5. *Peripheral layers*

In most experiments where the peripheral layers of the hypocotyl were removed the cotyledons were also removed. A small part of the peripheral layers was loosened with a razor blade, and the epidermal layers were peeled off with a pair of tweezers. This procedure was repeated until the right amount of peripheral layers had been removed.

Seedlings were bisected along the longitudinal axis of the right angle plane between the cotyledons.

During pretreatment and experiments the relative humidity was kept at at least 97%. Non-turgid seedlings were disregarded.

2.7. Light sources and light conditions

Prior to experimental treatments seedlings were illuminated with white light from day-light tubes, hanging approximately 1.5 m above the plants. Because of the small size of these tubes there was slight variation in the intensity of illumination. Intensity was measured both immediately below the tubes and at the farthest point at which plants were placed for light treatment. Unilateral illumination with white light or light of special wavelengths was obtained from white or coloured fluorescent tubes, with appropriate filters (Table II.1.).

All the light intensities were measured at plant height with an IL 150 Plant Growth Photometer, International Light Inc., Newburyport, Massachusetts, U.S.A. Light intensity was measured in the 3 regions of the spectrum which are known to be physiologically important, viz. blue (half broad band 110 nm, viz. 400–510 nm), red (half broad band 120 nm, viz. 620–740 nm) and far red (half broad band 100 nm, viz. 710–810 nm). Because the sensitivity of this instrument was rather low, 55 $\mu W/cm^2$ in the blue, 60 $\mu W/cm^2$ in the red, and 50 $\mu W/cm^2$ in the far-red region, the light intensities for blue, green, red and far-red irradiation were also measured with an optometer from United Detector Technology, model UD 80x PN, Santa Monica, California, U.S.A., which measured the total light energy. The intensities measured with this instrument were the same, within a limit of 8%, as those measured using the IL 150 Plant Growth Photometer, indicating that no contamination with other wavelengths occurred. The intensities for the light sources used are given in Table II.1. Traces of the transmission spectra of the different coloured layers are shown in Fig. II.5.

The light intensities which were obtained from the blue and the red fluorescent tubes with their appropriate filters had a lower intensity than the light from the day-light tubes. For this reason a white fluorescent tube was used as a control.

Table II.1. The light sources, the filters, and the light intensities used in this thesis.

light colour	light source	filters	used for	intensity ($\mu W/cm^2$)	remarks
white	day-light tubes Philips HPI/T 400 W	none	normal culturing	blue : 165– 99 red : 180–108 far red : 110– 66	The first figure indicates the intensity underneath the tube, the second figure indicates the intensity at the farthest distance from the tube.
white	fluorescent tube Philips 40 W/33	none	unilateral illumination	blue : 70 red : 64 far red : –	
			illumination from above	blue : 93 red : 85 far red : –	
red	fluorescent tube Philips 40 W/15	1 layer Cinamoid red no. 14	unilateral illumination	blue : – red : 65 far red : –	
			illumination from above	blue : – red : 86 far red : –	
blue	fluorescent tube Philips 40 W/18	2 layers Cinamoid blue no. 62	unilateral illumination	blue : 66 red : – far red : –	
			illumination from above	blue : 67 red : – far red : –	

green	fluorescent tube Philips 40 W/17	2 layers Cinamoid yellow, no. 46, and 1 layer Cinamoid blue, no. 62	safe light	blue red far red total	:– :– :– :19	total: measured with the optometer from United Detector Technology, see text.
far red	incandescent tube Philinea, Osram linestra, 120 W/ 6277 X	1 layer Cinamoid red, no. 14, and 2 layers Cinamoid blue, no. 62	unilateral illumination	blue red far red	:– :– :19	far-red: measured with the optometer from United Detector Technology, see text.
far red	8 incandescent tubes Philinea, Osram linestra, 60 W/dX2	1 layer plexiglass Blau 627, 1 layer plexiglass rot 501 and 2 layers Cinamoid red, no. 14	far-red experiments	blue red far red	:– :48 – – :350 – 150	The first intensities are measured directly under the filters, to determine the ratio, = 7.3. The second intensities are measured at plant height.
red	6 fluorescent tubes Philips 20 W / 15	1 layer plexiglass Rot 501.	far-red / red experiments	blue red far red	:– :160 :–	Far red could not be detected.

–: could not be detected.

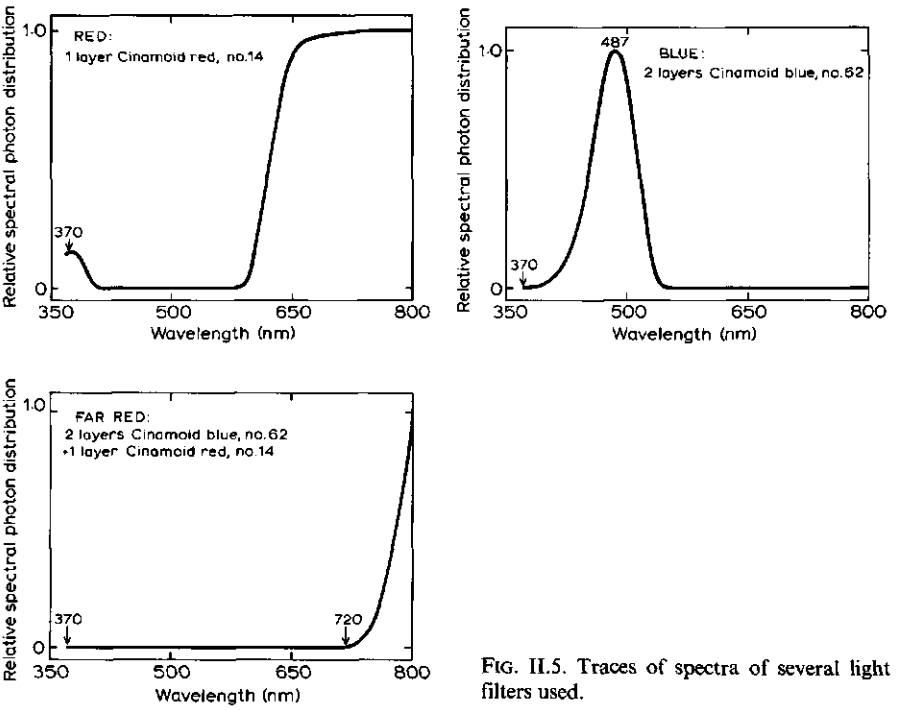


FIG. II.5. Traces of spectra of several light filters used.

Curvatures of seedlings grown in white light from day-light tubes or from fluorescent tubes were determined, and the average values were not significantly different. This justifies the choice.

2.8. SAMPLING PLANT MATERIAL FOR HORMONE DETERMINATIONS

The seedlings were cut off just above soil level, and the apex and cotyledons removed. During sampling the hypocotyls were kept on ice, and afterwards stored at –20 °C.

When bisected seedlings were sampled, the half hypocotyls were immediately frozen in liquid nitrogen, to avoid enzymatic reactions at the wounded surface, and stored at –20 °C.

2.9. EXTRACTION PROCEDURES

2.9.1. *IAA*

IAA was extracted according to the method described by KNEGT and BRUINSMA (1973).

2.9.2. *Xanthoxin*

The original method by FIRN et al. (1972) and THOMPSON and BRUINSMA (1977) was used, and modified in a later stage, as shown below.

Method 1:

Frozen plant material was macerated in a Braun mixer, in 2 volumes 100% methanol, 3 times for 10 seconds. The extract was filtered through a G3 and a G4 filter (sintered glass filters, from Schott Jena, Mainz, G.F.R.), and the residue suspended in 1 volume 80% methanol, and left overnight. The filtering procedure was repeated, and the 2 filtrates combined. All operations were carried out at 2–4 °C, under dim illumination. The filtrate was evaporated in vacuo, at 25 °C, and the aqueous residue made up to 80% methanol and shaken against an equal volume petroleum spirit, approximately 5 times. After evaporation in vacuo of the methanol layer, potassium phosphate buffer was added to the aqueous residue to 1 M (pH = 8.5) and partitioned against an equal volume of ethyl acetate, approximately 4 times. Each time after evaporation in vacuo the residue was vibrated ultrasonically to remove the whole residue from the wall of the flask. The in vacuo evaporated ethyl acetate layers were applied to an ethanol-washed Whatman no. 3 paper and developed in isopropan-1-ol : ammonia : water (10 :1 :1 by vol.), by descending chromatography. The paper with R_f 0.5–0.95 was eluted in ethyl acetate for 15 hours in the dark at 2 °C. The eluate was applied to 0.2 mm prewashed silica plates, and developed in ethyl acetate: hexane (3 :1 by vol.). The silica with a R_f value 0.2–0.4 was removed and transferred to a pasteur pipette with a small piece of cotton wool in the end. The xanthoxin was eluted from the silica with 0.5 ml ethyl acetate, 3 times. The solvent was collected in petri dishes, diameter 5 cm, and evaporated to the air.

Method 2:

This method was similar to method 1, except that after combining the filtrates from the G3 and G4 step, the whole filtrate was again filtered by a Sep-pack C18 cartridge (Waters Associates Inc., Etten-Leur, The Netherlands) in 70 to 80% methanol. The purification obtained by this procedure allowed the removal of the paper-chromatography step, which shortened the whole procedure by 2 days.

2.9.3. *GA*

Originally, the plant material was ground in a Braun mixer, in 1 volume 100% methanol, and the extract filtered through a G3 and a G4 filter. All operations were carried out at 2–4 °C. The filtrate was evaporated in vacuo, at 25 °C, and the aqueous residue was taken up in Sørensens phosphate buffer, pH = 8.2, and shaken against petroleum spirit. The aqueous layer was shaken against ethyl acetate, and the ethyl acetate layers were combined. The aqueous layer was acidified (pH = 2.5) and again partitioned against ethyl acetate. All the ethyl acetate layers were combined, evaporated in vacuo and stored at –20 °C.

Afterwards the residue of the plant material was extracted as described by BROWNING and SAUNDERS (1977), using the non-ionic detergent Triton X-100,

with some modifications. The ground material was stirred in a 2% Triton X-100 solution in Sørensens phosphate buffer (pH = 8.0), for 2 hours, in 2 volumes per fresh weight. The solution was placed on a Dowex 21-K column in formiate form, and washed with 2 volumes of Sørensens phosphate buffer (pH = 8.0). Afterwards the gibberellins were washed off the column, using 200 ml ethanol: formic acid (4:1 by vol.). Before the actual extraction was carried out, this elution volume was determined using radioactive GA_3. The eluate was stirred in a 10% P.V.P. solution (see GLENN et al., 1972) for 1 hour and passed through a G3 filter. The filtrate was acidified (pH = 3.0) and partitioned against ethyl acetate. The ethyl acetate layers were combined, evaporated in vacuo, and stored at -20°C.

2.10. DETERMINATION PROCEDURES

2.10.1. *IAA*

-Spectrofluorometrical determination. This procedure is described by KNEGT and BRUINSMA (1973).

-Bioassay. This procedure was used to determine the activity of possible other auxins than IAA, and of inhibiting compounds in the diffusates. The bioassay used is the straight-growth test of *Avena* coleoptile segments after NITSCH and NITSCH (1956). The vials used were plastic or glass ones, with a diameter of 1.3 cm. To determine activities in the diffusates, 1.5 ml diffusate and 0.5 ml buffer at 4 times the usual concentration were used instead of the normal buffer, sometimes with the addition of K-IAA standards. The *Avena* seeds used were Victory oat 1 (Swedish Seed Company Ltd., Svalöv, Sweden).

2.10.2. *Xanthoxin*

The original bioassay of TAYLOR and BURDEN (1970) was used in a modified way as described by FRANSSEN et al. (1979). Forty seeds of *Lepidium sativum* L., var. Holland select grof (Nunhems Zaden B.V., Hoelen, Holland) were sown in a 5 cm petri dish on a filter paper moistened with 1 ml water or aqueous solution, and incubated at 25°C in the dark at high air humidity. After 12 hours, and at 3 hour intervals during the subsequent 39 hours, the seeds showing rootlets of at least 1 mm were counted and removed. If necessary, the countings were continued, at intervals from 15 to 20 hours. The time at which 50% of the seeds had germinated was determined by interpolation.

2.10.3. *Gibberellin*

The dwarf rice elongation bioassay described by MURAKAMI (1968) was used. Seeds of *Oryza sativa* L., var. Tan-ginbozu dwarf, were sown in tap water for 48 hours at 25°C. The germinated seeds were transferred to 1% agar, and grown for 2 to 3 days, at high air humidity, in white light. A standard of K-GA_3 and the samples in ethanol were applied to the leaf axil of the second leaf and the length of the second leaf or the total length of the seedling was measured after 3 days.

The extracts of both the gibberellin extractions were applied separately. In the

results the total amount of gibberellin extracted is given.

2.10.4. *Ethylene*

Ethylene was determined by gas chromatography. This method is described by SAWAMURA et al. (1978). The gas chromatograph was equipped with an alumina column and a flame ionization detector.

2.11. COLLECTION OF DIFFUSATES

Derooted sunflower seedlings, hypocotyls only, or cotyledons only, were placed with their cut surfaces in twice distilled water or in 0.01 M phosphate buffer, pH = 5.9, containing 2% sucrose (w/v). Five seedlings or hypocotyls, or 10 cotyledons were placed in 1 ml of solution in blackened beakers or petri dishes to avoid destruction of IAA as much as possible. The relative humidity was kept near to 100% by placing the beakers or petri dishes in large petri dishes filled with water over which a glass bell was placed. After 6 hours the plants or organs were removed and the diffusates were filtrated through millipore filter, 0.45 nm, from Millipore, Brussels, Belgium, to exclude the involvement of microorganisms. The diffusates were used immediately, stored at 4°C for maximal 15 hours, or stored at –20°C for longer periods. This storage did not affect the activity of the diffusates.

2.12. RADIOACTIVITY

IAA-1-^{14}C (spec. act. 52 mCi/mmol), ^{14}C-xanthoxin (spec. act. 0.26 mCi/mmol) and 1-^{14}C-ABA (spec. act. 1.64 mCi/mmol) were used.

For recovery experiments the radioactive compound was added immediately after grinding and a small part of the extract at the end of the extraction procedure was counted. The same procedure was followed to determine the loss of activity of IAA during diffusion.

^{14}C-xanthoxin, in an ethyl acetate-twice distilled water or ethanol-twice distilled water mixture was applied to seedlings by injection or the microdrop method. At the end of the experiment the seedlings were washed, divided into segments and transferred to scintillation vials, in which they were cut in small pieces. One ml methanol was added and the pieces were left for 4 hours at 25°C. Lumagel scintillation fluid, Baker Analysed Reagent, J. T. Baker Chemicals B.V., Deventer, The Netherlands, was added and the radioactivity was counted in a L.S.C. Mark 1, model 6860, with external standard.

2.13. GENERAL

All experiments were repeated at least twice, unless mentioned otherwise.

2.14. Statistics

All statistics were carried out by the nonparametric method. The tests of Wilcoxon, of Kruskal Wallis, and of Jonckheere were used, at $P > 0.05$, as described by Lehman (1975).

2.15. Chemicals

K-GA_3 was purchased from Calbiochem, San Diego, Calif. 92112, CCC and IAA-1-^{14}C from BDH Chemicals Ltd., Poole, U.K., and ABA-1-^{14}C from Mallinkrodt, St. Louis, Mo., U.S.A.

A mixture of cis, trans- and trans, trans-isomers of xanthoxin, extracted compound and ^{14}C-xanthoxin were kindly provided by Prof. R. L. Wain and H. F. Taylor, Wye College, U.K., and the synthetized compound was a kind gift of F. Hoffman-La Roche and Co., Basel, Switzerland.

Seeds of *Oryza sativa* L. var. Tan-ginbozu dwarf, were kindly donated by M. Koornneef, Department of Genetics, Agricultural University, Wageningen, The Netherlands.

Plexiglass was obtained from Rohm and Haas, Darmstadt, G.F.R., and Cinamoid filters from Strand Electric, London, U.K.

3. FACTORS INFLUENCING THE EXTENT OF THE PHOTOTROPIC CURVATURE IN INTACT SEEDLINGS

3.1. INTRODUCTION

In order to study the phototropic response of the hypocotyl properly, it had to be distinguished from other interfering curvature reactions. Also the variability of the material owing to, for example, age and length of the seedlings was taken into account.

To distinguish between phototropism proper and these other processes, it is also necessary to study the latter in sunflower seedlings. For this reason factors like geotropism, circumnutation, the variability of the phototropic response in the course of the year, and the state of the hypocotyl in relation to its bending properties were investigated. The results of these experiments are described in this Chapter.

3.2. RESULTS

3.2.1. *Geotropism*

During unilateral illumination straight hypocotyls bent towards the light source. After 60 to 80 minutes they started to curve back. Most seedlings did not regain the vertical position; when almost vertical they again bent towards the unilateral light source (Fig. III.1.).

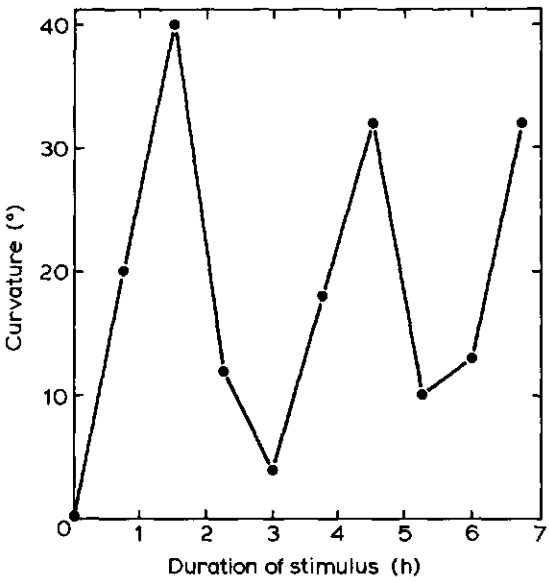


FIG. III.1. The course of curvature of a typical hypocotyl, continuously unilaterally illuminated with white light during 7 hours.

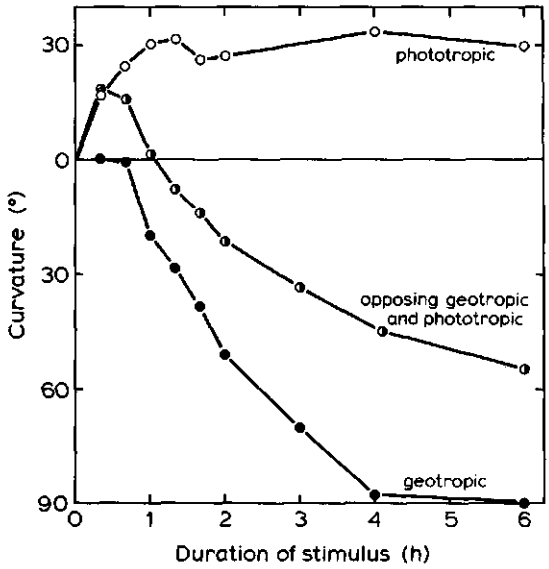


FIG. III.2. The influence of phototropic (○–○) or geotropic stimulus (●–●) alone and of contrary phototropic and geotropic stimuli (◑–◑) on the curvature. Average values of 12 seedlings.

It was investigated as to how far geotropism interfered with the phototropic movement. The fluctuation in curvature might result from geotropic stimulation of the deflected hypocotyl. This was analysed using seedlings in vertical and horizontal positions, some of which were unilaterally illuminated. As shown in Fig. III.2., the response to the phototropic stimulus occurred after a few minutes already, whereas the geotropic response took about 45 minutes to start. When seedlings were simultaneously exposed to opposite phototropic and geotropic stimuli, they started to curve towards the light source. This intensified the geotropic stimulation, so that its influence became apparent already after about 20 minutes. It took another 40 minutes before the seedlings actually started to bend geotropically, resulting in a predomination of geotropism after about 60 minutes (Fig. III.2.). The phototropic response, however, remained detectable in the total response; the seedlings bent geotropically, but to such an extent that the effects of the two stimuli can be considered to be additive.

When the phototropic and geotropic stimuli coincided the bending started earlier than with a geotropic stimulus alone (Fig. III.3.), apparently because the phototropic response occurred sooner. The ultimate extent of the curvature was not augmented by the phototropic component, the hypocotyls reaching the vertical position.

Although geotropic stimulation gradually increases during the phototropic curvature, its effect only develops after a considerable time. Whereas most of the individual seedlings reached their maximal response in about 60 min, some seedlings were delayed in the onset of their response. Accordingly the development of the geotropic effect will have been postponed. With such seedlings it took up to a 100 min to reach maximal curvature. In order to cope with this retardation, the maximal curvature of each individual seedling was determined

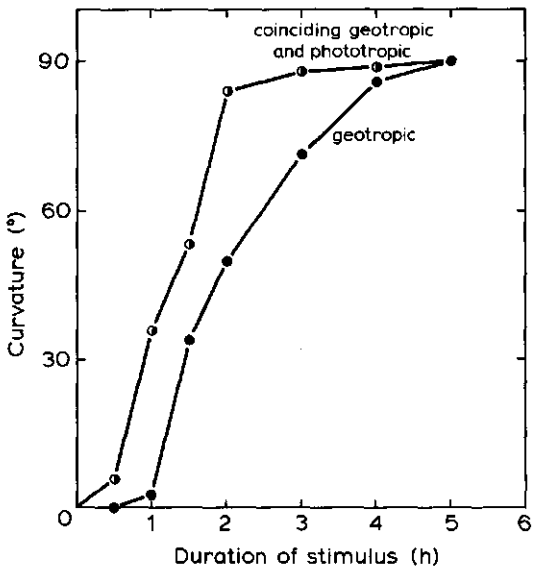


FIG. III.3. The influence of geotropic stimulus (●–●) and coinciding geotropic and phototropic stimuli (◑–◑) on the curvature. Average values of 10 seedlings.

during the first 2 hours of phototropic stimulation, and the average values are used in the results.

3.2.2. *Circumnutation*

Sunflower seedlings show a considerable measure of circumnutation which can act in all directions, more or less enlarging or decreasing the effect of the phototropic stimulus. The degree of circumnutation can be quite large, 16.6° ± 4.5°, with 8° and 27° as the extremes. Also the oscillation time varied from 3 to 9 hours, 6 hours on average.

To what extent circumnutation proceeds during unilateral illumination could not be measured, but some seedlings curved laterally indicating that nutation was still going on.

3.2.3. *Variability in the course of the year*

After two years of experimental work it was surmised that the maximal phototropic curvature of the seedlings was related to the time of the year. In Fig. III.4., the average maximal curvature of all the control seedlings used for the various experiments is plotted in fortnightly periods. From this Figure a large difference in maximal curvature can be noticed. The reduced responsiveness in the month of May in both years is conspicuous, however, a clear-cut annual rhythm cannot be detected.

The difference between the two years may be attributed to the fact that sunflower seeds of the harvest of the previous year were used. These seeds were grown under different climatic conditions, possibly causing a variance in endogenous factors involved in the phototropic response.

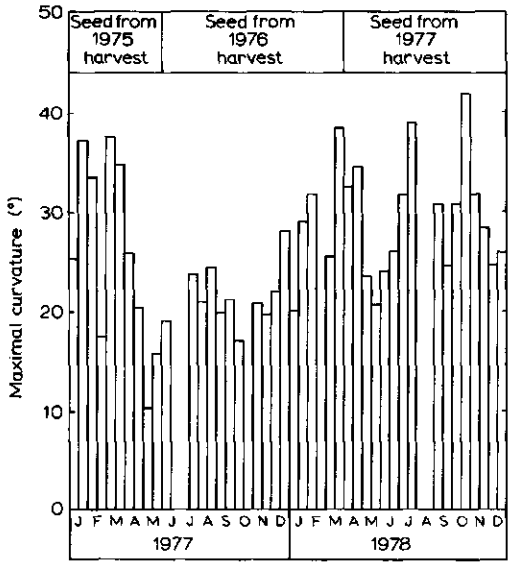


FIG. III.4. The maximal curvature of hypocotyls in the course of 2 subsequent years. Average values of samples of 20 to 90 seedlings.

3.2.4. *Age of the seedling*

Not only between experiments in the course of the year the bending response differed, but also seedlings within the experiments showed a considerable variation in phototropic curvature. This was connected with the age and length of the individual seedlings.

On the 5th or 6th day after germination most of the seedlings had reached a length of 40 to 60 mm, and most of them showed good curvature. About 15 to 20% of the seedlings, however, reached this length only at the 7th or 8th day (Table III.1.). Their hypocotyls very often showed a delayed response and a reduced curvature.

In the further experiments seedlings were only used on the 5th or 6th day after germination.

3.2.5. *Length of the hypocotyl*

Due to genetic heterogeneity not all the seedlings reached the same length

TABLE III.1. Relation between age of the hypocotyl and its phototropic curvature. Average values of 10 seedlings.

days after germination	average maximal curvature (°)		
	exp. 1	exp. 2	exp. 3
5	27.3 (a)	29.6 (a)	22.0 (ab)
6		25.6 (a)	24.2 (a)
7	7.3 (b)	9.2 (b)	12.1 (bc)
8		8.4 (b)	9.3 (c)

Figures within the column followed by different indices differ at $P < 0.05$.

TABLE III.2. Relation between length of the hypocotyl and its phototropic curvature. Average values of 10 seedlings.

average length (mm)	average maximal curvature (°)		
	exp. 1	exp. 2	exp. 3
30–40	11.8 (a)		12.0 (a)
40–50	22.2 (b)	19.3 (a)	23.4 (b)
50–60	21.0 (b)	23.6 (a)	25.6 (bc)
60–70	23.0 (b)	32.0 (b)	29.7 (bc)
70–80	34.0 (c)	41.2 (c)	35.2 (c)

Figures within the column followed by different indices differ at $P < 0.05$.

simultaneously. To investigate the influence of the length of the hypocotyl on its curvature, groups of seedlings with hypocotyl lengths of 30 to 40, 40 to 50, 50 to 60, 60 to 70 and 70 to 80 mm, were unilaterally illuminated and their curvatures measured. In hypocotyls smaller than 30 mm almost no curvature occurred.

From Table III.2. it can be concluded that hypocotyls smaller than 40 mm had a small response. Hypocotyls within the range 40 to 60 mm showed the same degree of bending. Hypocotyls with a length of 60 to 70 mm tended to show more curvature, and still longer hypocotyls bent significantly more.

In the further experiments only seedlings with a length of 40 to 60 mm were used.

3.2.6. *The region of bending during phototropic curvature*

Measurements were done to locate the region or the point of bending of the phototropically stimulated hypocotyl. After unilateral illumination the length of the hypocotyl and the length between the attachment of the cotyledons and the bending point were measured. Some hypocotyls showed a distinct bending point, others curved like an arc, and all intermediate forms occurred. Most of the seedlings curved in the middle of the hypocotyl. As the hypocotyls were longer, the bending point relatively shifted to the upper part of the hypocotyl, the absolute distance from the top tending to remain constant (Fig. III.5.). This had no further consequences for the accuracy of the measurements.

3.2.7. *Effects of seedling manipulation*

During the first experiments it was observed that seedlings that were transported and manipulated several times showed a lower bending response than untreated seedlings. In further experiments care was taken that the seedlings were not touched and translocated as little as possible. They had to be transferred, however, from the growth room to the place of pretreatment, in most experiments, and to the experimental box. This was done with utmost care.

In most experiments the phototropic curvature was measured every 20 minutes. This involved movement of the seedling before and after the measurement. This treatment did not reduce the curvature (Fig. III.6.).

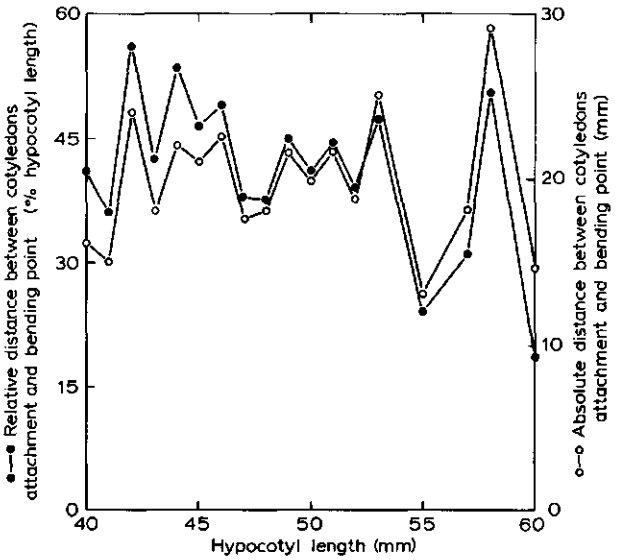


FIG. III.5. Relation between the length of the hypocotyl and the location of the bending point. Average values of samples of 6 to 9 seedlings.

Other series in which the plants had to be manipulated were those in which the hypocotyls or cotyledons had to be removed or covered with foil. To investigate the effect of covering, a control sample was included in which the organs concerned were covered with transparent plastic. As long as the manipulation was carried out with utmost care, it had no adverse effects (see also Chapter 5).

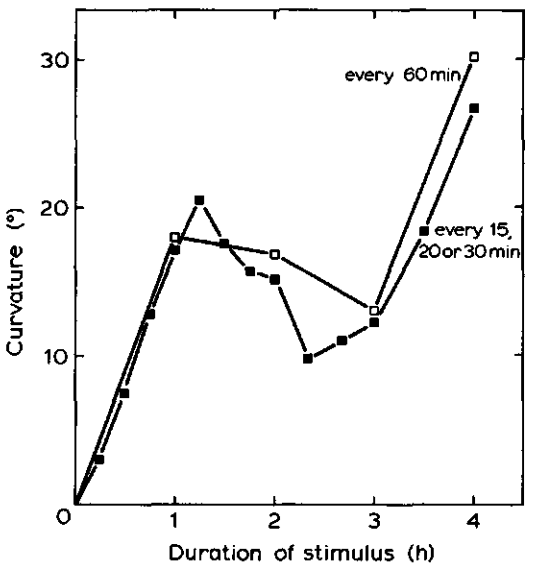


FIG. III.6. Influence of transfer of the seedlings for measurement on phototropic curvature. The bending was measured every 60 min (□–□), or every 15 min during the first two h, every 20 min during the third h, and every 30 min during the fourth h of the stimulus (■–■). Average values of 10 seedlings.

3.3. Discussion

The results show a rhythmic change between phototropic and geotropic reactions in the course of the unilateral illumination of the seedlings. That geotropism is involved is shown in Fig. III.2., which also demonstrates that geotropism eventually has a stronger influence on the seedling than phototropism. However, the lag time for phototropism is only a few minutes, whereas the geotropic reaction takes about 45 minutes, apparently because the perception mechanisms are entirely different. Moreover, the geotropic stimulus arises only gradually during the phototropic response, so that the first hour of curvature, which always falls within the first two hours of phototropic stimulation, can be considered to show proper phototropic curvature. The counteracting influence of geotropism on phototropism also is mentioned by Arisz (1915).

Another process involved in straightening of seedlings is autotropism (Firn and Digby, 1979). However, this reaction occurs only after a longer period of stimulation and can be left out of consideration here.

Nutations also occur in higher plants and interfere with geotropism (Brown and Chapman, 1977; Johnsson, 1977). A theory for nutation is given by Israelsson and Johnsson (1967), and is based on the lateral auxin transport, which is supposed to arise when a gravitational force component acts on the plant. Darwin (1880) was the first author to describe these circumnutations for a large number of plant species, and concluded that they are a universally occurring phenomenon. About sunflower seedlings he writes: 'The extreme actual distance travelled was at least .1 of an inch.' From his results, assuming that his sunflower seedlings bent similarly to those used in our experiments, a maximal curvature of 18 degrees can be calculated. Also Blaauw (1915) described nutations of 20 degrees for his sunflower seedlings. These figures agree well with our findings.

Arisz (1913) mentioned nutations as 'extremely difficult' in phototropic research. They may be expected to go on during phototropic curvature. Spurný (1976) stated the positive phototropic response of the nutating hypocotyl to be dependent on the phase in which the hypocotyl is exposed to the unilateral illumination. Pfeffer (1904) mentioned that in torsion of tendrils nutations can either co-operate with the direction of growth or antagonize it. The same might be expected for phototropic curvature: an enhancement or reduction in bending dependent on the accidental direction of nutation. As it is not possible to determine instantly the direction of nutation in straight hypocotyls, this might contribute to the considerable variability in curvature of seedlings within a sample.

Because of the large nutation, part of the seedlings could not be used. Depending on the time of the year between 25 and 65 % were rejected because they were not straight.

The heterogeneity in the material is also responsible for the fact that the curvature during unilateral illumination is maintained at about the same level in Fig. III.2., whereas it shows fluctuation in Fig. III.6. All individual seedlings

fluctuate in curvature during phototropic stimulation, but the bending time before the straightening up varies because of the reasons mentioned above. By taking the average values the individual fluctuation may disappear.

That nutation was already known and difficult to handle many years ago is evident by the following quotation from JOST (1904): 'Blicken wir zurück, so sehen wir in den autonomen Bewegungen Erscheinungen, die dem Physiologen bis jetzt nur wenig Freude machen, und die auch biologisch nur zum Teil verständlich sind. Das mag die Kürze ihrer Behandlung rechtfertigen'.

In phototropic studies, the length and/or age of the seedlings are generally mentioned, but the dependence of the curvature on these characteristics is not further investigated (BRENNAN et al., 1976; LAM and LEOPOLD, 1966; SHUTTLEWORTH and BLACK, 1977). As demonstrated in Tables III.1. and III.2., the age and length of the hypocotyl does affect its curvature. The experiments led us to select the seedlings according to age and length.

A number of authors described the influence of mechanical stress on growth (HAUGLAND et al., 1978; JAFFE, 1976; MITCHELL, 1977; STEUCEK and ARCHER, 1976). The ultimate effect of mechanical touching is a reduction in elongation growth, often coupled with an enhanced radial growth in accordance with a participation of ethylene in the response (JAFFE, 1976). We observed that slowly growing seedlings (40 to 60 mm on the 7th or 8th day) showed a reduced bending and enhanced radial growth. If the cause of curvature would be a lateral gradient of some growth-regulating substance, it might take more time to bring about such a gradient in more expanded seedlings or, alternatively, ethylene might interfere with its translocation as it does with auxin transport. Thus reduced growth might be coupled with reduced curvature.

The covering of cotyledons or hypocotyls, if done with care, need not give a stress effect owing to touching or to a decrease in the transpiration flow or other gaseous exchange. SHUTTLEWORTH (1977), doing the same kind of experiments, also came to this conclusion.

The opinion prevailed that in geotropism curvature starts in the upper part of the hypocotyl and subsequently proceeds to the more basal part (DOLK, 1936; IWAMI and MASUDA, 1974). Recent studies (FIRN et al., 1978; KÖHLER, 1978) show that all parts of the hypocotyl curve simultaneously, resulting in a more or less arc-curved hypocotyl. Also FIRN and DIGBY (1979) stated that the shift of the geocurvature from the apex to the lower zones is probably due to autotropism, subsequent on geotropism. In phototropism, ever since the observations of DARWIN (1880) it is thought that translocation of a substance occurs from the first leaves or cotyledons to the hypocotyl (BRUINSMA et al., 1975; DIEMER, 1961; LAM and LEOPOLD, 1966; SHIBAOKA and YAMAKI, 1959). This will further be discussed in the next Chapter.

With the seedlings used in this study different types of curvature were seen. When the phototropic stimulus was given longer than 2 hours some hypocotyls curved a second time in a more basal part resulting in a S-curved hypocotyl. In shorter experiments the degree of distinction of the bending point did not interfere with the accuracy of the determination of the curvature.

4. INFLUENCES OF THE DIFFERENT ORGANS OF THE SEEDLING AND OF THE EPIDERMIS OF THE HYPOCOTYL ON PHOTOTROPIC CURVATURE

4.1. INTRODUCTION

Already in 1880 DARWIN postulated an influence of the upper parts of the seedling on phototropic bending: 'when seedlings are freely exposed to a lateral light, some influence is transmitted from the upper to the lower part, causing the latter to bend'. After him several other authors, all working with green sunflower seedlings, affirmed this theory (BRUINSMA et al., 1975; LAM and LEOPOLD, 1966; SHIBAOKA and YAMAKI, 1959). SHUTTLEWORTH and BLACK (1977), on the contrary, reported that phototropic curvature also takes place when the cotyledons are fully covered, so that the perception of the stimulus must take place in the hypocotyl. BRENNAN et al. (1976) investigating the phototropic response of mung bean, concluded that the first leaves are not important in the phototropic response.

FIRN and DIGBY (1977), investigating an alternative of the CHOLODNY-WENT theory for shoot geotropism, proposed a model in which it is speculated that the peripheral cell layers are the site of both geoperception and -response.

It is investigated in this Chapter to which extent the cotyledons, the top, the roots, and the peripheral cell layers play a role in hypocotyl phototropism of green seedlings.

4.2. RESULTS

4.2.1. *Influence of the cotyledons*

When intact seedlings are unilaterally illuminated, they can be placed with the cotyledons either perpendicular or parallel to the light source. From Table IV.1. it can be seen that there is no difference in curvature of seedlings between these two positions.

TABLE IV.1. The influence of the position of the cotyledons on phototropic curvature. Average values of 10 seedlings in exp. 1 and exp. 3, and of 14 seedlings in exp. 2.

position of cotyledons to the light source	average maximal curvature (°)		
	exp. 1	exp. 2	exp. 3
parallel	20.4 (a)	24.4 (a)	14.4 (a)
perpendicular	18.7 (a)	25.6 (a)	14.2 (a)

Figures within the column followed by different indices differ at $P < 0.05$.

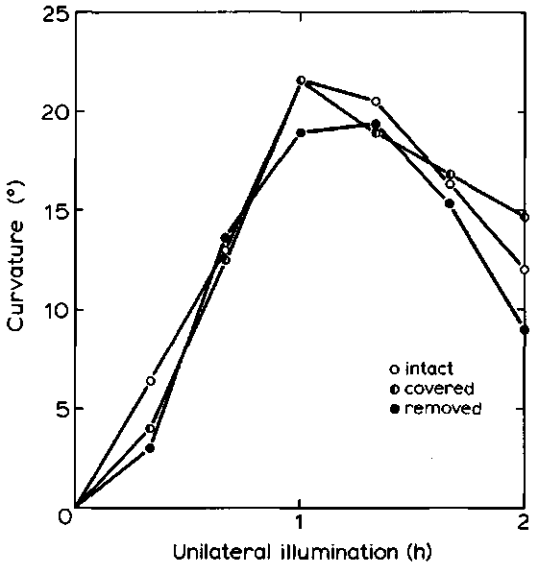


FIG. IV.1. Influence of covering (◐–◐) or removal (●–●) of cotyledons on hypocotyl curvature and their intact control (○–○). Average values of 10 seedlings.

To investigate whether the cotyledons are required for the perception of the phototropic stimulus, they were covered with aluminium foil immediately before unilateral illumination. Fig. IV.1. shows that darkening of the cotyledons had no influence on the curvature. Because it is still possible that the cotyledons provide the hypocotyl with a growth substance that is acting in the hypocotyl during its unilateral illumination, the cotyledons were removed immediately before unilateral illumination. However, the phototropic response remained unimpaired (Fig. IV.1.). This clearly indicates that the cotyledons are not required for the phototropic response.

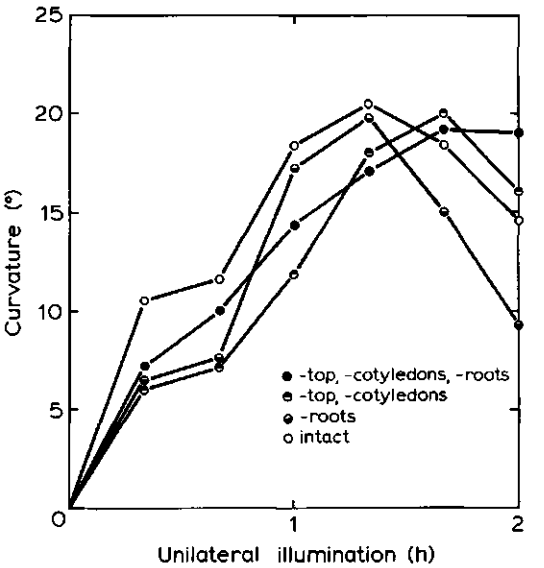


FIG. IV.2. Influence of removal of top (◒–◒) or all organs (●–●) on hypocotyl curvature and their intact control (○–○). Average values of 10 seedlings.

4.2.2. *Influence of top and roots*

Because it was technically impossible to remove the apex with the first, still undeveloped leaves without damaging the cotyledons, and the latter had no direct influence on phototropic curvature, top and cotyledons were removed together immediately before unilateral illumination to study the influence of the apex. It can be concluded from Fig. IV.2. that also the apex had no influence on phototropic curvature. In some experiments the curvature of seedlings without top and cotyledons was reduced, although not significantly. This might be due to stress as a result of the removal (cf. Chapter 3).

Also removal of the roots immediately before unilateral illumination had no influence on the curvature, as is shown in Fig. IV.2.

From these data it is evident that none of the organs is responsible for perception of the phototropic stimulus except the hypocotyl. This was confirmed by the removal of all organs prior to illumination of the isolated hypocotyl. The results show that an isolated hypocotyl still has the full capacity to bend (Fig. IV.2.). The curvature during unilateral illumination was sometimes slightly reduced; since this could be corrected by removing the organs about half an hour prior to the unilateral illumination, the reduction can be ascribed to a stress effect.

4.2.3. *Influence of the peripheral cell layers of the hypocotyl*

To study the influence of the epidermis on curvature an attempt was made to remove the epidermis from the hypocotyl. Microscopic examination revealed that together with the epidermis 1 or 2 cortical cell layers were also removed. These outer 2–3 cell layers are referred to as peripheral cell layers.

Since it was technically easier to remove these peripheral cell layers from seedlings of which the cotyledons were removed, and removal of the cotyledons has no influence on curvature, the effect of the peripheral cell layers was studied using hypocotyls without cotyledons. Such seedlings have no capacity to curve under the influence of a phototropic stimulus (Fig. IV.3.). To investigate whether damaging of the underlying cortical cells was responsible for this effect, several vertical cuts were made in unpeeled hypocotyls. Though a small effect owing to this damaging occurred, this could only be responsible for a small part of the loss of phototropic response of the peeled hypocotyls. This stress effect was further analysed by measuring the evolution of ethylene from peeled and non-peeled hypocotyls. The ethylene evolution was similar in both groups (Fig. IV.4.).

It was further analysed whether the role of the epidermis was a direct one on phototropism, involved in perception, or an indirect one, via the elongation growth of the hypocotyl. Therefore hypocotyls were longitudinally split or the epidermis was peeled off at one side only. These hypocotyls were placed with their epidermis towards the unilateral light or not, and curvatures were measured.

When one side of the epidermis was peeled off, or when hypocotyls were longitudinally split, the hypocotyl elongated immediately in that part of the seedling, with as a consequence a curving with a concave peeled side. Sometimes the seedlings were not totally rigid just after peeling. This effect was much larger

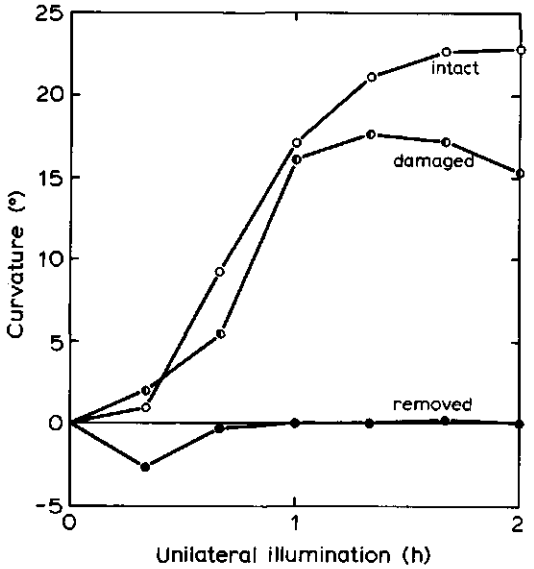


FIG. IV.3. Influence of damage (◐–◐) or removal (●–●) of the hypocotyl epidermis on the curvature of seedlings without cotyledons and their control (○–○). Average values of 10 seedlings.

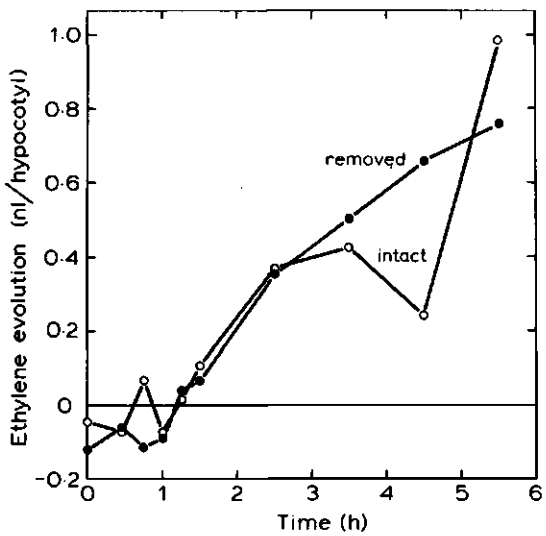


FIG. IV.4. Evolution of ethylene by hypocotyls with (○–○) or without (●–●) epidermis. Of both groups the top, cotyledons and roots were removed. Average values of 10 seedlings.

when the hypocotyls were bisected. For this reason the hypocotyls were split 15 hours, or half-peeled 2 hours before the phototropic experiment. During this time the seedlings became rigid again; seedlings with half their epidermis stripped off became almost vertical again, whereas bisected hypocotyls stayed curved; non-rigid seedlings were removed. Care had to be taken that the relative humidity remained very high.

Partly-stripped hypocotyls, illuminated from above, showed a small non-phototropic curvature owing the effect of a growing epidermis versus a growth-

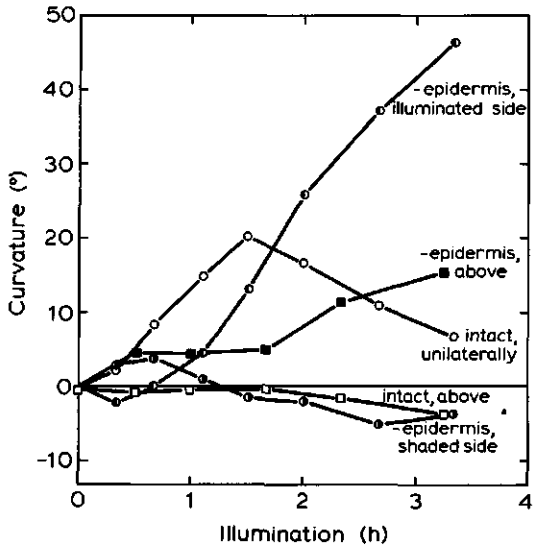


FIG. IV.5. Curvature of cotyledonless seedlings, intact (○-○) or with the epidermis removed on the illuminated (◐-◐) or the shaded (◑-◑) side during unilateral illumination, or of intact (□-□) or with a partly removed epidermis (■-■) illuminated from above. Average values of 9 seedlings.

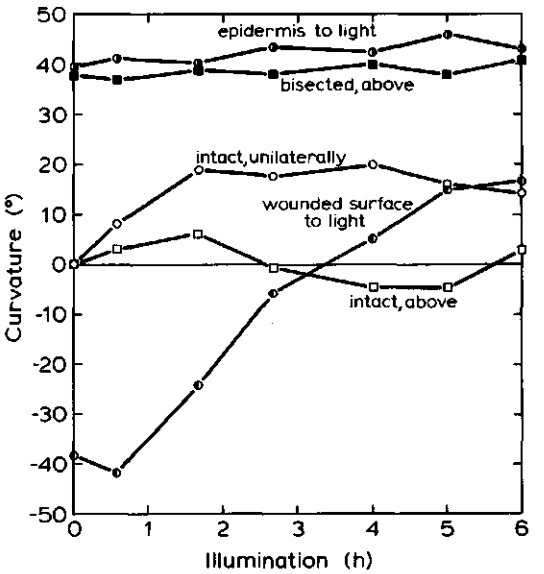


FIG. IV.6. Curvature of intact (○-○) or bisected seedlings with one remaining cotyledon with the wounded surface (◐-◐) or with the epidermis side (◑-◑) towards the unilateral light, or of intact (□-□) or bisected seedlings with one remaining cotyledon (■-■) illuminated from above. Average values of 8 seedlings.

inhibited peeled side (Fig. IV.5.). Seedlings with the epidermis side to the unilateral light source did not respond to a phototropic stimulus. The small negative curvature is not significantly different from the small positive curvature. On the contrary, when the peeled side was phototropically stimulated, the hypocotyls responded well after a reproducable lag time. Part of this response is probably due to the pure growth response, also occurring in seedlings illuminated from above (Fig. IV.5.). Also bisected seedlings bent only when phototropically stimulated at the wounded side (Fig. IV.6.) If the remaining cotyledon was

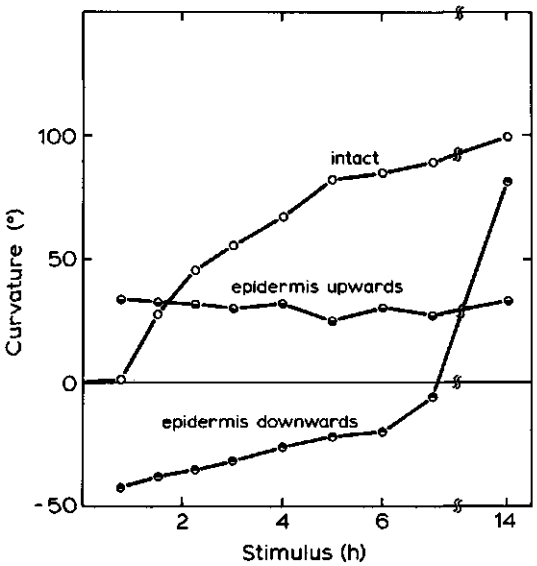


FIG. IV.7. Geotropic curvature of horizontal, intact (○-○) or bisected seedlings with their epidermis side directed upwards (◒-◒) or downwards (◓-◓). Average values of 10 seedlings.

removed from the halved seedling, the phototropic response almost vanished (Table IV.2.): in this Table halved seedlings curving less than 10 degrees within a 7 hours period of unilateral illumination were considered to be non-responsive. The experiments could be extended to 7 hours because the epidermis-less lower side of the curved halved seedlings made them non-responsive to the geotropic stimulus (Fig. IV.7.).

4.3. DISCUSSION

No influence of the cotyledons could be detected during phototropic curvature, except when they were removed many hours prior to the phototropic experiments. This was the case with the halved seedlings (Table IV.2.), where the required recovery demanded removal of the cotyledon 15 hours before the

TABLE IV.2. Phototropic responsiveness of bisected seedlings with or without a cotyledon.

cotyledon	wounded surface to the light	epidermis side to the light
intact	n (total) = 26 (3 exp.) responding :66% non-responding :34% mean response :44°	n (total) = 25 (3 exp.) responding :13% non-responding :87% mean response :17°
removed	n (total) = 37 (4 exp.) responding : 5% non-responding :95% mean response :18°	n (total) = 35 (4 exp.) responding :17% non-responding :83% mean response :17°

proper experiment. The loss of response during such a prolonged period can be ascribed to shortage of food supply from the cotyledon to the hypocotyl. This is in agreement with Chapter 5. In accord with the independence of the phototropic response of the presence of the cotyledons is the irrelevance of the position of the latter towards the source of unilateral illumination (Table IV.1.).

The conclusion that the cotyledons are not directly involved in the phototropic response is in agreement with the results of SHUTTLEWORTH and BLACK (1977), who reached the same conclusion for de-etiolated cucumber and sunflower seedlings, but not with those of several other authors (BRUINSMA et al., 1975; LAM and LEOPOLD, 1966; SHIBAOKA and YAMAKI, 1959). LAM and LEOPOLD (1966) and SHIBAOKA and YAMAKI (1959), however, used seedlings of 10 to 15 days old. Furthermore, LAM and LEOPOLD (1966) did not use unilateral illumination; they measured the curvature due to the covering or removal of one cotyledon. As SHUTTLEWORTH and BLACK (1977) pointed out, this phenomenon is not connected with phototropism; the growth rate of the hypocotyl can be inhibited under the influence of red light, perceived by the cotyledon.

DARWIN (1880), who postulated the effect of the upper parts of the seedling in curvature, described that he did not cover top or cotyledons, but the upper part of the hypocotyl itself. This could be the cause of this disagreement. In older literature it is indeed clearly shown, that the removal of the apex may exert a negative influence on phototropic curvature. These experiments, however, were done with totally different material, namely etiolated coleoptiles (e.g. DARWIN, 1880; WENT, 1928).

SHIBAOKA and YAMAKI (1959) stated that 'The seedlings grown in the open air for 15 days were kept in darkness for 40 hours, and from these seedlings all cotyledons and all leaves were removed'. If not done directly before unilateral illumination, removal of the storage food substances may have reduced the curvature. Otherwise, rough manipulation prior to unilateral illumination might affect the responsiveness by stress (Chapter 3).

The difference between our results and those of BRUINSMA et al. (1975) cannot be explained. The seedlings used in these experiments were of the same age and variety, but in spite of extensive experimentation the results of BRUINSMA et al. could not be reproduced.

Removal of the shoot tip did not influence the bending rate either, although sometimes the reaction time was more prolonged than in intact seedlings. This may be due to stress: when the removal took place about half an hour before unilateral illumination, no effect was found. BRUINSMA et al. (1975) found only a small effect of removal of the apex; BRENNAN et al. (1976) reported that the top of the mung bean plant is one of the primary zones of perception for phototropic curvature of the stem.

Several authors collected diffusates from phototropically stimulated hypocotyls, which necessitated cutting off the roots (BRUINSMA et al., 1975; LAM and LEOPOLD, 1966). If the roots were important for curvature, these diffusates might give irrelevant information about the processes occurring during phototropic

curvature. Our findings that root removal does not affect the curvature removes this objection.

The experiments on organ removal turned out to be completely reproducable when a blue irradiation was given instead of the white light used in the experiments shown in the present Chapter.

It is evident that the peripheral cell layers of the hypocotyl are required for curvature; their removal causes total loss of responsiveness (Fig. IV.3.). It is unlikely, however, that the epidermis is the site of light perception: in the experiments with peeled and halved hypocotyls phototropic curvature only occurred when the epidermis was at the shaded side (Fig. IV.5. and IV.6.).

In view of the different perception mechanisms for photo- and geotropic stimuli, it is not remarkable that the phototropic one is not perceived in the epidermis as is the case with geotropism according to FIRN and DIGBY (1977). The often very large curvatures of peeled and halved seedlings are partially not of a phototropic nature. The region where the epidermal layer is present tends to grow faster than the side at which the parenchymous tissue is exposed to the unilateral light source. Increased evaporation at the surface of the wounded tissue may well be involved.

These experiments can be carried out for a longer period of time, because eventually no straightening up of the seedlings due to geotropism can occur (Fig. IV.7.). FIRN and DIGBY (1977) concluded that geocurvature was only possible when the lower epidermis of hypocotyl sections is present. Our findings with intact plants agree with their results. In the initial bending of the halved hypocotyls with their wounded tissue exposed to the unilateral light source geotropism may be involved, although this straightening up does not occur in bisected seedlings illuminated from above. However, as soon as the hypocotyls actually are straightened up and show positive curvatures, no geotropism can be involved. On the one hand the absence of a fluctuating phototropic response in these experiments points to the absence of geotropic stimulation. On the other hand the absence of such a fluctuation in these geotropically not responding seedlings allows us to ascribe the usually fluctuating response of intact seedlings in Fig. III.1. to an interaction between photo- and geotropic stimuli, indeed.

Stress may well be involved in covering or removing parts of the seedling, and this often involves the production of ethylene (HYODO, 1978; SALTVEIT and DILLEY, 1978). No difference in ethylene evolution could be measured between isolated intact and peeled hypocotyls, indicating that ethylene does not play a major role in the reduction of curvature in the present experiments.

5. INFLUENCE OF LIGHT OF DIFFERENT WAVELENGTHS ON HYPOCOTYL PHOTOTROPISM

5.1. INTRODUCTION

Important factors affecting the activity of unilateral illumination on a phototropic stimulus are wavelength and duration of the stimulus. BRIGGS (1963c) summarized the literature on etiolated coleoptiles. The dose-response curve of coleoptiles, made for *Avena sativa*, is well known, showing a first positive, a first negative, and a second positive curvature. Although etiolated coleoptiles are studied far more extensively than dicotyledonous seedlings, some dose-response curves are known for this material also. STEYER (1967) compared four mono- and twelve dicotyledonous seedlings, all etiolated, for their phototropic reaction. She found that only *Avena sativa* showed the negative reaction, all the other plants only showed the two positive reactions. In her experiments *Helianthus annuus* showed less curvature than other seedlings, with a first positive response of only four degrees. During the first positive reaction the total amount of energy is important, i.e. both time and intensity, during the second positive curvature only the duration of the stimulus counts.

EVERETT (1974) described the difference between light-grown and etiolated radish seedlings. In green seedlings, the first positive curvature had disappeared; etiolated seedlings, however, showed this first reaction. Both groups of seedlings showed the second positive curvature to the same extent.

In 1887, SACHS had already shown that blue light, but not red, green, or yellow, was responsible for the phototropic curvature in various plant species. In 1969 CURRY presented an accurate action spectrum for the first positive curvature of etiolated *Avena sativa* coleoptiles. He found peaks at 445 and 475 nm, and a small peak at 375 nm. When unilateral light with a wavelength longer than 500 nm was presented no curvature occurred.

In this Chapter the effects of wavelength and duration of the actinic illumination on the phototropic response of light-grown and etiolated seedlings are described.

5.2. RESULTS

5.2.1. *Unilateral illumination*

Experiments were carried out to investigate whether a short phototropic stimulus was sufficient for curvature. Fig. V.1. shows the curvature of seedlings with a continuous stimulation, and seedlings with a phototropic stimulation of only a few minutes. The curvature of shortly phototropically stimulated seedlings is reduced with respect to that of continuously stimulated ones, but the duration of the unilateral irradiation given for the short periods only, plays

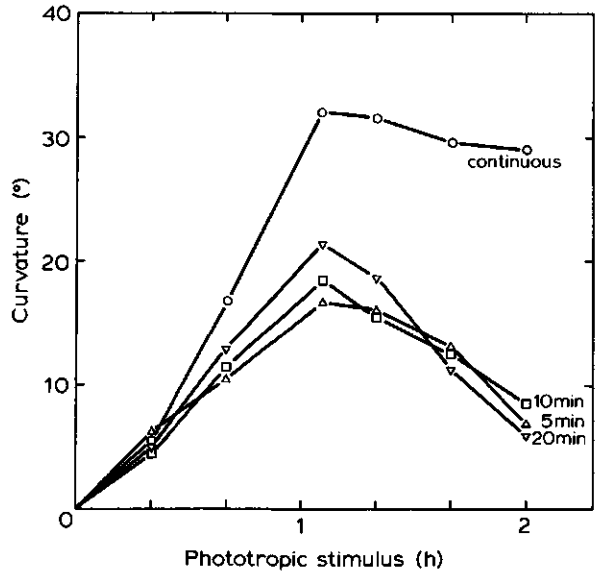


FIG. V.1. Effect of 5 (△-△), 10 (□-□), 20 min (▽-▽) or continuous (○-○) unilateral illumination with white light on the phototropic curvature of light-grown seedlings. Average values of 10 seedlings.

hardly a role. Also it was observed that the continuously stimulated hypocotyls curved again after the first dip in curvature. Briefly stimulated hypocotyls stayed vertical after their first curvature, indicating that the continuation of the stimulus is an important factor.

Also the wavelength range required for phototropic curvature was analysed. The amounts of energy in the blue, the red and the far-red regions were chosen in such a way that they matched with the amounts of these regions in the white light (see also Chapter 2). From Fig. V.2. it can be concluded that the wavelength

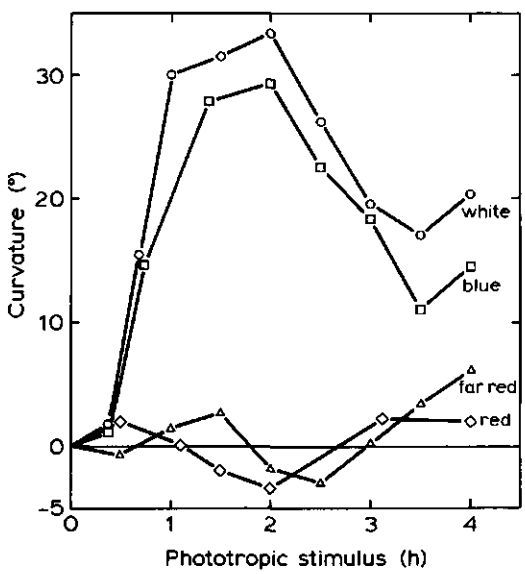


FIG. V.2. Phototropic response of green seedlings to an unilateral illumination with white (○-○), blue (□-□), red (◇-◇) or far-red (△-△) irradiation. The data are derived from 4 different experiments. Average values of 10 seedlings.

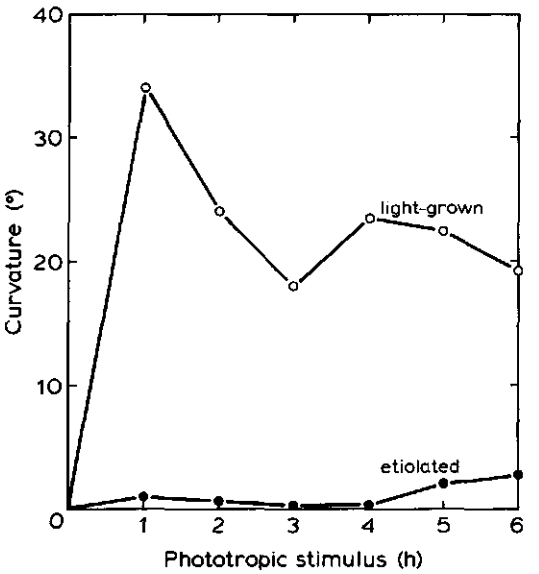


FIG. V.3. Phototropic response of light-grown (○-○) and etiolated (●-●) seedlings to continuous unilateral illumination with white light. Average values of 10 seedlings.

range required is in the blue region, although the blue unilateral irradiation gave only 85% of the curvature obtained with a white phototropic stimulus. This difference is not significant at the $P < 0.05$ level. However, in every experiment the average values of the seedlings treated with white light exceeded those of the ones treated with blue light. Unilateral illumination with red or far red had no phototropic effect.

5.2.2. *The difference in response between light-grown and etiolated seedlings*

In contrast to light-grown seedlings, etiolated seedlings do not respond to unilateral light (Fig. V.3.). During the first two hours of unilateral illumination the etiolated seedlings show no curvature at all. Age and length of the etiolated seedlings are not involved. After about two hours the hypocotyls begin to develop some response; the greening and opening of their cotyledons indicates that de-etiolation occurs. The curvature after about two hours can be ascribed to the developing sensitivity of a de-etiolating seedling.

5.2.3. *Phototropic sensitivity of seedlings intermediate between etiolated and light-grown*

To investigate the light requirement of etiolated seedlings to render them sensitive to the phototropic stimulus, etiolated seedlings were illuminated from above for different periods of time. Subsequently they were unilaterally illuminated and their curvatures measured. Only white light was used. The opposite was done with light-grown seedlings; after their last growth period of six hours light they were placed in darkness for different intervals to examine whether their phototropic sensitivity decreases in prolonged darkness.

The length of the seedlings, before putting them in the light or in the dark, was

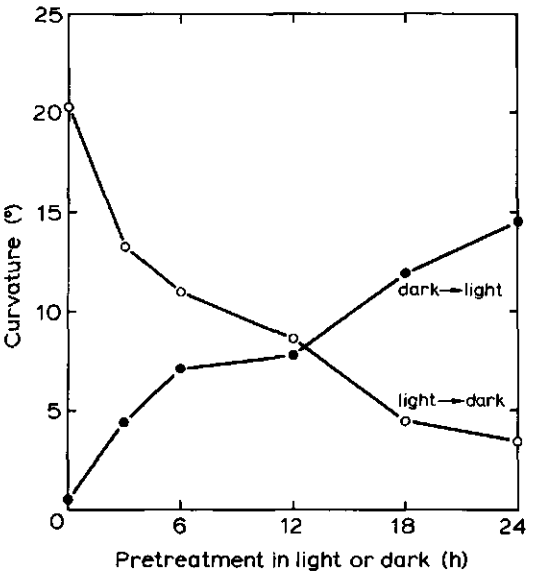


FIG. V.4. Phototropic sensitivity of etiolated seedlings during de-etiolation (●–●), and of light-grown seedlings during prolonged darkness (○–○). The curvature was measured after 1 h unilateral illumination with white light. Average values of 10 seedlings.

well below 40 mm in order to have them at the right length for the proper phototropic experiments. The curvatures were measured after one hour of unilateral illumination, at which point of time most samples had reached their maximum curvature. The few exceptions did not influence the overall picture presented.

From Fig. V.4. it can be concluded that phototropic sensitivity starts to develop shortly after de-etiolation, and increases gradually during a 24 hours period. After this period of illumination the etiolated seedlings look completely de-etiolated: the hypocotyl has thickened and looks greenish-yellow, the cotyledons are expanded and green. The same period of time is required to reduce the curvature of light-grown seedlings to almost zero. The light-grown seedlings do not look etiolated after 24 hours in darkness, and during unilateral illumination they need less time to regain phototropic responsiveness than the etiolated ones.

Normally the seedlings received 14 hours light per day. 24 hours continuous white light was given to etiolated seedlings. If this amount of light was given to light-grown seedlings, their sensitivity could not be further enhanced. In the experiments presented the sensitivity of de-etiolated seedlings after a 24 hours light treatment was still less than that of green seedlings; in others the sensitivity matched. The measure of reduction in phototropic responsiveness by a dark treatment of plants grown in 14 hours white light per day may vary in the course of the year.

5.2.4. *Perception of light during the pretreatment*

In order to test whether the hypocotyl and/or the cotyledons are responsible for the perception of the illumination during pretreatment, the hypocotyls or the tops of etiolated seedlings were covered with aluminium foil during 18 hours

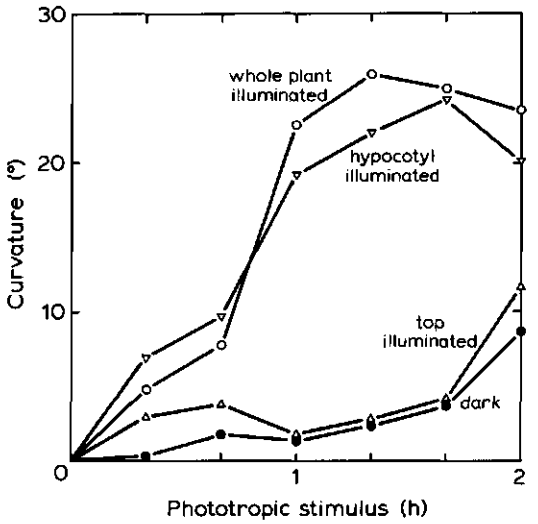


FIG. V. 5. Phototropic response of etiolated seedlings after 18 h white light on the whole seedling (○–○), on the hypocotyl only (▽–▽), on the top only (△–△), or after continuous darkness (●–●). Average values of 10 seedlings.

pretreatment with white light from above. After this period the foil was removed and the curvature upon unilateral illumination with white light measured. From Fig. V.5. it can be concluded that the perception of the light during pretreatment is in the hypocotyl only. This also holds for light-grown seedlings; when these seedlings had their cotyledons darkened during an 18 hours pretreatment, they still fully responded to the phototropic stimulus (Fig. V.6.).

It might be argued that by covering and uncovering the hypocotyl this organ might be so stressed that no curvature could occur. For this reason controls were used in which the hypocotyls were covered with transparent plastic. From the

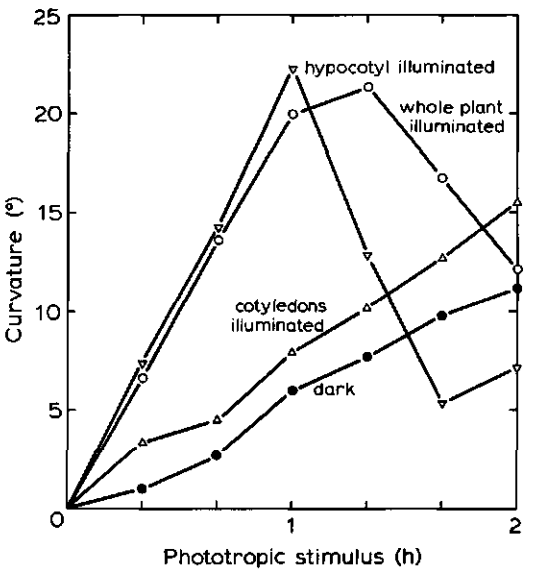


FIG. V.6. Phototropic response of green seedlings after 18 h white light on the whole seedling (○–○), on the hypocotyl only (▽–▽), on the cotyledons only (△–△) or after darkness (●–●). Average values of 10 seedlings.

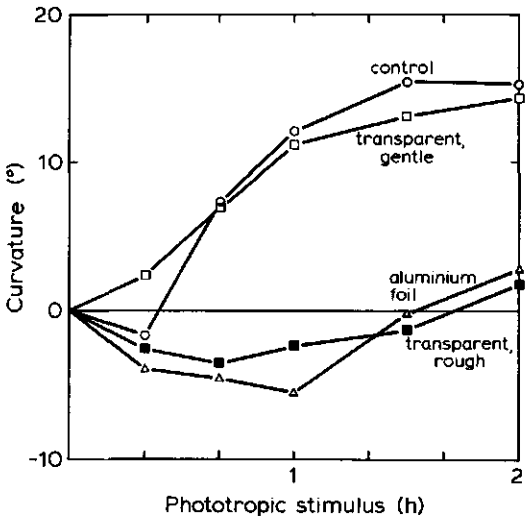


FIG. V.7. Phototropic response of green seedlings after 18h white light on whole seedlings (○-○), hypocotyls covered with aluminium foil (△-△) hypocotyls covered with transparent plastic, gently treated (□-□) or roughly treated (■-■). Average values of 10 seedlings.

results (Fig. V.7.), it is evident that the hypocotyls must be covered and uncovered very carefully, the difference between careful and rude covering being considerable. Especially the decovering of the hypocotyls turned out to be important in this matter. This was done immediately before the unilateral illumination, leaving the seedlings no time to recover from possible stress. When done carefully, however, the treatment of the hypocotyl did not affect the curvature (Fig. V.7.).

Also the effect of removal of the cotyledons 18 hours before unilateral illumination was investigated with respect to curvature. Although darkening of the

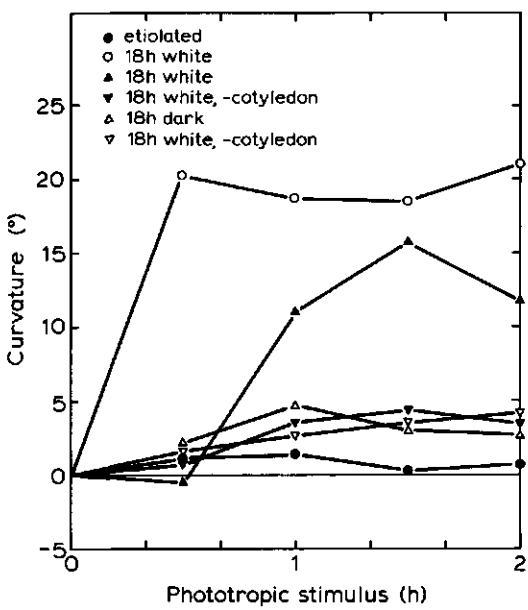


FIG. V.8. Phototropic response of etiolated seedlings after 18 h darkness (●-●), white light (▲-▲), and white light, cotyledons removed (▼-▼), and of green seedlings after 18 h white light (○-○), darkness (△-△), and white light, cotyledons removed (▽-▽). Average values of 10 seedlings.

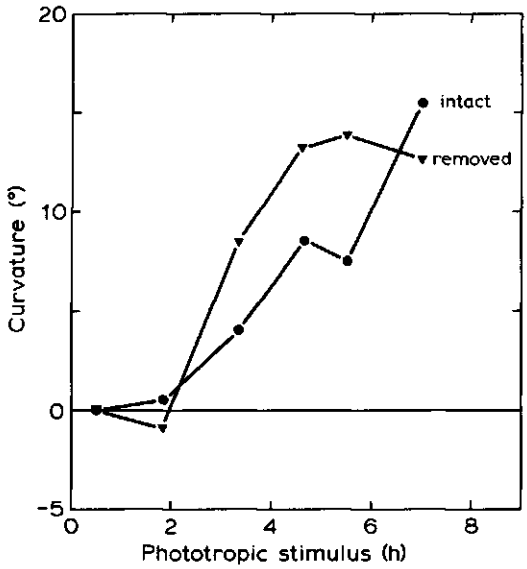


FIG. V.9. Phototropic response of etiolated seedlings with (●–●), and without (▲–▲) cotyledons. Average values of 14 seedlings.

cotyledons had no influence, their removal strongly reduced the subsequent phototropic response of green seedlings and prevented that of de-etiolating seedlings (Fig. V.8.). This might be due to the lack of substrates from the cotyledons. Whether or not the darkened cotyledons still provide the hypocotyl with food substances needed for curvature was investigated by removing the cotyledons of etiolated seedlings. Together with a control group, these seedlings were unilaterally illuminated for a longer period of time and their curvatures measured. Since no significant difference in curvature could be detected between these groups, a direct influence of the cotyledons in phototropic curvature can be ruled out (Fig. V.9.).

5.2.5. *Effect of the wavelength during pretreatment on the phototropic sensitivity*

The effect of wavelength on the development of phototropic sensitivity in de-etiolating seedlings was investigated. In Fig. V.10. it is shown that the active region is in the blue. Seedlings pretreated with red light showed only a very poor phototropic reponse. Light-grown seedlings with a pretreatment of 18 hours blue or red light, or seedlings cultured with a photo-periodism of 14 hours blue or red light/10 hours dark showed similar differences in behaviour. It is concluded that the final 18 hours light are decisive for phototropic sensitivity.

In some experiments the de-etiolated seedlings pretreated with blue light curved somewhat less than those pretreated with white light. However, the average value of curvature of seedlings pretreated with blue light was 19.1° ± 9.2° of 116 seedlings, and 19.4° ± 9.1° of 108 seedlings pretreated with white light. This difference is not significant indicating that blue light alone is responsible for the development of phototropic sensitivity.

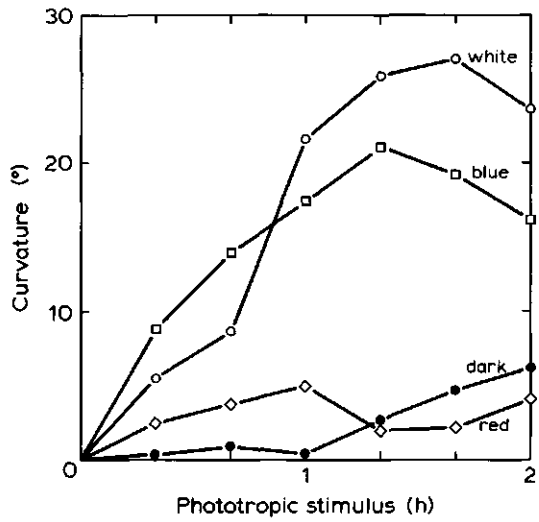


FIG. V.10. Phototropic response of de-etiolated seedlings after 18 h white light (○–○), blue light (□–□), red light (◇–◇) and of etiolated seedlings (●–●). Average values of 10 seedlings.

Dim green light was used as a safe light. To what extent this light was really safe was investigated by culturing seedlings under a rhythm of 14 hours green safe light/10 hours dark. The phototropic curvature of these seedlings was compared with that of fully etiolated ones. The seedlings cultured in green light curved to an appreciable and significant extent (Fig. V.11.). For this reason the 'safe' light was only used when inevitable and for as little as possible.

In order to check whether both the development and the effect of the phototropic stimulus were confined to blue light perceived by the hypocotyl during pretreatment and/or unilateral illumination, etiolated seedlings pretreated with 18 hours blue light, with darkened cotyledons, were unilaterally illuminated with blue light. When the curvature of these seedlings was compared with that of seedlings grown in, and unilaterally illuminated with, white light, no difference in curvature could be detected.

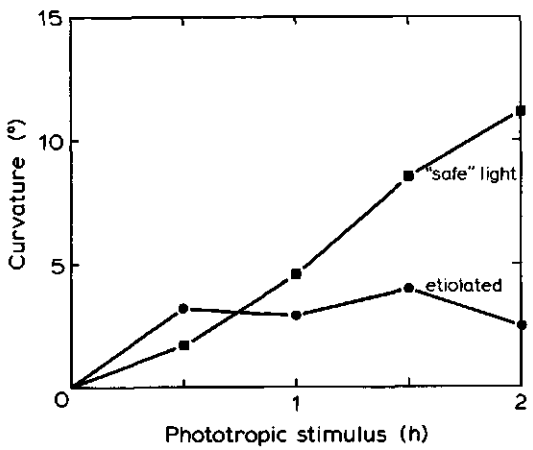


FIG. V.11. Phototropic response of etiolated seedlings (●–●) and seedlings cultured in green 'safe' light (■–■). Average values of 10 seedlings.

5.2.6. *Morphological state and growth rates of the seedlings*

The experiments described above were done with seedlings that were morphologically dissimilar due to their different pretreatments. Etiolated seedlings treated with blue light look intermediate between those grown in white light and etiolated ones; the cotyledons of the plants were coloured, but not as green and expanded as those of seedlings cultured in white light. Seedlings treated with red light had well expanded, dark green cotyledons and their hypocotyl had thickened.

No measurements of growth rates were made, but the differences were quite obvious. Etiolated seedlings grew more rapidly than those grown in white light. Seedlings treated with blue light had only a slightly inhibited growth rate compared to the etiolated ones, whereas seedlings treated with red light were much reduced in growth.

The growth rate during the phototropic experiments was measured by determining the length of the seedlings before and at the end of the phototropic experiment. Seedlings pretreated with white, blue, or red light grew approximately 1.0, 1.3, and 0.7 mm/hour, respectively; seedlings with their cotyledons removed 18 hours before the phototropic experiment almost ceased growth with a growth rate of 0.2 mm/hour.

5.3. Discussion

The experiments show that continuous stimulation of the seedlings is desirable, a short stimulation giving a reduced effect. The wavelength region involved is mainly, if not exclusively, in the blue. In all experiments, however, the curvature of seedlings unilaterally illuminated with white light slightly exceeded that of those phototropically stimulated with blue light. Shuttleworth and Black (1977) found for *Helianthus annuus* a very small effect of unilateral illumination with red light on curvature, but they consider this possibly due to a small contamination of blue light in the red. Another effect, however, might be involved. Red light, perceived by the cotyledons, exerts an inhibiting effect on the growth of the hypocotyl (Black and Shuttleworth, 1974). If the cotyledons are directed perpendicular towards a white light source, the cotyledon near the phototropic stimulus will perceive more light than the one at the shaded side, and a small, non-phototropic, difference in growth rate might be caused. This would explain the difference between unilateral illumination with blue and white.

The result that only light with a wavelength region below 500 nm is active in phototropism, is not only obtained for mono- and dicotyledonous seedlings, but also for lower organisms, e.g. *Vaucheria geminata* (Kataoka, 1977), *Griffithsia pasifaca* (Waaland et al., 1977), and *Phycomyces blakesleeanus* (Curry and Gruen, 1959). For *Phycomyces* and *Vaucheria*, practically identical actions spectra were made as for *Avena*, indicating that the phototropism of these organisms occurs through a common initial process. However, a discussion of the pigment involved in the perception of the phototropic stimulus falls outside the scope of

the present study. The possible role of far-red and red irradiation, indicating phytochrome as an accessory pigment, is analysed in Chapter 6.

Etiolated coleoptiles readily respond to a phototropic stimulus (ARISZ, 1915; WENT, 1928). Most etiolated or light-grown dicotyledons, pretreated with a prolonged dark period, also respond to unilateral illumination (EVERETT, 1974; KANG and BURG, 1974; VAN OVERBEEK, 1933), and *Helianthus* did not seem to be an exception (SHIBAOKA and YAMAKI, 1959; STEYER, 1967). The seedlings used by SHIBAOKA and YAMAKI (1959), however, were 10 days older than the ones used in these experiments, and STEYER (1967) did her investigations in seedlings of only 3 days old. The curvature she found was only 9°, and this might well be nutation. The etiolated *Helianthus* seedlings used in the present investigation did not respond to a phototropic stimulus at all, as was also found by DELA FUENTE and LEOPOLD (1968a), also working with seedlings of 5 days old. Therefore, seedlings of this age are a suitable material for the study of the mechanism of phototropism.

Etiolated sunflower seedlings became sensitive to unilateral illumination during a 24 hours period of blue or white light, perceived by the hypocotyl, just as light-grown seedlings pretreated with 18 hours blue or white light, and seedlings cultured in blue or white light. Seedlings treated with red light show a strong deetiolation: the dark green cotyledons are well expanded and the growth of the hypocotyl has decreased in longitudinal and increased in lateral direction. Nevertheless, their phototropic sensitivity is very poor, although significant (Fig. V.10.). This might indicate a possible involvement of red light in the development of the phototropic sensitivity. On ground of Fig. II.5. a contamination of red light by blue can be excluded. Moreover, no bending could be obtained by unilateral illumination with red light. If the red light source was contaminated with blue, a small response should occur.

Seedlings cultured in green safe light also respond to a unilateral illumination. Although the response was poor, it was significant, and too large to be neglected. It might be that the green light is contaminated with blue at a low energy level. Another possibility is that green light has a slight photomorphogenetic activity, as was stated by KLEIN (1979) and SHEN-MILLER (1978).

The cotyledons have no influence on the phototropic curvature. As is already shown in Fig. IV.1., they do not play a role during the curvature itself. In the experiments described in this Chapter, evidence is given that they are also not involved during the pretreatment. Darkening of the cotyledons has no influence; removal, on the contrary, has a detrimental effect. This is possibly due to the lack of food substances provided by the cotyledons to the hypocotyl. When etiolated seedlings have their cotyledons removed immediately before the unilateral illumination, no difference in response after several hours irradiation could be detected (Fig. V.9.)., indicating that the cotyledons are not involved in the development of the phototropic mechanism. Therefore, the blue light had to be perceived by the hypocotyl itself. This result is in agreement with those obtained by BLACK and SHUTTLEWORTH (1974) and JOSE (1977).

The growth rate of the seedlings has usually been considered to be an impor-

tant factor for the phototropic response. BLAAUW (1915) explained the phototropic curvature of *Helianthus globosus* as completely resulting from the differences in growth rate between light- and dark-treated seedlings, and VAN DILLEWIJN (1925) concluded the same for *Avena sativa*. The growth rate changes in dicotyledons due to pretreatment with light of different wavelengths has been studied extensively, although more in etiolated than in light-grown seedlings (BLACK and SHUTTLEWORTH, 1974; GABA and BLACK, 1979; JOSE, 1977; MEIJER, 1968; MOHR and WEHRUNG, 1960; VINCE-PRUE, 1977; WILDERMANN et al., 1978). Blue light inhibits growth much sooner than red light (COSGROVE and GREEN, 1978; GABA and BLACK, 1979). Also darkening or removal of the cotyledons and/or the top are involved in the growth rate (BLACK and SHUTTLEWORTH, 1974; BRUINSMA et al., 1975; VAN OVERBEEK, 1933).

For phototropic curvature, the growth rate before unilateral illumination is not important. Curvature can be described as a difference in growth rates between the illuminated and the shaded side, induced by the phototropically active, blue light. Red light, phototropically inactive, perceived by the cotyledons, only induces curvature due to the inhibition of growth, not properly phototropic (LAM and LEOPOLD, 1966; SHUTTLEWORTH and BLACK, 1977). These experiments were repeated and confirmed. The inhibition of growth by blue light is not very pronounced in *Helianthus* seedlings. Therefore, another mechanism besides the growth reaction must be involved in phototropism.

Neither the overall growth of the seedlings during unilateral illumination seems to be involved in phototropism as already indicated by BRIGGS (1963c). EVERETT (1974) describes that fully etiolated seedlings and etiolated seedlings which received 1 day red light curve at the same rate, although the length of the seedlings treated with red light differed significantly from that of the etiolated ones. To investigate if the growth rate during the phototropic curvature is involved in the response, the lengths of the plants were measured just before stimulation and at the end of the experiment. The growth rate during unilateral illumination was slightly higher in seedlings treated with blue light than in seedlings treated with white light, curving to the same degree. The difference in growth rate between seedlings treated with blue or white light, and seedlings treated with white or red light was about the same. The last group did not show much phototropic response. This indicates that elongation growth and phototropism are different processes and that they are less strongly coupled than is usually considered.

6. EFFECT OF PRETREATMENT WITH FAR-RED IRRADIATION ON THE PHOTOTROPIC RESPONSE

6.1. INTRODUCTION

Influences of far-red irradiation on physiological processes are well known. Roughly, these effects can be divided in two groups, the low-energy reactions, L.E.R., and the high-energy reactions, H.E.R. (SMITH, 1975). The L.E.R. are mostly phytochrome effects; red light induces a reaction by converting phytochrome into its active form. This effect can be reversed by far-red irradiation. Also pure far-red effects are described; these are mostly H.E.R. According to SMITH (1975) these effects are more common in nature, where plants are exposed to long periods of irradiation. SMITH (1975) summarized the literature on far-red effects and stated that high dosages of far-red irradiance can enhance the effect of a short period of red light, reversible by a low dosage of far-red. This indicates different patterns for the mechanism of the activity of far-red. Both the L.E.R. and the H.E.R. are involved in, e.g., seed germination, de-etiolation, and stem elongation (SMITH, 1975). In this Chapter it is investigated whether far-red effects are involved in the phototropism of *Helianthus* seedlings.

TABLE VI.1. The influence of varying pretreatments with far red on the phototropic sensitivity to continuous blue light. Average values of 10 seedlings.

pretreatment	average maximal curvature (°)							
	exp. 1	exp. 2	exp. 3	exp. 4	exp. 5	exp. 6	exp. 7	exp. 8
white-light control	34.8 (a)	21.7 (a)	37.4 (a)	20.9 (a)	28.2 (a)	47.0 (a)	39.1 (a)	28.5 (a)
dark pretreatment:								
60 min		20.8 (a)						
120 min			25.4 (b)			39.0 (a)	33.1 (a)	24.5 (a)
150 min	23.3 (a)							
180 min				20.6 (a)	23.1 (a)			
240 min						44.5 (a)		
FR pretreatment:								
5 min	30.3 (a)							
20 min	31.6 (a)							
60 min		22.3 (a)						
90 min					26.2 (a)			
120 min			19.3 (c)			26.7 (b)	19.1 (b)	16.5 (b)
150 min	16.4 (b)							
180 min				13.4 (b)	11.4 (b)			
240 min						25.0 (b)		

Figures within the column followed by different indices differ at $P < 0.05$.

6.2. Results

6.2.1. *Effect of different pretreatments with far-red irradiation on curvature*

Table VI.1. summarizes results of giving different periods of far-red irradiation, immediately followed by unilateral illumination, to green seedlings. The unilateral illumination used in these experiments always was with blue light because with an unilateral illumination with white light part of the far-red effect would be lost owing to the red region in the white light. It is evident (Table VI.1.) that 2 hours far-red is required to significantly reduce the phototropic sensitivity, so that the reaction seems to be a high energy one (H.E.R.). Prolonged pretreatment had the disadvantage that in some experiments also the dark treatment reduced phototropic sensitivity, although not significantly. This may be expected on account of fig. V.4., in which it is shown that pretreatment in darkness reduces the phototropic sensitivity. Probably the effect of pretreatment in darkness depends on the period of the year; in the season in which these experiments were carried out the reducing effect of a few hours pretreatment with darkness was relatively small.

To check whether the response to far-red irradiance was a H.E.R., indeed, seedlings were treated with shorter periods of far-red with additional darkness until 2 hours (Table VI.2). Shorter periods of far red down to even 1 minute also gave significant reduction in curvature, if followed by an additional dark period up to 2 hours, indicating that the response, in fact, is a L.E.R.

Table VI.3. shows that the far-red effect vanishes if the interval between application and phototropic treatment is reduced below 2 hours.

6.2.2. *Reversibility of the far-red effect by red light*

To examine whether phytochrome is involved in the reduction of phototropic sensitivity by far-red irradiance, the effect of short periods of red light was also investigated. It is obvious, that the response of green seedlings was unaffected by

Table VI.2. The influence of different periods of far red with additional darkness until 2 hours on phototropic sensitivity to continuous blue light. Average values of 10 seedlings.

pretreatment	average maximal curvature (°)				
	exp. 1	exp. 2	exp. 3	exp. 4	exp. 5
white-light control	21.3 (a)	24.2 (a)	30.4 (a)	37.4 (a)	38.3 (a)
2 hr darkness	21.7 (a)	21.5 (a)	31.5 (a)	25.4 (ab)	27.0 (ab)
FR dark					
120 min + 0 min	15.7 (a)	16.1 (a)	20.5 (b)	19.3 (bc)	
25 min + 95 min	24.6 (a)				
15 min + 105 min		17.9 (a)	18.4 (b)	16.7 (c)	
5 min + 115 min	14.7 (a)				
1 min + 119 min	12.3 (a)				20.5 (b)

Figures within the column followed by different indices differ at $P < 0.05$.

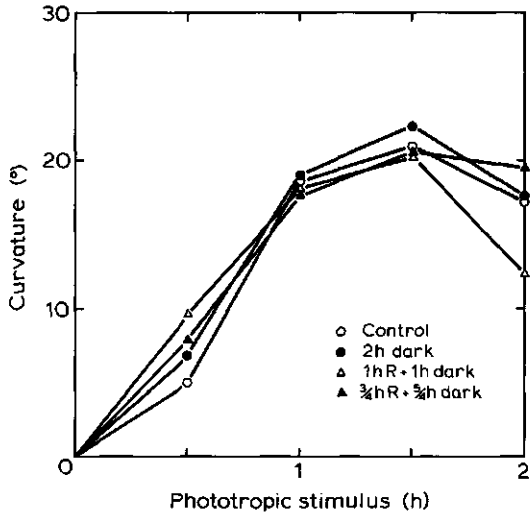


FIG. VI.1. Phototropic response of green seedlings to continuous blue light after a pretreatment with 2 h dark (●–●), 1 h R and 1 h dark (△–△), 3/4 h R and 5/4 h dark (▲–▲), and the white light control (○–○). Average values of 10 seedlings.

TABLE VI.3. Influence of different periods of darkness after 1 min far-red irradation on phototropic sensitivity to continuous blue light. Average values of 10 seedlings.

pretreatment	average maximal curvature (°)
white-light control	38.3 (a)
2 h dark	27.0 (b)
1 min FR + 120 min dark	20.5 (c)
1 min FR + 60 min dark	23.5 (bc)
1 min FR + 30 min dark	38.1 (a)

Figures within the column followed by different indices differ at $P < 0.05$.

TABLE VI.4. The influence of pretreatment with red light on the curvature of etiolated seedlings. Average values of 16 seedlings.

pretreatment	maximal curvature (°)	
	exp. 1	exp. 2
untreated control	3.1	–0.6
15 min R	2.1	1.1
15 min R + 2 h dark	2.5	2.9
15 min R + 4 h dark	1.3	0.8
15 min R + 6.75 h dark	4.4	1.3
15 min R + 8 h dark	2.5	4.7
15 min R + 12.75 h dark	1.4	0.8

There are no differences within the column differing at $P < 0.05$.

TABLE VI.5. The influence of red light on the far-red effect. Average values of 10 green seedlings, phototropically stimulated with continuous blue light.

pretreatment	average maximal curvature (°)			
	exp. 1	exp. 2	exp. 3	exp. 4
white-light control	46.5 (a)	28.5 (a)	39.1 (a)	37.7 (a)
2 h darkness		25.0 (a)	33.1 (a)	33.6 (ab)
4 h darkness			21.7 (bc)	
2 h FR	27.7 (b)	16.0 (b)	18.9 (b)	26.1 (bc)
2 h FR + 2 h dark				21. 2 (c)
2 h FR + 5 min R		14.1 (b)	25.1 (c)	
2 h FR + 20 min R		13.4 (b)	21.6 (bc)	
2 h FR + 75 min R		15.7 (bc)	24.2 (bc)	
2 h FR + 120 min R	44.5 (a)	24.0 (a)	20.6 (bc)	35.3 (a)
2 h FR + 150 min R		22.7 (ac)	23.5 (bc)	

Figures within the column followed by different indices differ at $P < 0.05$.

red-light pretreatments up to at least 1 hour (Fig. VI.1.). Also red light itself does not affect the lack of curvature of phototropically insensitive etiolated seedlings (Table VI.4.). Even prolonged unilateral illumination of these seedlings caused no difference in curvature whether they were pretreated with red light or not.

Although short periods of red light do not change the phototropic sensitivity, it is obvious that prolonged red illumination can reverse the effect of far-red irradiance. In Table VI.5., different periods of red light were given immediately after a 2 hours irradiation with far red. The restored phototropic sensitivity of the seedlings pretreated with irradiation with far red and prolonged red indicates involvement of phytochrome. The amount of red light required varied between the experiments. In experiment 3, 2 hours far red gave a significant reduction compared to the 2 hours dark treatment. When a pretreatment of 2 hours far red plus 2 hours red was given, the curvature of these seedlings equalled that of the seedlings pretreated with 4 hours dark. This is probably an effect of the dark period, namely lack of blue light, rather than of the far-red/red system. In experiment 4, for example, this effect of darkness is shown with the seedlings pretreated with 2 hours far red plus 2 hours dark. In this experiment, the red-light treatment fully restored the phototropic sensitivity.

6.2.3. *Influence of a pretreatment of far-red irradiation after white, blue or red light*

As shown in Chapter 5, dark-grown seedlings, de-etiolated with 18 hours white or blue light, respond to a phototropic stimulus, whereas after a pretreatment with 18 hours red light they only show a poor curvature. Far-red irradiation during 2 hours before unilateral illumination reduced the curvature not more than darkness, except in seedlings treated with white light (Table VI.6.).

TABLE VI.6. The influence of a pretreatment with white, blue, or red light on the far-red effect. Average values of 10 seedlings, grown in 14 h white light per day.

first pretreatment during 18 hours	second pretreatment during 2 hours	maximal curvature (°)			significance
		exp. 1	exp. 2	exp. 3	
white	white	19.2	16.9	13.4	a
	dark	15.0	14.6	11.0	a
	far red	6.3	1.9	4.2	b
blue	blue	14.4	16.6	20.1	
	dark	17.3	13.0	12.5	n.s.
	far red	11.8	15.5	13.4	
red	red	8.6	5.5	7.4	
	dark	5.5	3.2	8.2	n.s.
	far red	9.8	5.5	8.7	

Figures within the column followed by different indices differ at $P < 0.05$.

6.2.4. *Site of the far-red perception and growth rate of the seedlings*

To investigate the site of perception of the far-red irradiation, hypocotyls or cotyledons were covered with aluminium foil during the far-red irradiation; afterward the foil was removed and the phototropic response to continuous blue light measured. To determine the effect of the covering, other hypocotyls or cotyledons were covered with transparent plastic; no effect of this covering could be detected. From the first 2 experiments in Table VI.7., it can be concluded that the perception of the far-red is located both in the hypocotyl and in the cotyledons. Since it might be possible that some irradiation still penetrated to the covered hypocotyls or cotyledons, the latter were removed. In this way the perception of far-red by the hypocotyl alone could be investigated. From the last 2 experiments in Table VI.7. it can be seen that the far-red perception occurs in the hypocotyl. The overall curvature was reduced due to the removal of the cotyledons; during

TABLE VI.7. The locus of perception of the far-red irradiation. Average values of 10 green seedlings.

pretreatment	average maximal curvature (°)			
	exp. 1	exp. 2	exp. 3	exp. 4
white-light control	29.1 (a)	26.9 (a)	30.4 (a)	42.8 (a)
2 h dark	25.3 (a)	25.0 (a)	31.5 (a)	41.4 (a)
2 h FR	17.9 (b)	13.7 (b)	20.5 (b)	33.2 (ac)
2 h FR on hypocotyl only	15.5 (b)	13.0 (b)		
2 h FR on cotyledons only	13.4 (b)	17.2 (b)		
2 h white, cotyledons removed			17.8 (b)	
2 h dark, cotyledons removed			19.5 (b)	27.1 (bc)
FR treatment, cotyledons removed			12.3 (c)	13.6 (d)

Figures within the column followed by different indices differ at $P < 0.05$.

the phototropic experiment the growth rate of the hypocotyls was reduced from 0.6 mm/hour in intact seedlings to 0.3 mm/hour in cotyledonless seedlings. A significant difference between 2 hours dark and 2 hours far red remained to be seen.

An experiment in which the cotyledons were covered immediately upon emergence and remained so during far-red pretreatment, so that they were fully etiolated at the onset of unilateral illumination, indicated that green cotyledons are not required for the far-red sensitivity of the hypocotyl, although the growth rate of the seedlings with their cotyledons covered was higher than that of untreated seedlings. However, the illuminated hypocotyls looked also etiolated.

It might be argued that the reduction in curvature due to far red is the result of a change in growth rate of the seedlings rather than a direct effect on phototropic sensitivity. For this reason in each experiment the length of the seedlings was measured just before and after 2 hours of unilateral illumination. No difference in growth rate between well and less responding seedlings could be detected; on an average the seedlings elongated 1.2 mm/hour during the phototropic treatment.

6.3. Discussion

It is evident from the results that far-red irradiation reduces the phototropic sensitivity. This effect can be antagonised by low-energy red light, indicating that phytochrome is involved. According to Smith (1975), phytochrome responses should be low-energy reactions, red/far red reversible. Our system meets these criteria.

Phytochrome has an influence on geotropism. Kang and Burg (1972) described an enhancement of geocurvature 8 hours after a brief exposure of pea stems to red light. They explain this as a result of the inhibition of ethylene production, resulting in a more readily lateral auxin transport. Shen-Miller et al. (1976) pointed in the same direction: maize coleoptiles require red light for geocurvature. By such an illumination an easier transport of substance(s) through the cuticular layer of the cap-root juncture takes place. These results indicate a change in membrane permeability (see also Smith, 1975), as was found by Rasul (1976), describing the involvement of phytochrome in the geotropism of oat coleoptiles. McArhur and Briggs (1979) actually measured the phototransformation of phytochrome in pea epicotyls and found that 10 times more light was required to produce a spectrophotometrically detectable transformation of phytochrome than for a significant change in the geotropic response.

Also in phototropism phytochrome may be involved in the first and second positive response of etiolated coleoptiles. Chon and Briggs (1966) found that red light enhances the second positive response of maize coleoptiles and that this effect is reversed by far-red irradiation. They also demonstrated an agreement between the physiological and spectrometric states of phytochrome (Briggs and Chon, (1966). Also Kang and Burg (1974) described enhancement of the

phototropic response of etiolated pea seedlings due to red light; they did not try to reverse this effect with far red.

In the present experiments no enhancement of the phototropic sensitivity by red light could be detected, neither in etiolated seedlings nor in light-grown ones. On the contrary, red light, although having a de-etiolating effect, reduces the phototropic sensitivity of light-grown seedlings (see Chapter 5.). It is possible that the normal curvature of green seedlings is already maximal. FARR and ELLIOTT (1975) stated that the growth of etiolated maize seedlings is inhibited by red light, the red effect being reversed by far red. These seedlings treated with red light do not respond to a second treatment with red light. In our experiments seedlings treated with white light, in which red light occurs, do not respond to further red light, but they do respond to far red. When seedlings are pretreated with blue light, no reaction towards far red could be detected, indicating a red/far-red system.

Due to blue light the phytochrome content in mung bean hooks was increased (TANADA, 1977). KENDRICK (1972), and KENDRICK and FRANKLAND (1969) reported a loss of phytochrome following exposure to blue light in dicotyledonous seedlings. In our sunflower seedlings phytochrome was not measured spectrophotometrically; therefore it is not possible to decide whether the lack of response of seedlings pretreated with blue light to far red is the result of the absence of phytochrome. From the literature it is known that blue light is involved in the phytochrome system (DEUTCH, 1976; FURUYA, 1977; WILDERMANN et al., 1978). The precise action of blue light in this system is not yet fully understood. Also the age of the seedlings plays an important role in the phytochrome response (BLACK and SHUTTLEWORTH, 1974; FURUYA, 1977; SMITH, 1975).

According to BLACK and SHUTTLEWORTH (1974), working with cucumber hypocotyls, the red and far-red irradiations are perceived by the cotyledons; part of the red light exerts its effect on the hypocotyl. These experiments were done to investigate the growth rate. In our experiments, far red was mainly perceived by the hypocotyl directly, indicating that there are two different systems acting, namely the perception of red and far red irradiation by the cotyledons, involved in the growth rate, and the perception of far red by the hypocotyl directly, involved in curvature. GEE and VINCE-PRUE (1976) described that in *Phaseolus mungo*. L. seedlings the hook is the light-sensitive region for phytochrome responses; because in covering experiments the upper parts of the hypocotyl are always difficult to keep covered, this part might play an important role.

Several authors attributed the change in photo-or geocurvature to morphogenetic growth responses (ELLIOTT and SHEN-MILLER, 1976; KANG and BURG, 1974). Much research has been done on the involvement of phytochrome in the growth of de-etiolating seedlings (BLACK and SHUTTLEWORTH, 1974; HODDINOTT, 1977; GARRISON and BRIGGS, 1975; VINCE-PRUE, 1977). In our experiments, no change in growth rate between far-red treated and untreated seedlings could be detected, possibly because the time interval between pretreatment and the phototropic experiment was too short (GABA and BLACK, 1979). Far-red

treated seedlings, however, were reduced in their phototropic sensitivity. From this, together with the fact that far red acts immediately on the hypotocyl, it can be surmised, that the far-red irradiation influences the phototropic sensitivity directly rather than by mediating the photomorphogenetical growth response of the hypocotyl.

SMITH (1975) lists the lag periods between red-light irradiations and the onset of detectable responses of different processes (his Table 3.4.). From this Table it is clear that a lag phase of several hours is no exception: in most processes a considerable lag phase occurred. Although the changes listed by SMITH are induced by red light, the effect of far red, described in this Chapter, fits well in this enumeration. The lag phase indicates that the ultimate events are quite remote from the initial changes induced by the irradiation. The reasons for this are not yet clear.

7. INVOLVEMENT OF HORMONES IN PHOTOTROPISM

7.1. INTRODUCTION

For about 50 years, since the CHOLODNY-WENT theory was proposed, the involvement of hormones in phototropism, particularly of IAA, seems to be an established fact. Many authors have published experiments to affirm this theory, collecting diffusates from unilaterally illuminated organs (BOYSEN-JENSEN, 1933; DUBUY, 1933; CHOLODNY, 1927; LAM and LEOPOLD, 1966; OPPENOORTH, 1939; VAN OVERBEEK, 1932; WENT, 1928), or applying IAA to one side of an organ (DELA FUENTE and LEOPOLD, 1968b; GARDNER et al., 1974; HAUPT, 1972; KANG and BURG 1974; KRIEGER, 1978; PICKARD and THIMANN, 1964: ULLRICH, 1978). In collecting diffusates, however, not only IAA may be obtained, but also other diffusible compounds present in the organ, for instance growth-inhibiting substances. The IAA-activity was mostly determined in a bioassay, without exclusion of other activities. In applying exogenous IAA to the plant it is implied that this IAA has the same properties, for instance in transport and compartmentation, and the same effect on metabolism, as the endogenous molecules.

The CHOLODNY-WENT theory has been modified several times in the last 30 years. GALSTON and BAKER (1949) showed that IAA is destroyed by light in the presence of riboflavin, resulting in an explanation of phototropism as a result of photooxidation. Also an altered sensitivity of the tissue due to light or other compounds, for instance other hormones, is mentioned (JINDAL et al., 1974; KANG and BURG, 1974; VAN OVERBEEK, 1932; RECHES et al., 1974).

At present, physical-chemical methods are available to determine the endogenous amount of IAA and other hormones in plant extracts (HILLMAN, 1978). BRUINSMA et al. (1975) found an equal distribution of endogenous IAA in phototropically stimulated sunflower seedlings. The presence of a growth inhibitor was suggested (BRUINSMA, 1977), analogous to the probable function of ABA in root geotropism (PILET, 1977). THOMPSON and BRUINSMA (1977) demonstrated the presence of a growth inhibitor, xanthoxin, in light-grown seedlings and the absence of this inhibitor in etiolated ones, that fail to respond phototropically (see Chapter 5.). Also BAYER (1961) described the activation of an inhibiting system in sunflower hypocotyls by light. The role of this inhibitor is investigated in this Chapter.

Also gibberellins are mentioned in relation to phototropism. PHILLIPS (1972) suggested an asymmetric distribution of gibberellins in phototropically and geotropically (PHILLIPS and HARTUNG, 1976) curved sunflower shoots. FIRN et al. (1977) considered it very unlikely that gibberellins are involved in geotropism. In this Chapter a possible role of gibberellins in phototropism is studied.

7.2. Results

7.2.1. *Auxin*

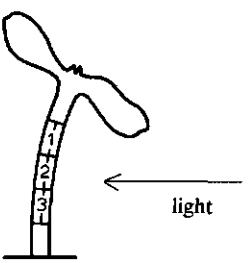
To complete the results of Bruinsma et al. (1975), which showed that IAA was not asymmetrically distributed over the two sides of phototropically curving sunflower hypocotyls, an additional experiment was carried out to determine the distribution of IAA in curving parts of plants. The curving parts of 415 hypocotyls after 30 to 45 min unilateral illumination were divided in 6 sections, 3 illuminated and 3 shaded ones. The results of Table VII.1. confirm that in curving hypocotyls no asymmetrical distribution of IAA occurs.

Since the amount of diffusable IAA may be more important than the amount of extractable IAA, the IAA activity in diffusates from light-grown straight, curving, and curved hypocotyls was studied in the *Avena* straight-growth bioassay. However, no IAA activity at all could be detected in these diffusates. Two possibilities were investigated which might account for this lack of IAA activity, namely destruction of IAA during its transport into the diffusate and codiffusion of IAA-inhibiting substances.

7.2.2. *Destruction of IAA*

These experiments were carried out by adding a physiological amount of ^{14}C-labelled IAA to the buffer before the onset of the experiment, and calculating the percentage recovery of this IAA after 6 hours diffusion. Also the weight of the hypocotyls was measured at the beginning and end of each experiment to check whether the seedlings were healthy and, therefore, grew during the experiment. Part of the loss of radioactivity from the diffusate might be ascribed to the uptake of liquid because of this growth. The average growth of the seedlings during the experiment equalled the average loss of the liquid. For this reason the amount of dpm/ml is used. The results are shown in Table VII.2. Although the method used is not highly precise, it is obvious that decarboxylation of IAA in the diffusate owing to the influence of the hypocotyls is only small: the average recovery was 83.3 %, versus 92.9 % in the absence of hypocotyls.

Table VII.1. The distribution of IAA in curving parts of hypocotyls after 30 to 45 min unilateral illumination. Number of curving hypocotyls: 415; curvature: 16.9° ± 6.1.



segment number	shaded side			illuminated side		
	fresh weight (g)	ng IAA / g	%	fresh weight (g)	ng IAA / g	%
1	9.60	7.3	53.7	9.66	6.3	46.3
2	9.16	6.9	46.0	10.85	8.1	54.0
3	9.77	7.1	56.3	10.89	5.5	43.7

TABLE VII.2. The influence of the cut surfaces of sunflower hypocotyls on the decarboxylation of radioactive IAA during diffusion.

exp. no.	no hypocotyls			25 hypocotyls		
	dpm/ml t = 0	dpm/ml t = 6	% recovery	dpm/ml t = 0	dpm/ml t = 6	% recovery
1	2386	2152	91.2	2386	2066	86.6
2	2386	2201	92.2	2386	1988	83.3
3	4089	3608	88.2	4082	2856	70.0
4	2875	2888	100.5	2419	2300	95.1

7.2.3. *Inhibiting substances*

To test whether compound(s) inhibiting-IAA activity was (were) present in the diffusate, various concentrations of IAA were added to the diffusate before testing it in the *Avena* straight-growth bioassay. The results in Table VII.3. show the presence of such inhibiting activity. The possible involvement of this inhibiting activity in phototropism was investigated. Diffusates were made from light-grown seedlings, able to curve under the influence of unilateral light, and from etiolated seedlings, which lack this ability. During the diffusion period the hypocotyls were illuminated or kept in the dark. Also diffusates from whole plants, hypocotyls only, and cotyledons only (which play no role in phototropism, see Chapter 4, Fig. IV.1.) were collected, and after addition of 3.10^{-6} M IAA to the diffusates the percentage recovery of IAA activity was calculated from the *Avena* straight-growth bioassay. From the results in Tables VII.4. and VII.5. it can be concluded that all diffusates contain inhibiting activity. Diffusates collected from the illuminated and shaded sides of phototropically stimulated hypocotyls did not show different amounts of inhibiting activity.

7.2.4. *Xanthoxin*

THOMPSON and BRUINSMA (1977) found that etiolated sunflower seedlings

TABLE VII.3. The activity of IAA-inhibiting compounds in diffusates of light-grown sunflower seedlings, determined in the *Avena* straight growth bioassay.

exp. no.	%IAA recovery			
	diffusate undiluted		diffusate diluted 5×	
	$+10^{-6}$ M IAA	$+3.10^{-6}$ M IAA	$+10^{-6}$ M IAA	$+3.10^{-6}$ M IAA
1	26	58	51	52
2	30	28	36	16
3	21	13	58	50
4	13	71	58	71
5	56	15	100	20

TABLE VII.4. The activity of IAA-inhibiting compounds in diffusates from light-grown and etiolated seedlings, collected in the light or in the dark, determined in the *Avena* straight-growth bioassay, after addition of 3.10^{-6} M IAA.

exp. no.	% IAA recovery in the bioassay diffusates from			
	light-grown		etiolated	
	diffusion in light	diffusion in dark	diffusion in light	diffusion in dark
1	13	28	8	22
2	15	54	34	30
3	17	7	7	14

TABLE VII.5. The activity of IAA-inhibiting compounds in diffusates from light-grown whole plants, hypocotyls only, and cotyledons only, determined in the *Avena* straight-growth bioassay, after addition of 3.10^{-6} M IAA.

exp. no.	% IAA recovery in the bioassay diffusates from		
	whole plants	hypocotyls	cotyledons
1	58	38	62
2	28	28	45
3	2	3	4

TABLE VII.6. The amount of extracted xanthoxin, determined by bioassay, from the first, second and third extraction, as percentage of the total amount extracted.

exp. no.	% xanthoxin in extraction		
	1	2	3
1	68	28	4
2	83	15	2

contain no xanthoxin, whereas light-grown ones do. Experiments were carried out to determine the involvement of this inhibitor in phototropism.

7.2.5. *Extraction and determination procedure*

The extractions were carried out according to both methods described in Chapter 2. The frozen tissue was extracted twice as described; a third extraction of the tissue was not necessary as the results of Table VII.6. indicate. In the second extraction procedure a C18 filter was used. The recovery of radioactive xanthoxin, syringed by this filter, was 100% when the described method was followed. The recovery of radioactive xanthoxin, added to the frozen tissue in methanol before grinding, was 18 ± 4% in the first extraction procedure, and 42

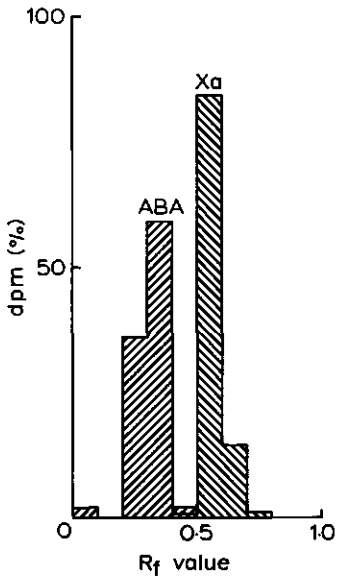


FIG. VII.1. Distribution of radioactive ABA and xanthoxin on TLC in the solvent system ethyl acetate:hexane = 3:1.

± 8% in the second. The results are corrected for these percentages.

Although the whole procedure was directed towards the extraction of neutral compounds, the possibility of contamination of extract with ABA, also active in the determination procedure used, was investigated. Experiments with labelled ABA and labelled xanthoxin show that both compounds are separated well on TLC in the solvent system used. ABA has a R_f value of 0.2–0.4 and xanthoxin of 0.5–0.7 (Fig. VII.1.). When a plant extract was mixed with ABA or xanthoxin, the inhibiting zones, measured in the cress seed germination assay, matched when xanthoxin was added, and not when ABA was added (Fig. VII.2.). These data, together with those of THOMPSON and BRUINSMA (1977), namely that

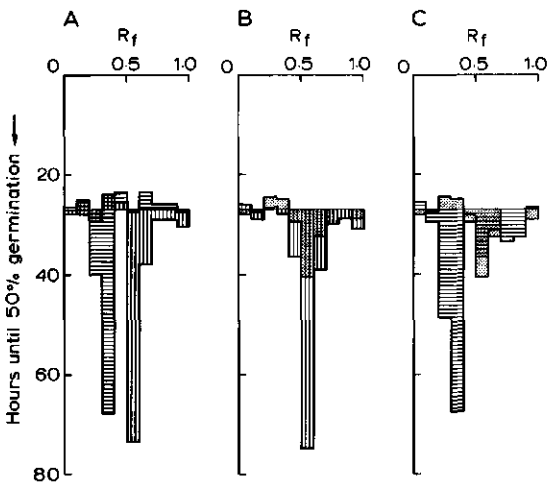


FIG. VII.2. The inhibiting zones in the cress seed germination assay of A: ABA ( ) and xanthoxin ( ), B: plant extract ( ) and plant extract mixed with xanthoxin ( ) and C: plant extract ( ) and plant extract mixed with ABA ( ). Solvent system used: ethyl acetate : hexane = 3:1.

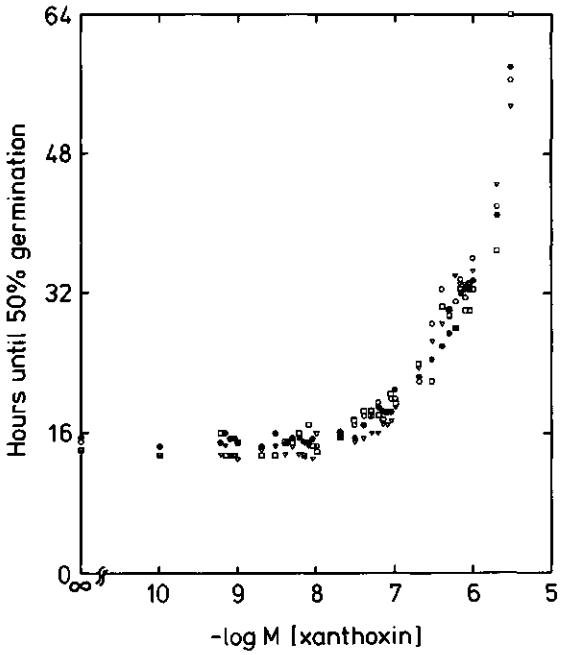


FIG. VII.3. Four standard series of xanthoxin in the cress seed germination assay, independently obtained.

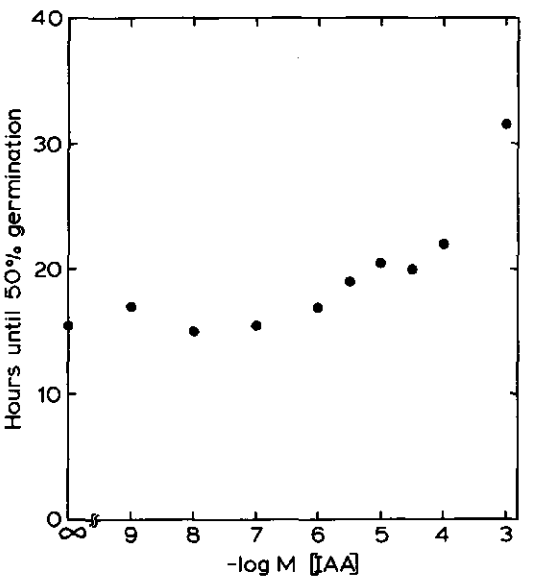


FIG. VII.4. Activity of IAA in the cress seed germination assay.

xanthoxin is 10 times more active in this bioassay than ABA, exclude an involvement of ABA in the described experiments.

Because it was not possible to determine xanthoxin by GLC, the cress seed germination bioassay had to be used. This determination procedure was greatly improved by extending the number of determinations of the germination rate. Instead of only measuring the percentage of germination after 48 hours, the seeds showing rootlets of at least 1 mm were counted and removed every 3 hours from 12 hours to 39 hours after the onset of the bioassay. After this period the counting was continued at intervals from 15 to 20 hours. The point of time of 50% germination could be determined by interpolation. This resulted in a xanthoxin determination over a broader concentration range. In Fig. VII.3., four standard series of xanthoxin are plotted against the time of 50% germination. This figure indicates that the standard curve is very reproducible. IAA is only slightly active in this bioassay, at concentrations higher than 10^{-6} M (Fig. VII.4.).

It was also investigated how much fresh weight was needed to give a distinct inhibition in the bioassay, and whether the relation between the amount of fresh weight and the quantity of inhibitor extracted was linear. For this reason a large amount of light-grown material was extracted, and after the paper-chromatography step the extract was divided into portions of 10, 20, 30, 40, 50 and 60 g equivalents fresh weight. These portions were treated separately during the subsequent steps of the extraction procedure and in the bioassay. The results in Fig. VII.5. show that xanthoxin can be determined well between 10 and 30 g fresh weight. When more than 30 g material was extracted the xanthoxin was not fully obtained, and less than 10 g material contained not enough inhibitor to give a distinct inhibition.

The xanthoxin used as a reference was actually obtained by extraction itself. In the final month of this research project, however, a chemically synthetized product became available. The activities of both the extracted and chemically

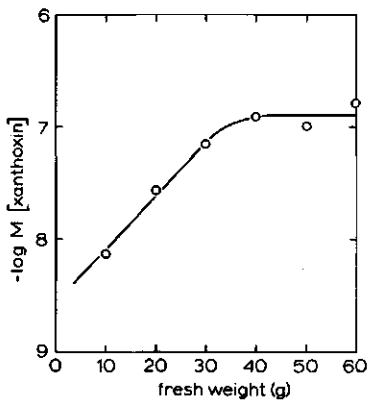


FIG. VII.5. Relation between the amount of fresh weight extracted and the amount of inhibitor obtained as determined in the cress seed germination assay.

synthetized xanthoxin were compared in the cress seed germination bioassay. Fig. VII.6. shows that the synthetized product is about 3 times as active as the extracted one. The results are corrected for this percentage.

7.2.6. *Involvement of xanthoxin in phototropism*

The amount of xanthoxin was determined in bisected straight hypocotyls, and in the illuminated and shaded halves of curving and curved light-grown hypocotyls. Only the bent parts of the hypocotyls during and after curvature were used for extraction. The results in Table VII.7. show an equal distribution of xanthoxin in straight hypocotyls, but an asymmetrical distribution in the curving and curved ones, especially in the bending seedlings. More xanthoxin was present in the illuminated than in the shaded side.

Subsequently, the xanthoxin levels were determined in etiolated seedlings pretreated with 18 hours white, blue, or red light, or kept in darkness, to investigate a possible relationship between the amount of curvature and the xanthoxin level in the hypocotyl. The results of Table VII.8. show that well-bending hypocotyls, treated with white or blue light, contain a high amount of xanthoxin, while the dark-treated plants contain far less. The xanthoxin level in seedlings treated with red light varied in the two experiments.

Attempts to induce curvature by exogenous application of xanthoxin failed. No curvature could be induced in phototropically stimulated etiolated seedlings treated with xanthoxin, or in light-grown seedlings to which xanthoxin was unilaterally applied. The uptake of radioactivity from a radioactive xanthoxin sample by the hypocotyl was investigated. From the results of Table VII.9. it can be seen that the uptake was somewhat higher in the light-grown seedlings than in etiolated ones. Xanthoxin was applied in an ethyl acetate-water mixture or in diluted ethanol, 125 ng/plant.

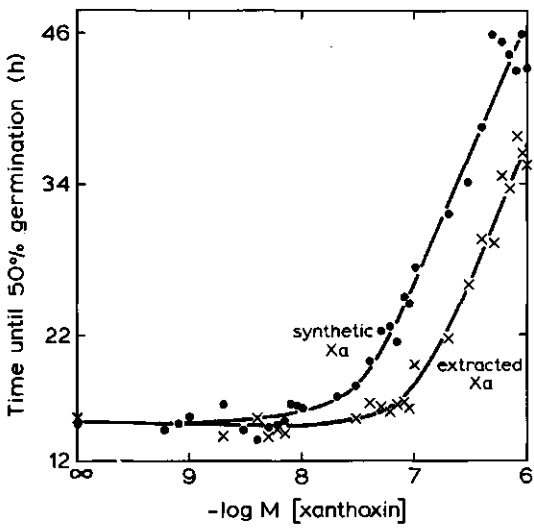


FIG. VII.6. Standard curves of extracted (×–×) and synthetized (●–●) xanthoxin in the cress seed germination assay.

TABLE VII.7. Distribution of xanthoxin in straight, curving, and curved green hypocotyls, sampled before and after 30–45 and 60–80 min continuous phototropic stimulation with white light, respectively.

	exp. no.	half 1 %	half 2 %	n	curvature (°)
straight hypocotyls t = 0	1	*48*	*52*	208	0
	2	*49*	*51*	235	0
		shaded side %	light side %		
curving hypocotyls t = 30–45 min	3	*28*	*72*	395	14.4 ± 4.6
	4	*39*	*61*	475	16.5 ± 6.5
curved hypocotyls t = 60–80 min	5	*38*	*62*	422	34.4 ± 8.5
	6	*43*	*57*	433	34.4 ± 6.8

TABLE VII.8. The amount of xanthoxin in seedlings pretreated with 18 h white, blue, or red light, or darkness. Data from samples of 100 hypocotyls.

pretreatment with	xanthoxin (ng/hypocotyl)	
	exp. 1	exp. 2
white light	14.1	11.4
blue light	14.4	10.2
red light	15.6	6.0
darkness	5.4	5.1

TABLE VII.9. The percentage radioactivity recovered after 2 or 6 hours from the hypocotyl segments of etiolated or light-grown seedlings. Average values of 3 seedlings.

exp. no.	percentage recovery			
	etiolated		light-grown	
	t = 2 h	t = 6 h	t = 2 h	t = 6 h
1			87	80
2	57	44	89	76
3		60		
4			98	

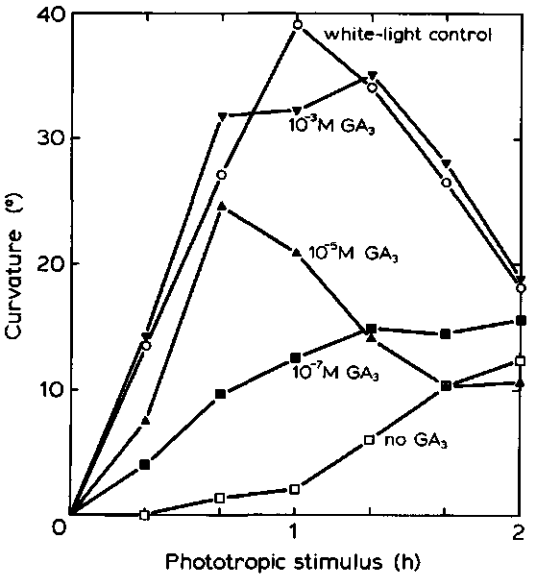


FIG. VII.7. The influence of 100µl 10^{-3}M (▼–▼), 10^{-5}M (▲–▲), and 10^{-7}M (■– ■) GA_3 on the phototropic curvature of seedlings pretreated with red light (□–□), and the phototropic curvature of seedlings grown in white light (○–○). Average values of 10 seedlings.

7.2.7. *Gibberellin*

As already shown in Chapter 5, Fig. V.10., seedlings treated with red light do not respond to unilateral illumination. However, if Ga_3 was applied to the top of the seedlings 1 hour before phototropic stimulation, the seedlings did respond (Fig. VII.7.). The effect of the red pretreatment was completely removed by applying 10^{-3}M, i.e. 48 µg GA_3 per plant. GA_3 had no influence at all on the curvature of plants grown in white light.

To determine whether endogenous gibberellins were involved in phototropic bending, seedlings were treated daily with chlormequat, and their lengths and phototropic responses were measured. The growth of these plants was significantly reduced compared with untreated seedlings but could be completely restored by exogenous GA_3 (Table VII.10.). Their phototropic bending, however, was not affected (Fig. VII.8.).

TABLE VII.10. The average length of seedlings treated daily with 1 ml chlormequat (CCC) on the soil, with or without 50 µl GA_3 on the plant top.

treatment	exp. 1			exp. 2		
	length (cm)	age (days)	n	length (cm)	age (days)	n
control	4.4 ± 1.4	5	137	7.3 ± 1.8	7	26
10 mM CCC	3.6 ± 1.1	5	91			
50 mM CCC	2.5 ± 0.8	5	95	5.7 ± 0.8	7	23
50 mM CCC + 1 mM GA_3				7.3 ± 0.9	7	24

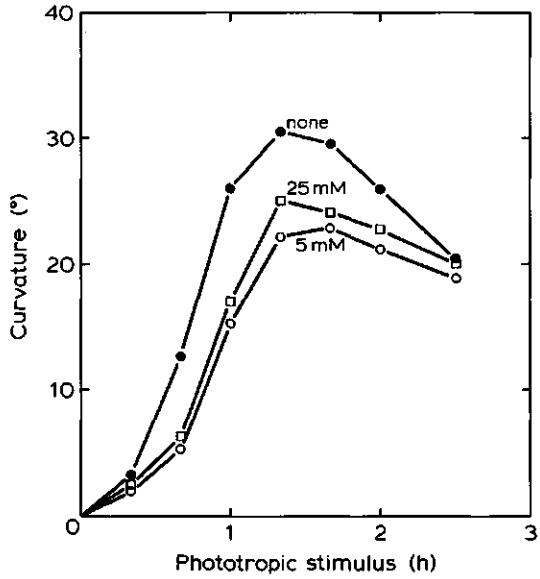


FIG. VII.8. Phototropic curvature of seedlings grown in white light treated daily with 1 ml 5mM (○–○) or 1 ml 25 mM (□–□) chlormequat on the pot soil; untreated controls: (●–●). Average values of 10 seedlings.

The amount of gibberellins which can be extracted from seedlings treated with red light is about the same as for seedlings grown in white light, namely 49 μg GA_3 equivalents/g fresh weight from control seedlings and 66 μg GA_3 equivalents/g fresh weight from material treated with red light.

7.3. DISCUSSION

The finding that IAA is not asymmetrically distributed in phototropically curving sunflower seedlings (Table VII.1.) agrees with the results of BRUINSMA et al. (1975), and renders a direct role of IAA in sunflower phototropism highly improbable. As already pointed out, many authors stated such a direct involvement in phototropism (DU BUY, 1933; DELA FUENTE and LEOPOLD, 1968b; GARDNER et al., 1974; VAN OVERBEEK, 1932; PICKARD and THIMANN, 1964; WENT, 1928). THIMANN and CURRY (1960) reviewed the role of auxin in phototropism and concluded that the effect of low-intensity light is exerted not on the auxin itself, but on the system which produces and/or transports it. In this review they also gave some evidence for an equal distribution of applied, isotopically labelled, IAA in coleoptiles after unilateral illumination, and used this as evidence for the involvement of the auxin-producing system in phototropism. An alternative hypothesis was not mentioned.

As BRUINSMA et al. (1975) stated, it is unlikely that IAA-decarboxylating enzymes, present at the cut surface of the hypocotyl (AASHEIM and IVERSEN, 1971; IVERSEN and AASHEIM, 1970; SCOTT and BRIGGS, 1962; STEEVES et al., 1953; ZENK and MÜLLER, 1964), could account for the total absence of IAA activity in the diffusates. In the experiments described in this Chapter only up to 20 % of the

exogenous IAA was inactivated. Because this IAA was not transported by the hypocotyls, the possibility still exists that endogenous IAA is inactivated in the wounded tissue before actually reaching the cut surface. A more plausible explanation, however, is the presence of substance(s) inhibiting IAA transport or activity. The presence of such substance(s) is clearly shown in Tables VII.3., VII.4. and VII.5. This inhibiting activity, however, occurs in potentially curving and non-curving seedlings, and in diffusates of hypocotyls and cotyledons, the latter playing no direct role in phototropism (Fig. IV.1.). Therefore, this activity need not be directly correlated with phototropism and might be due to the interference of, e.g., phenolic compounds (LINSKENS et al., 1964; MARIGO and BOUDET, 1977).

Also non-acidic growth inhibitors in dicots are known (GOTO, 1978; HIGGINS and BONNER, 1974), some of which are activated by light (BAYER, 1961; SHIBAOKA, 1961). LAM and LEOPOLD (1966), in investigating the asymmetry of auxin activity in green sunflower seedlings in relation to phototropism, found a growth inhibitor markedly effective in inhibiting IAA transport. This growth inhibitor has the same R_f value as xanthoxin in the solvent system used. However, they did not correlate this activity with phototropism.

Most of the older results in agreement with the CHOLODNY-WENT theory were obtained with the technique in which small agar blocks were used to collect diffusates of illuminated and shaded halves of phototropically stimulated organs. It was assumed that the difference in auxin activity was a measure for the difference in IAA responsible for the bending. A growth inhibitor, collected in this way, would be another explanation and will probably give the same results, the more so as the diffusates were not tested physicochemically, but by bioassay.

Several methods are described for the detection and estimation of xanthoxin in plants (BÖTTGER, 1978; FIRN et al., 1972; TAYLOR and BURDEN, 1970). Because a reliable physicochemical method for its determination could not be developed by lack of a sufficient amount of pure material, the cress seed germination assay of TAYLOR and BURDEN (1970) was improved and used as a bioassay for xanthoxin determination. The standard curves in this bioassay were very reproducible (Fig. VII.3.), although the duration of germination sometimes varied (compare Fig. VII.3. and VII.2.). Therefore, for every xanthoxin determination a standard curve was included.

According to TAYLOR and BURDEN (1970), xanthoxin can be formed from the xanthophyll, violaxanthin, at last in vitro, and occurs naturally in the shoots of most higher and some lower plants (FIRN et al., 1972). Therefore, this substance could be involved in phototropism, as also stated by WAIN (1975). The results of Table VII.7. show a distinctly uneven distribution of this compound in phototropically stimulated hypocotyls. The relative accumulation at the illuminated side agrees with the expectation if this inhibitor should be involved in the phototropic curvature. DIGBY and FIRN (1976) argued that a difference in distribution of auxin of about 30% cannot account for the difference in growth rate necessary for the curvature observed. However, the dose-response curve for xanthoxin is about linear, in contrast to the logarithmic curve for auxin activity, and the established difference in concentration may well account for the photo-

tropic response. Whether this distribution is the result of lateral transport, synthesis, and/or conversion of xanthoxin, cannot be concluded from the present data.

THOMPSON and BRUINSMA (1977) reported a scarcely detectable amount of xanthoxin in etiolated sunflower seedlings, and BURDEN et al. (1971) found a marked difference in xanthoxin content between etiolated and light-grown dwarf-pea seedlings. Table VII.8. shows similar results. Also in hypocotyls treated with blue light a high amount of xanthoxin could be detected. These data are in agreement with the phototropic sensitivity of seedlings treated with white or blue light. TAYLOR and SMITH (1967) suggested that blue light might produce xanthoxin in plants by a photolytic process, and therefore be involved in phototropism. According to ANSTIS et al. (1975), and BURDEN et al. (1971), however, xanthoxin can also be formed under red light. One experiment presented in Table VII.8. is in agreement with these data, the other experiment, however, shows a rather low amount of xanthoxin in hypocotyls treated with red light. The latter result supports the view that xanthoxin is involved in the ability to bend. It might be possible that, although seedlings treated with red light contain a high amount of xanthoxin, this xanthoxin cannot be divided asymmetrically during the phototropic stimulus due to altered membrane properties by red light. Whether or not compartmentation is involved in the lack of phototropic sensitivity after red illumination must await further analyses of xanthoxin contents and distribution, preferably using a physicochemical method that is less time-consuming than the bioassay used in the present study.

Attemps to induce curvature by exogenous application of xanthoxin failed, although part of the radioactivity was taken up by the hypocotyl. Several reasons can account for this failure. The growth inhibitor is not soluble in water, and therefore other solvents had to be used, which may have damaged the tissue. Another reason might be an impurity of the sample of the radioactive xanthoxin. Fig. VII.6. shows that the extracted and the synthetized compounds differ in activity by a factor 3. The labelled xanthoxin sample may also be impure which renders it impossible to determine the amount of xanthoxin taken up by the hypocotyl. Moreover, the xanthoxin was applied immediately underneath the cotyledons. Because the phototropic reaction of the hypocotyl does not necessarily require a basipetal transport of a hormone (Chapter 4), the xanthoxin may not be transported to the bending part, about halfway the hypocotyl (Chapter 3).

KANG and BURG (1974) found a promotive effect of GA on phototropic curvature, without interference with a possible lateral auxin transport. PHILLIPS (1972) suggested an asymmetrical distribution of gibberellins after phototropic stimulation. The results in this Chapter show that GA_3 does not enhance the phototropic bending of normal, light-grown plants, but removes the inhibition of curvature of seedlings treated with red light (Fig. VII.7.). No large difference could be detected between the amounts of gibberellins in seedlings treated with red light or white light, and hypocotyls of plants treated with chlormequat, an inhibitor of gibberellin biosynthesis (JONES and PHILLIPS, 1967; DENNIS et al.,

1965), showed the same phototropic sensitivity as untreated plants. This indicates that endogenous gibberellins are not directly involved in phototropism. It is possible that gibberellin influences membrane properties, altered by illumination with red light and, thereby, the phototropic sensitivity. Alternatively, both red light and gibberellin are known to influence several metabolic processes, and these changes might affect the curvature of seedlings (BOEKEN and VAN OOSTVELDT, 1977). They also have much influence on the growth rate, especially in dwarfism (KÖHLER, 1977a, b). Growth rate and phototropism have often been considered as closely related processes (see also Chapter 5). The results of Fig. VII.8., however, show an unimpaired phototropic reaction by seedlings, the growth of which was considerably reduced according to Table VII.10. Moreover, no difference could be detected between the growth rates of seedlings, pretreated with red light or red light and GA_3, during phototropic stimulation. This is further evidence against a direct relationship between rate of elongation growth and phototropic curvature.

8. GENERAL DISCUSSION

In Chapter 3 the extent to which pure phototropism can be studied before interference from geotropism occurs, has been investigated. It was concluded, that this is only possible during the first hour after the onset of curvature. Because of the variability in the onset of the response the experimental period had to be extended to two hours after the beginning of the phototropic stimulation in order to determine the individual maximal curvatures of the seedlings. In the study of seedlings pretreated with an irradiation with red, blue, or far red, these pretreatments might have influenced the geotropic response. The effect of red light on the geotropic response, described by HILD (1977), HUISINGA (1976), MCARTHUR and BRIGGS (1979), and WILKINS and GOLDSMITH (1964), however, is irrelevant in this matter, because seedlings pretreated with red light only show a poor phototropic curvature, so that they are not subjected to the geotropic stimulus. Irradiation with far red does not alter the geotropic response in coleoptiles, except for antagonizing the effect of red light (WILKINS and GOLDSMITH, 1964). Blue light decreases the geotropic response in coleoptiles of *Zea mays* (WILKINS and GOLDSMITH, 1964). In all these literature data the treated seedlings are compared with etiolated seedlings. ANKER (1962) wrote in his review on ortho-geotropism in shoots and coleoptiles that white light delays the geotropic response. Because the effect of red light is sometimes described as increasing geotropic curvature (HILD, 1977; MOHR and PICHLER, 1960; QUAIL and BRIGGS, 1978; TEPFER and BONNET, 1972), and sometimes as decreasing this response (MCARTHUR and BRIGGS, 1979; WILKINS and GOLDSMITH, 1964), it is hard to predict how sunflower seedlings will react, and therefore not possible to say whether the insignificant difference in bending between seedlings pretreated with blue and with white light is a result of a shift in geosensitivity.

Taking this into account, we may conclude that the present experiments show the proper phototropic responses of seedlings of the right age and size (Chapter 3). They confirm that the main actinic region is in the blue (Chapter 5), and demonstrate that the perception of the phototropic stimulus occurs within the responding organ, the hypocotyl (Chapter 3). Because the cotyledons are only involved as suppliers of nutrients and phytochrome responses (Chapters 4 and 6), the phototropic curvature does not require the basipetal translocation of a hormone. Moreover, as already shown by BRUINSMA et al. (1975), no asymmetric distribution of the endogenous auxin, IAA, could be detected between the illuminated and shaded halves of phototropically stimulated sunflower seedlings (Chapter 7).

These findings rule out the CHOLODNY-WENT theory as an explanation for the phototropic curvature of sunflower seedlings. Many experiments investigating the involvement of IAA in phototropism were done with exogenously applied IAA (see Chapter 1). It would be interesting to know if seedlings curving under the influence of unilateral blue light and seedlings curving due to the asymmetri-

cal application of IAA would show the same growth kinetics. It was already indicated by DU BUY and NUERNBERGK (1930), experimenting with *Avena* coleoptiles, that this is not the case. If their observation is true, this indicates that experiments done with exogenously applied IAA to prove the validity of the CHOLODNY-WENT theory might be based on an artefact. Actually, many of the graphs of DU BUY and NUERNBERGK (1929) show a cessation in growth at the illuminated side after phototropic stimulation. This points to the involvement of a growth inhibitor rather than that of a growth promotor. The older experiments on the distribution of endogenous auxin in phototropically curved organs have only been made with bioassays, that cannot distinguish between differences in contents of growth promoting and growth inhibiting substances.

The alternative theory on phototropic curvature, presented by BLAAUW (1915), explains the phenomenon as an unilateral inhibition of growth by light. If this would imply that the measure of phototropic curvature would depend on the rate of elongation growth, this theory, too, would be invalid to describe the present experiments. For instance, we showed that seedlings cultured for 18 hours in white, blue, or red light, or in darkness, show different growth rates, whereas the bending falls into two distinct groups (Chapter 5). It is remarkable that the two extremes in growth rate, namely the etiolated ones and the ones pretreated with red light, show no or little curvature. Apparently blue light not only serves as the actinic region but is also required to bring the seedlings in a responsive state (Chapter 5). BLAAUW did his experiments with white light, not excluding other wavelengths from the blue. Further evidence for the independence of curvature from elongation growth is furnished by the experiments involving gibberellic acid, GA_3. Seedlings pretreated with red light and GA_3 do show phototropic curvature, in contrast to seedlings pretreated with red light only. No difference in growth rate could be measured, however (Chapter 7). A pretreatment of irradiation with far red reduced the bending and again, no change in growth rate could be detected (Chapter 6). Seedlings grown in white light and pretreated with the inhibitor of gibberellin biosynthesis chlormequat, CCC, show a decrease in growth, but no difference in curvature (Chapter 7).

However, althought curvature requires elongating cells, the measure of curvature need not necessarily be correlated with the rate of growth if it is a matter of inhibition. Whereas the rate of cell elongation is governed by auxin and gibberellin, (a) growth-inhibiting substance(s) may reduce this rate to an extent relatively independent of this rate. Growth at the irradiated side may even be completely arrested (FRANSSEN et al., in press), so that nutrients become available for an even accelerated elongation at the shaded side.

It has been frequently reported that illumination of plant parts induces occurrence of (a) growth-inhibiting substance(s) (BAYER, 1961; SHIBAOKA, 1961; HIGGINS and BONNER, 1974; THOMPSON and BRUINSMA, 1977; KESSLER and MUIR, 1978). In this thesis it is shown that this abscisin, xanthoxin, is asymmetrically distributed between the illuminated and the shaded halves of phototropically responding hypocotyls, more xanthoxin being present in the illuminated

than in the shaded half (Chapter 7). The amount of xanthoxin present in the phototropically sensitive seedlings pretreated in white or blue light, was sufficiently high to account for curvature, whereas etiolated seedlings, that are phototropically insensitive, contain far less (see also THOMPSON and BRUINSMA, 1977). Although the asymmetrical distribution of xanthoxin may well cause the bending further evidence is required, for instance, from a comparison between the xanthoxin levels in etiolated seedlings pretreated with white light, to render them phototropically sensitive, and vice versa (see Chapter 5). Also exogenous application needs further study, to test the hypothesis (Chapter 7). Both BRUINSMA (1976) and JACOBS (1959) remarked that experiments on endogenous levels of growth substances should go together with application of these substances to the plant, producing similar effects. If one of the systems fails, the hypothesis needs further consideration.

TAYLOR (1968) showed that xanthoxin can be derived from carotenoids *in vitro*. WALLACE and HABERMANN (1959) described phototropic curvature in an albino mutant of *Helianthus annuus*, which lacks carotenoids. This mutant, however, contains a low amount of carotenoids and shows a delayed phototropic response (THIMANN and CURRY, 1960). PRESTI et al. (1977) found phototropism in *Phycomyces* mutants lacking β-carotene. The use of Sandoz preparations, inhibiting the formation of carotenoids (JABBEN and DEITZER, 1979), might be useful to give further information on the relationship between these substances and phototropism. However, the biosynthetic pathway of xanthoxin is still unknown and may also occur through ABA which is derived through the mevalonic pathway.

The facts that xanthoxin may play a direct role in phototropism, and that the perception of the stimulus occurs within the responding organ, still leaves the question whether flavonoids or carotenoids are involved in the perception of the stimulus open. There is as yet no evidence that the site of perception and the site of action are the same.

Seedlings pretreated with red light show only a poor phototropic response (Chapter 5), and yet may contain xanthoxin (Chapter 7). Irradiation with red or far red changes the membrane properties, namely leads to changes in transmembrane transport (SMITH, 1976). If red light prevents lateral movement of xanthoxin, the seedlings pretreated with red light would not be able to bend, although they contain xanthoxin. A similar, membrane-located phenomenon, could play a role after irradiation with far red, or after application of GA_3 to the seedlings. For a full understanding of these phenomena more experimentation is necessary.

SUMMARY

In this thesis the phototropic bending of hypocotyls of sunflower seedlings, *Helianthus annuus* L., is investigated.

Chapter 1 gives the reasons for this project. Although phototropism has been studied extensively over the past 100 years, the understanding of the mechanism is far from clear. During this century two main hypotheses were developed, namely the theory of BLAAUW (1915), explaining phototropism as an effect of growth inhibition by light, and the CHOLODNY-WENT theory (1927, 1928), in which the lateral distribution and basipetal transport of the growth-promoting substance, auxin, are involved. Especially the latter received the attention of many investigators, probably because it deals with the first phytohormone discovered. The research on this subject has been mainly concentrated on the properties of auxin and the way in which it influences the pattern of curvature in etiolated coleoptiles. Much experimentation has been done with the exogenous application of diffusate from coleoptile tips or IAA, the auxin, to etiolated monocotyledonous seedlings, without measuring the endogenous levels of this growth substance in the plants themselves. In 1975 BRUINSMA et al., investigating the levels of IAA in illuminated and shaded halves of phototropically stimulated, green sunflower seedlings, showed that this hormone is equally distributed between both halves. This discovery, and the fact that the evidence for both hypotheses mentioned above is still inconclusive, led to the present investigation.

As experimental material sunflower seedlings, *Helianthus annuus* L., were chosen. Chapter 2 lists the material and methods used for the experiments described in the subsequent Chapters.

Chapter 3 describes the influence of other processes on phototropism. It is shown that geotropism acts strongly on the deflected hypocotyl. Only the first hour after the beginning of the phototropic response can be considered to show pure phototropism. Because the delay in the onset of the response, the maximal degree of curvature is determined for each individual seedling during the first 2 hours of phototropic stimulation. The influences of the age of the seedling and length of the hypocotyl were studied as well. Seedlings of 4 to 5 days old and 40 to 60 mm long bend as uniformly as possible, and were used in the experiments described in this thesis. Careful manipulation of the seedlings did not influence the response.

Chapter 4 deals with the influence of the other organs of the green seedling on the bending of the hypocotyl. It is concluded that neither the cotyledons and the shoot tip, nor the roots are essential for the response, although a stress effect due to the removal of these organs may cause a delay. The epidermis of the hypocotyl is indirectly involved; the peripheral layers are not necessary for the perception of the unilateral light, but probably control the growth, without which curvature cannot occur.

In Chapter 5 the results of treatments with different wavelengths are shown.

Unilateral blue light is the main active region, although the response caused by blue light is always slightly less than when caused by unilateral white light. In contrast to seedlings grown in white light, etiolated ones are not phototropically sensitive. Pretreatment of dark-grown seedlings with white or blue light renders them able to curve, and only illumination of the hypocotyl is necessary. The role of the cotyledons, that cannot be removed during this treatment without decreasing the bending, is probably to supply the substrates essential for the growth of the hypocotyl. Pretreatment with darkness of seedlings grown in white light makes them phototropically unresponsive. Again, only the hypocotyl needs this treatment and this indicates that the mechanism for phototropism is located within the hypocotyl. Green seedlings pretreated with red light only show poor curvature, but the growth rate of the seedlings is not related to the bending capacity.

Chapter 6 presents the results of the effect of pretreatment with far-red irradiation on the phototropic response. It is shown that far red, more than a treatment in darkness, decreases the curvature in seedlings grown in white light and that the response is a low-energy reaction (L.E.R.). Red light did not affect the lack of bending of etiolated plants, but prolonged red illumination could reverse the effect of far-red irradiance in green seedlings. It is concluded that the perception of the far-red irradiance is located in both the hypocotyl and the cotyledons. Again, no correlation between the growth rate and the curvature of plants pretreated with far red can be detected.

The involvement of hormones in the phototropic response is studied in Chapter 7. IAA is not unilaterally distributed in curving parts of the seedlings that were 30 to 45 min phototropically stimulated. No IAA could be detected in diffusates from the seedlings, and this absence is not due to destruction of IAA at the cut surface. However, a growth-inhibiting activity was found in the diffusates, both from hypocotyls and from cotyledons, and this activity was not asymmetrically distributed in bending plants.

The role of the growth inhibitor, xanthoxin, was investigated. Experiments are described to show that xanthoxin is not contaminated with ABA after the extraction procedure, and that a bioassay can be properly used for the determination of xanthoxin. In straight hypocotyls xanthoxin is equally distributed, but in curving and curved ones more inhibitor can be extracted from the illuminated side than from the shaded one. From seedlings pretreated with white or blue light, which are phototropically sensitive, a higher amount of xanthoxin can be extracted than from etiolated, phototropically unresponsive, plants. In seedlings pretreated with red light, which show only a poor bending, a variable amount of xanthoxin was detected. The results of experiments in which xanthoxin was exogenously applied to seedlings were inconclusive.

Gibberellic acid, GA_3, applied to the seedlings pretreated with red light 1 hour before the onset of the phototropic experiment, increases the curvature, whereas GA_3 applied to plants grown in white light has no effect. The growth rate of the treated plants does not differ from that of the control group. Seedlings treated daily with various concentrations of the growth retardant CCC show different

lengths after four days. Their phototropic curvature, however, is the same, again demonstrating that the extent of curvature is not related to the elongation growth.

In the General Discussion (Chapter 8) the CHOLODNY-WENT theory is rejected as an explanation of phototropism in the sunflower seedling. Instead the theory of BLAAUW is modified by ascribing the phototropic reaction to a growth inhibition at the irradiated side, caused by light-induced relative accumulation of xanthoxin at that side. This may account for a curvature independent of the growth rate regulated by auxin and gibberellin.

SAMENVATTING

Positieve fototropie, het zich richten van planten of plantedelen onder invloed van licht naar deze stimulus, is al bekend van voor Christus (MÖBIUS, 1937).

Het onderzoek omtrent fototropie is in de vorige eeuw goed op gang gekomen, mede door de ontwikkeling van de andere natuurwetenschappen. Uit het werk van vele onderzoekers aan het eind van de vorige en begin deze eeuw, waarvan vooral DARWIN en SACHS genoemd moeten worden, zijn twee theorieën ontwikkeld, nl. die van BLAAUW (1915) en die van CHOLODNY (1927) en WENT (1928). BLAAUW verklaarde fototropie als een groeieffect, waarbij licht de groei van de plant remt. Wanneer eenzijdig belicht wordt, wordt de plant slechts aan één zijde geremd in groei waardoor een verschil in groeisnelheid ontstaat resulterende in kromming. In de CHOLODNY-WENT theorie wordt auxine, een groeibevorderend hormoon, onder invloed van eenzijdige belichting asymmetrisch verdeeld in de top van de plant, en vervolgens basipetaal getransporteerd. Deze theorie is opgesteld na onderzoek aan geëtioleerde coleoptielen. De aandacht van het latere onderzoek is grotendeels uitgegaan naar deze laatstgenoemde theorie, waarschijnlijk omdat deze de ontdekking van het eerste plantehormoon met zich meebracht. Veel experimenten zijn uitgevoerd door diffusaat uit coleoptieltoppen, en in een later stadium het auxine indolazijnzuur, IAA, asymmetrisch toe te dienen aan kiemplanten en op deze manier kromming te induceren. Met behulp van radioactief IAA werd bepaald hoe dit exogeen toegediende IAA zich verdeelt in de kiemplant. Hierbij werd dikwijls vergeten dat diffusaat, naast auxine, ook andere stoffen kan bevatten, b.v. een groeiremmer, en dat exogeen toegediend IAA niet dezelfde eigenschappen hoeft te hebben als de IAA moleculen in de plant zelf.

Alhoewel deze theorie ontwikkeld is voor geëtioleerde coleoptielen, wordt ze ook gebruikt om fototropie te verklaren in bijvoorbeeld dicotyle groene planten. Het endogene IAA in groene zonnebloemkiemplanten wordt echter niet asymmetrisch verdeeld onder invloed van eenzijdige belichting (BRUINSMA et al., 1975). Deze ontdekking, en het nietsluitende bewijsmateriaal voor beide bovengenoemde theorieën vormde de aanleiding tot het hier beschreven onderzoek (Hoofdstuk 1).

Het onderzoek is verricht aan kiemplanten van de zonnebloem, *Helianthus annuus* L. In hoofstuk 2 worden de kweekwijze van de planten en de methoden en technieken gebruikt in de experimenten beschreven.

Hoofdstuk 3 geeft de resultaten van de interferentie met andere processen die een rol spelen tijdens de fototrope kromming. Zodra de kiemplanten fototroop krommende zijn, worden zij onderworpen aan een geotrope prikkel, die de fototrope kromming tegenwerkt. Zuivere fototropie kan slechts bestudeerd worden tot een uur na het begin van de fototrope reactie. Omdat deze reactie bij individuele kiemplanten soms vertraagd begint werd van elke kiemplant de maximale kromming gedurende twee uur na het begin van de fototrope belichting bepaald.

De kromming is mede afhankelijk van de leeftijd en lengte van de kiemplanten. Kiemplanten met een leeftijd van 4 tot 5 dagen en een lengte van 40 tot 60 mm werden het meest geschikt bevonden. Het manipuleren, mits gedaan met grote zorgvuldigheid, had geen effect op de kromming.

De rol, die de andere organen van de kiemplant spelen in de fototrope kromming van het hypocotyl, wordt bestudeerd in Hoofdstuk 4. De cotylen en de top, evenals de wortels, blijken geen essentiële rol te spelen in de krommingsreaktie, indien direct verwijderd voor het begin van de eenzijdige belichting, alhoewel een stress effect als gevolg van het verwijderen van deze organen een vertraging in de response veroorzaakt. De epidermis van het hypocotyl speelt geen rol bij de perceptie van de fototrope stimulus; wel zijn de buitenste cellagen betrokken bij de groeiregulatie van het hypocotyl en oefenen op deze manier indirect hun invloed op de fototrope reaktie uit.

Hoofdstuk 5 behandelt het effect van licht van verschillende golflengten. In dit Hoofdstuk is bevestigd dat eenzijdige belichting met blauw kromming veroorzaakt, hoewel planten fototroop geprikkeld met wit in ieder experiment iets beter kromden dan wanneer gestimuleerd met blauw licht. In tegenstelling tot kiemplanten opgekweekt in wit licht, vertonen die opgekweekt in donker geen kromming onder invloed van eenzijdige belichting. Wanneer deze geëtioleerde planten gedurende een bepaalde periode worden voorbehandeld met een alzijdige belichting met wit of blauw en vervolgens eenzijdig belicht, reageren zij wel. Een tijdsduur van 18 uur blijkt nodig voor een goed waarneembare kromming. Alleen het hypocotyl moet gedurende deze periode belicht worden; belichting van de cotylen heeft geen effect hoewel deze niet verwijderd kunnen worden. Het omgekeerde effect treedt ook op: het plaatsen van groene kiemplanten in donker gedurende 18 uur maakte deze ongevoelig voor de fototrope stimulus. Ook hier zijn alleen de hypocotylen gevoelig voor de behandeling. Een behandeling met rood licht, gegeven voor de eenzijdige belichting, heeft slechts een zeer geringe kromming ten gevolge. De groeisnelheid van de planten tijdens de eenzijdige belichting is niet gerelateerd aan hun vermogen tot kromming.

Het vermogen te krommen van planten, opgekweekt in wit licht, blijkt duidelijk afgenomen na een bestraling met verrood, meer dan na een donkerbehandeling (Hoofdstuk 6). Dit effect is een lage-energie reaktie, L.E.R., d.w.z. dat slechts een korte periode bestraald hoeft te worden om een effect te veroorzaken. Wel blijkt een induktieperiode nodig: een bestraling van 1 min heeft geen invloed, een bestraling van 1 min gevolgd door een 2 uur durende donkerperiode geeft een duidelijk effect. Dit verrood effect kan teniet gedaan worden door een bestraling met rood licht. Rood licht zelf heeft geen effect op het krommingsvermogen van geëtioleerde kiemplanten. Verrood wordt zowel door de cotylen als door het hypocotyl gepercipieerd. Wederom kan geen verband gelegd worden tussen de krommingsreaktie en de groeisnelheid.

De rol van hormonen in de krommingsreaktie is bestudeerd in Hoofdstuk 7. IAA blijkt niet asymmetrisch verdeeld in fototroop krommende hypocotyldelen. In diffusaten van kiemplanten werd geen IAA aangetoond, en dit is niet het

gevolg van afbraak van deze stof aan het wondvlak. Wel wordt de aktiviteit van aan diffusaten toegevoegd IAA geremd. Deze remmende werking wordt echter zowel gevonden in diffusaten van hypocotylen als ook van cotylen, en blijkt gelijkelijk verdeeld in diffusaten uit de belichte en de schaduwhelften van krommende planten.

De groeiremmer xanthoxine is niet asymmetrisch verdeeld in niet gestimuleerde, ongekromde kiemplanten, maar duidelijk asymmetrisch verdeeld in fototroop gestimuleerde, krommende en gekromde kiemplanten. Meer xanthoxine wordt geëxtraheerd uit de belichte dan uit de schaduwhelften. Onderzocht werd of het krommingsvermogen gerelateerd is aan de totale hoeveelheid xanthoxine in de planten. Geëtioleerde kiemplanten, voorbehandeld met een belichting van bovenaf met wit of blauw, dus in staat tot kromming, bevatten meer xanthoxine dan geëtioleerde planten, ongevoelig voor de fototrope stimulus. De hoeveelheid xanthoxine in met rood licht voorbehandelde planten, in geringe mate tot kromming in staat, was variabel. Het induceren van kromming in kiemplanten door toediening van xanthoxine had geen resultaat.

Zoals reeds vermeld, krommen planten voorbehandeld met rood licht slechts in geringe mate. Wanneer echter aan deze planten gibberellinezuur, GA_3, wordt toegediend één uur voor de fototrope stimulus, wordt het krommingsvermogen herwonnen. GA_3 toegediend aan normale, lichtgekweekte planten heeft geen effect op de kromming. Tussen de groeisnelheden van rood en rood + GA_3 voorbehandelde planten wordt geen verschil gevonden. De remmer van de gibberelline biosynthese, chlormequat, toegediend aan lichtgekweekte planten, geeft een duidelijk verschil in lengtegroei te zien met de controle groep, maar de fototrope gevoeligheid van beide is gelijk.

In Hoofdstuk 8 wordt de CHOLODNY-WENT theorie verworpen als verklaring voor fototropie in de zonnebloemkiemplant. In plaats hiervan is de theorie van BLAAUW uitgebreid door de fototropie reactie toe te schrijven aan een groeiremming aan de belichte zijde, veroorzaakt door een door licht geïnduceerde, relatieve ophoping van xanthoxine in die zijde. Dit zou een kromming onafhankelijk van de groeisnelheid, gereguleerd door auxine en gibberelline, verklaren.

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