PHOTOTROPISM IN LIGHT-GROWN MUSTARD (*SINAPIS ALBA* L.) SEEDLINGS

A thesis submitted for the degree of Doctor of Philosophy,
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QUOTATIONS

"Consistency is the last resort of the unimaginative"

Oscar Wilde

but

"Consistency is all I ask!"

Tom Stoppard

ABSTRACT

This thesis is an investigation of the mechanisms of blue-light-mediated phototropism in higher plants. Phototropism was analysed in light-grown mustard (Sinapis alba L.) seedlings under low pressure sodium (SOX) lamps to minimise the involvement of phytochrome. Light-grown mustard seedlings do not show a blue-light-mediated inhibition of axis extension growth and the Blaauw theory must therefore be rejected as an explanation of phototropism. Phototropic curvature was established by an inhibition of growth on the illuminated side of the hypocotyl accompanied by an equal but opposite acceleration on the shaded side, with little or no change in net growth. This pattern of differential growth can be modified by light-growth responses separate from those involved in phototropism. Preliminary results from a biophysical analysis of cell growth using micro-pressure probes indicates phototropic growth responses are caused by changes in wall rheological properties, possibly by wall extensibility alone. There is a complex relationship between the kinetics of phototropism and stimulus fluence rate. The lag time is independent of fluence rate. The initial rate of curvature was directly proportional to log fluence rate between threshold and saturation fluence rates. Following this initial phase, gravitropic compensation and autotropic straightening modify curvature, which can no longer be used as a quantitative measure of phototropism. Implications for measurement of the response and selection of appropriate photobiological conditions are outlined. The magnitude of the internal light gradient influences the rate of curvature. The experimental results are discussed in relation to other knowledge of the phototropic transduction chain. The results are not inconsistent with the Cholodny-Went theory of phototropism. Preliminary data indicating phytochrome-mediated phototropism are presented, and the comparison of lag times in plant physiology is also discussed.

LIST OF CONTENTS

ABBREVIATIONS AND SYMBOLS

B	Blue light
C	Curvature proportionality constant
FR	Far-red light
K	Transmission coefficient
L	Hydraulic conductance
O	Orange light
P	Cell turgor pressure
Pfr	Far-red light absorbing form of phytochrome
Pr	Red light absorbing form of phytochrome
Ptot	Total amount of phytochrome
Pwall	Hydrostatic pressure of cell wall
ref.	Fluence rate of reference light source
SOX	Low pressure sodium lamp light
s.p.d.s.	Spectral photon distribution scan
UV	Ultra-violet light
V	Volume of cell
V_o	Volume of cell at zero turgor pressure
var.	Fluence rate of variable light source
WL	White light
Y	Yield threshold
$\overline{II}_{cell}$	Osmotic potential of cell
$\Delta\ \overline{II}$	Difference in osmotic pressure between cell and water source
σ	Solute reflection coefficient
ϕ	Apparent wall extensibility
$\bar{\phi}$	Phytochrome photostationary state
ψ	Water potential

PREFACE

The research presented in this thesis, with the exception of Chapter 4, has been carried out under the supervision of Professor H. Smith in the Botany Department, University of Leicester between September 1983 and August 1986. The work in Chapter 4 was carried out in collaboration with, and under the direct supervision of, Dr A.D. Tomos in the Department of Biochemistry and Soil Science, University College of North Wales, Bangor during Spring 1986.

The general plan of this thesis is based on publication of the results chapters. Chapter 2 has already been published (Rich, Whitelam & Smith, 1985). Chapters 3, 4, 5 and 6 are either in review or in preparation. Appendix II is in press (Rich & Smith, 1986) and as not directly related to phototropism, has been left essentially in its published format. The results in Appendix I are not publishable but are considered to be of sufficient interest to merit inclusion.

CHAPTER 1

INTRODUCTION, DEFINITIONS AND SCOPE

This thesis is primarily concerned with the mechanisms of blue-light-mediated phototropism in higher plants.

Phototropism is one of a number of light-induced plant movements. The terminology of these movements is imprecise with words being used in a variety of rather loose senses, the term phototropism itself being no exception. In the absence of widely accepted, precise definitions, the situation is simplified here by dividing the movements into four categories according to whether they require a directional light stimulus, and to whether they are mediated by differential growth or turgor changes in the plant; similar responses can then be grouped together irrespective of their nomenclature:-

1. Turgor-mediated movements which do not require a directional light stimulus (e.g. nyctinasty, *sensu* Satter 1979; leaflet movement in *Oxalis*, Bjorkman & Powles, 1981).
2. Differential growth-mediated movements which do not require a directional light stimulus (e.g. epinasty, *sensu* Kang, 1979).
3. Turgor-mediated movements which require a directional light stimulus (e.g. solar tracking in *Lavatera*, Schwartz & Koller, 1978; and in *Lupinus*, Vogelmann & Bjorn, 1983).
4. Differential growth-mediated movements which require a directional light stimulus (e.g. phototropism in *Avena* and *Phycomyces*, *sensu* Dennison, 1979).

The term phototropism is used here specifically only to include the latter category of movements, and is hence defined more specifically as a directional growth response to a directional light stimulus (Smith, 1975). The term

'growth' requires further qualification as it is also used in a variety of ways; here it is considered to be primarily an increase in cell length. It should be noted other authors do not necessarily use these definitions; for instance, Schwartz & Koller (1978) describe their turgor-mediated solar tracking as phototropism. For the purposes of this thesis, only phototropic responses conforming to the above definition are considered.

Directional growth responses to directional light stimuli have been reported in many plant groups including fungi, algae, bryophytes, pteridophytes and higher plants, and it is evident a number of different mechanisms operate. Photoregulation of growth movements may be exerted through three information-transducing photoreceptors (which are themselves probably groups of similar pigments) independent of the photosynthetic pigments, which, however, provide the energy needed for growth and development. The two main information-transducing photoreceptors are phytochrome and a blue-light-absorbing pigment (Smith, 1982) and there is also evidence of a specific UV-light-absorbing photoreceptor (e.g. Hashimoto & Tajima, 1980; Hashimoto et al., 1984; Steinmetz & Wellmann, 1986) which has not yet been characterised in detail. Growth itself may also be of two types; it may either involve a uniform expansion of the entire cell wall (e.g. Avena coleoptiles, Castle, 1955) or be restricted to the tip of the growing cell (e.g. bryophyte protonemata, Haupt, 1965).

In fungal sporangiophores (Page, 1968; Dennison, 1979) phototropic responses can be induced by blue and UV light. Extension growth of the chitin cell wall is related to the amount of light such that the more light, the faster the growth rate. Hence a difference in light quantity established across the sporangiophore by a directional light stimulus (ie. unilateral light) results in localised differential growth causing curvature of the

organ, and hence lateral movement. In blue light there is a strong focusing effect within the sporangiophore (Shropshire, 1962) which results in the concentration of light on the far side of the sporangiophore and curvature occurs towards the light stimulus. In UV-light there is strong attenuation by the tissue; less light reaches the far side and curvature occurs away from the light source.

In the protonemata of bryophytes which grow exclusively from the tip, a different mechanism operates (Hartmann, 1984). Unilateral irradiation of the apical region causes a reorientation of the growth centre towards the light which results in a swelling and bulging of the tip followed by a redirection of growth towards the light source. Responses in bryophytes are usually phytochrome-mediated (Nebel, 1968; Hartmann et al., 1983) but in one liverwort at least (Steiner, 1967), responses are mediated by a blue-absorbing photoreceptor. In general the phototropic responses in ferns are similar to those in bryophytes, but differ in that blue light is usually also effective in addition to phytochrome (Etzold, 1965; Kadota et al., 1982). In both these groups the directional light stimulus is detected by a dichroic orientation of photoreceptors on the plasma membrane.

In the multicellular tissue of higher plants there are different mechanisms again. Phototropic responses to blue and UV light have been extensively investigated (for recent reviews see Galston, 1959; Thimann & Curry, 1960; Briggs, 1963; Curry, 1969; Dennison, 1979; Firn & Digby, 1980; Gressel & Haupt, 1983; and also Firn, 1983; Dennison, 1984; Pickard, 1985; Firn 1987) and it has recently been demonstrated that phytochrome can also mediate phototropic responses (Iino, Briggs & Schafer, 1985). However, it is evident that the phototropic responses of higher plants are very complex and there are numerous gaps and inconsistencies in our knowledge of the mechanisms involved:

we simply do not know how phototropism is brought about. Firn & Digby (1980), in their critical review of the establishment of blue-light-mediated phototropic curvatures in plants, pointed out that there are simply insufficient data available from which to produce an adequate description of phototropism in higher plants. This review was the starting point of the work documented in this thesis; the aim therefore was to characterise in detail the blue-light-mediated phototropic responses of a single higher plant species in order to provide the necessary "firm foundations" (Firn & Digby, 1980) on which to build theories and test hypotheses about the mechanisms involved. Five main problems were therefore selected to provide pertinent information relating to the development of the phototropic response.

First, what is the form of differential growth? Firn & Digby (1980) pointed out that there are five potential ways of achieving differential growth, and that any mechanism must be capable of producing a pattern of differential growth consistent with that measured in the responding organ. Inconsistencies between predicted and observed patterns of differential growth can be used to distinguish between models, and this approach has been used to test the Blaauw theory of phototropism in Chapter 2. Analysis of growth can also provide information relating to where curvature takes place in the organ and to whether the cells behave in a coordinated fashion or not.

Although patterns of differential growth can be used in this way for particular experimental conditions, the variety of published results suggest caution in interpreting their significance on a wider scale. Different patterns of differential growth have been reported in the same species on a number of occasions e.g. Avena; Franssen et al., (1981) versus Curry, (1969); Zea, Franssen et al., (1981) vs. Iino & Briggs (1984); Lepidium, Franssen et al., (1981) vs. Hart, Gordon & MacDonald (1982); Sinapis alba, Franssen et al., (1981) vs. Rich et al., (1985). It is not possible,

therefore, to draw clear conclusions about what growth rate changes actually occur specific to phototropism. Macleod et al. (1986) even concluded that phototropism was not caused by a consistent pattern of differential growth. In Chapter 3 the pattern of differential growth has been examined in mustard using two different techniques to investigate possible explanations of the inconsistencies in published data.

Second, which biophysical parameters of cell growth change to cause the phototropic growth response? Cell extension growth is generally accepted to be due to yielding of the cell wall in response to turgor pressure (Ray, Green & Cleland, 1972), and the rate of growth is usually described as a function of four biophysical parameters relating to wall extension and water uptake. The differential growth rates that give rise to curvature are themselves caused by changes in the biophysical parameters describing cell extension, but exactly which parameter(s) change(s) has not been investigated for phototropism. Knowledge of the changes would be a useful advance in understanding the mechanisms as any potential mechanism must again predict biophysical changes consistent with those observed. For instance, as Cosgrove (1985) showed the effect of auxin on straight extension growth rate can be fully accounted for by changes in wall extensibility, a phototropic mechanism involving auxin would also therefore be expected to be mediated by changes in wall extensibility. The results of a preliminary investigation of the biophysics of phototropic growth are presented in Chapter 4.

Third, what are the kinetics of phototropism? Kinetics can provide useful information about the development of the response because any potential mechanism must be able quantitatively to account for the observed time courses. For instance, a comparison of lag times is a widely used means of determining potential mechanisms involved in a response, but

due to the poor statistical basis of most estimates of lag times in plant physiology (Appendix II), care must be taken in interpreting their significance. Firn & Digby (1980) cite the discrepancy between lag times and auxin action as evidence against the Cholodny-Went theory, but at present there are simply insufficient data to draw firm conclusions. Indeed, the only true mean lag times (sensu Appendix II) reported for both phototropism and auxin-induced growth in the same tissue are 22 minutes (Baskin et al., 1985) and 16 minutes (Kutschera & Schöpfer, 1985) in maize which are not inconsistent with the Cholodny-Went theory. Although some phototropic lag times (e.g. Ellis, 1984) are faster than the currently accepted lag times for auxin-induced growth (e.g. Evans 1974, 1985), measurements of both responses must be made in the same tissue before the Cholodny-Went theory can be assessed on this basis in more detail. A further criticism of hormonal explanations of phototropism based on comparative kinetics by Firn & Digby (1980), i.e. that "there is no convincing evidence that large enough gradients of any known hormone are established during the latent period", is misleading because the lack of convincing evidence is simply due to the fact that nobody has measured the redistribution within the lag time (e.g. Gressel & Horwitz 1983). This is more a criticism of scientists than theories, but again, more information is required.

A fourth problem to be investigated relates to how the light dose affects the response kinetics. The principal importance of analysis of different light treatments is to establish conditions within which other experiments should be designed; for example, investigation of reciprocity or action spectra must be carried out under appropriate photobiological conditions. The relationship between dose and response can also provide information on which steps in the transduction chain are influenced by light, which is also of importance in building a model of the response.

Fifth, what is the importance of the light gradient? The light gradient plays a key role in phototropism because it determines the direction of the response and influences the extent of curvature. It was selected for study here primarily because a number of recent studies have described the characteristics of the light gradient but there have been no attempts to relate the gradient back to the phototropic response. Hence in Chapter 6 an attempt was made to draw together the physical measurements with physiological responses to investigate how the plant integrates the light signal.

An additional problem relates to phytochrome and phototropic responses; for many years there has been an apparent anomaly as to why phytochrome regulates extension growth but does not also mediate phototropic responses. The relationship between phytochrome, extension growth and phototropism is investigated briefly in Appendix I.

CHAPTER 2

TESTING THE BLAAUW THEORY OF PHOTOTROPISM

INTRODUCTION

Firn & Digby (1980) critically reviewed the establishment of tropic curvatures in plants and compared the relative merits of the Blaauw and Cholodny-Went theories of phototropism. They concluded there were insufficient data in the literature to decide which theory provided the best description of phototropism, and that careful studies on the exact timing, location and magnitude of differential phototropic growth were needed to resolve the problem. Firn *et al*. (1983) pointed out that as the two theories predict different patterns of differential growth, analysis of the changes in growth rate that give rise to curvature should provide a means of distinguishing between them.

The Blaauw model of phototropism (Blaauw, 1914, 1915) was based on the relationship between straight extension growth rate and light quantity observed in *Helianthus*, such that the more light, the greater the inhibition of growth. He related extension growth to phototropism by suggesting that a gradient of light quantity across a plant axis should cause differential inhibition of growth and therefore curvature. The theory predicts that differential growth should be established by a large inhibition of growth on the illuminated side of the organ with less or no inhibition on the shaded side (Firn *et al*., 1983).

The Cholodny-Went theory of tropic curvatures in plants was proposed following the investigations of the role of an endogenous plant growth regulating substance (auxin) in coleoptile extension. The most explicit formulation of the theory is that of Went & Thimann (1937):

> "Growth curvatures, whether induced by internal or external factors, are due to an unequal distribution of auxin between the two sides of a curving organ. In the tropisms induced by light and gravity the unequal auxin distribution is brought about by a transverse polarization of the cells, which results in lateral transport of the auxin".

This theory predicts (Firn et al., 1983) that curvatures should be established by an inhibition of growth on the illuminated side of the axis accompanied by an acceleration on the shaded side.

As a phototropic stimulus ultimately leads to differential growth, the exact relationship between light treatment and axis extension is central to the mechanism of phototropism. In the majority of plant species examined to date, light control of axis extension growth has been shown to be mediated by two distinct photoreceptors, phytochrome and a blue-light-absorbing pigment (e.g. Shuttleworth & Black, 1977; Thomas & Dickinson, 1979; Gaba & Black, 1979). One exception to this general rule is de-etiolated mustard (Sinapis alba L.) seedlings, where hypocotyl growth is controlled by phytochrome alone (Wildermann et al., 1978; Thomas, 1980). As classical phototropism in higher plants is usually considered to be mediated by the blue-light-absorbing photoreceptor, this species presents an interesting opportunity to test the Blaauw theory of phototropism.

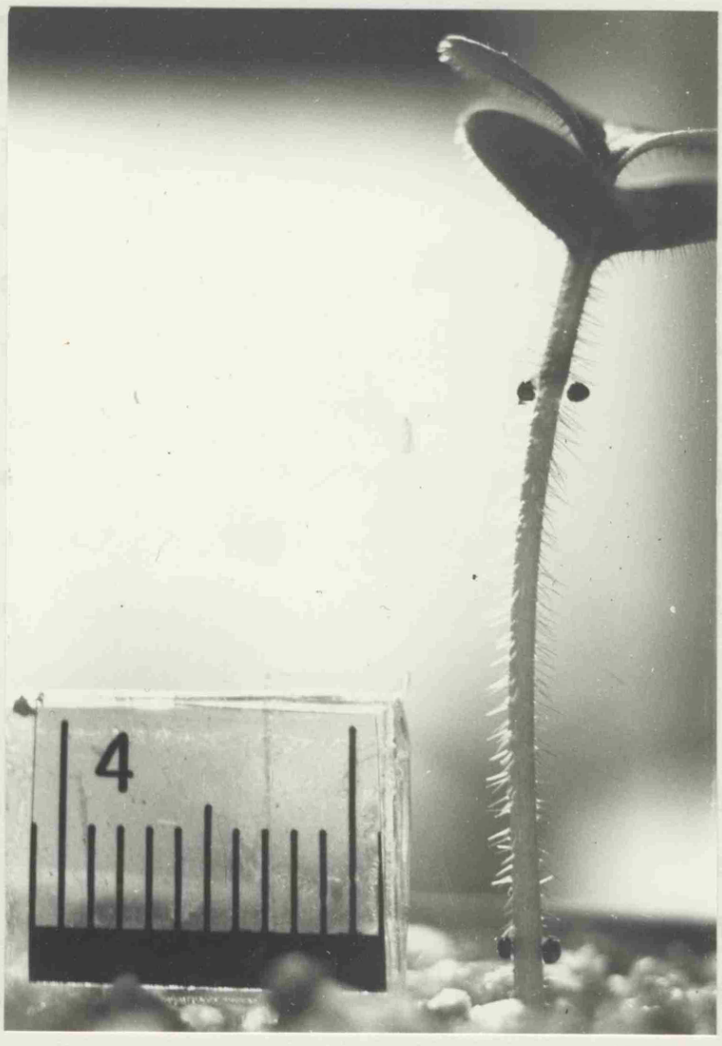
Because phytochrome absorbs blue light, however, any blue light treatment to induce phototropism will also affect phytochrome in the tissue. This may alter the axis growth rate, or perhaps even induce an additional phototropic response as Iino, Briggs and Schäfer (1984) have demonstrated phytochrome-mediated phototropism in maize (see also Appendix I). Experimental conditions should therefore be selected to minimise the involvement of phytochrome in the response. A suitable technique to achieve this in green plants is the low pressure sodium lamp (SOX) technique of Thomas & Dickinson (1979). The SOX lamp acts as a high intensity

pseudo-red light source in terms of the phytochrome response; it generates primarily narrow band radiation centred at 589 nm, which is preferentially absorbed by the red-light-absorbing form of phytochrome (Pr), resulting in the saturation of the photoconversion of phytochrome giving a similar photoequilibrium (ϕ) to that established by red light. Small amounts of blue light, which is considerably less efficient at photoconversion than SOX light (Pratt & Briggs, 1966), can then be added to the SOX background without significantly increasing the total fluence rate or changing the phytochrome photoequilibrium (Thomas & Dickinson, 1979). By selecting appropriate SOX and blue light fluence rates, it should therefore be possible to analyse the phototropic responses under conditions independent of phytochrome. The SOX lamp also provides a background of light for photosynthesis during the course of the experiments.

MATERIALS AND METHODS

Seeds of mustard (*Sinapis alba* L. subspecies *alba*; identification confirmed by the Botanical Society of the British Isles Cruciferae expert) were obtained from Asmer Seeds Ltd (Leicester). The same batch of seeds was used for all the experiments. Seeds were sown directly into small pots of moist, fine grade, horticultural vermiculite and grown in a constant environment room for 6 days under 113 μmol m^{-2} s^{-1} continuous, fluorescent white light at 20°C. Seedlings were watered each day with tap water. Seedlings were then selected for straightness of hypocotyl and uniformity of height (*c.a.* 1.5-2.0 cm), and the top and base of the hypocotyl marked with seeds of *Scrophularia auriculata* attached with stopcock grease (Dow Corning Ltd) (Plate 1). The seedlings were then transferred to an experimental room (20 ± 2°C) and left overnight in a background of SOX light (118 μmol m^{-2} s^{-1}) to equilibrate. During

PLATE 1

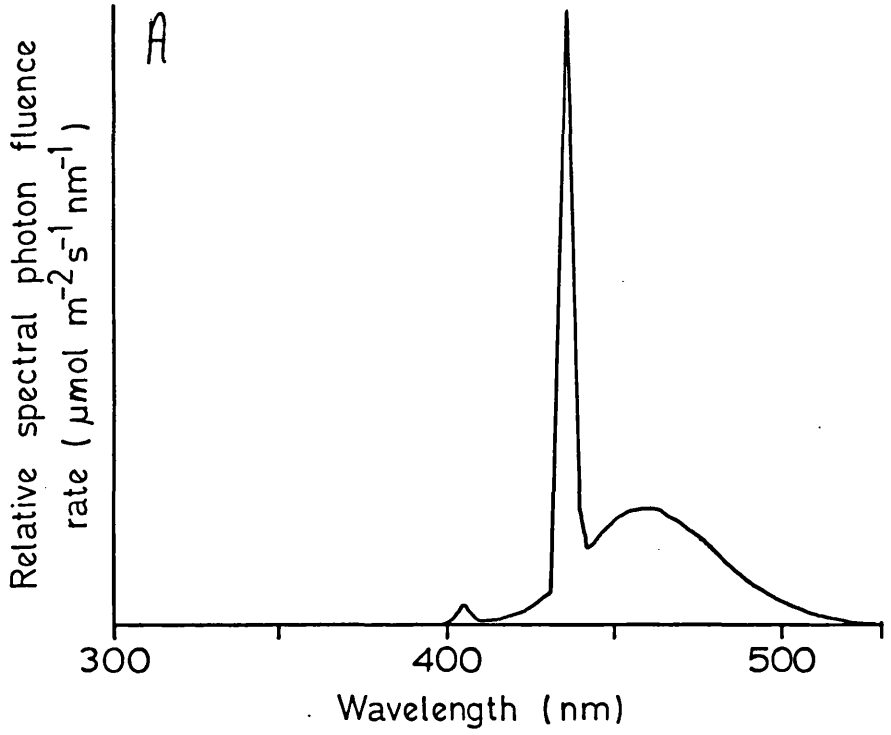


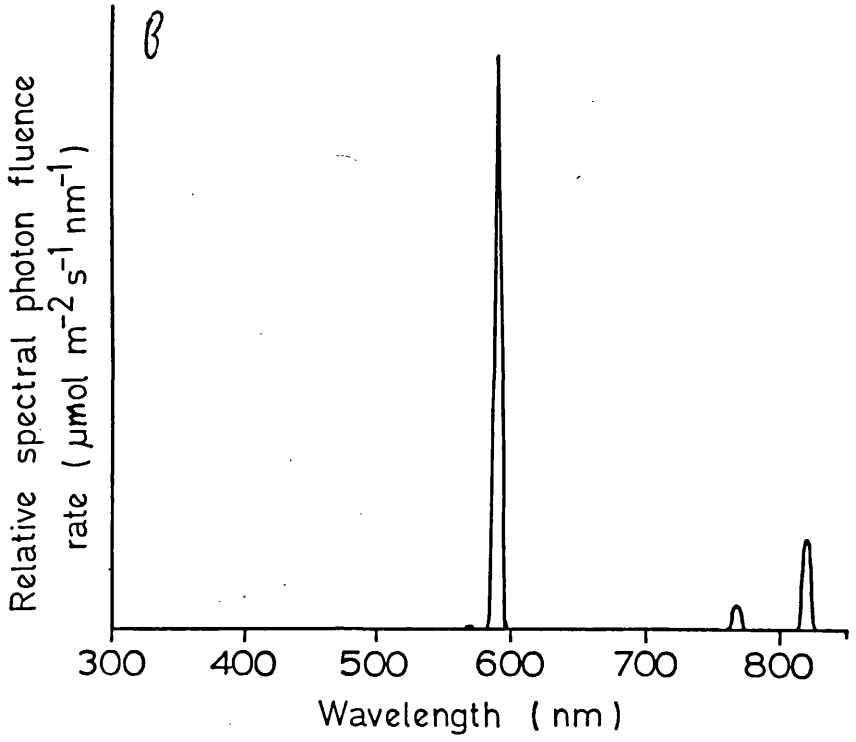
Mustard seedling with markers used to measure growth rates during phototropism. The 1 cm scale beside the plant was used to ensure standard enlargement of photographic negatives during analysis.

the experiments plants were placed in a shallow tray of water to maintain an adequate water supply. Treatment was started the following day, and plants were photographed every hour (the background SOX light providing sufficient light) and analysed from enlarged negatives. Continuous, low fluence rate blue light was added bilaterally (total fluence rate 1.72 μmol m^{-2} s^{-1}), or unilaterally (fluence rate 0.86 μmol m^{-2} s^{-1}). Plants were orientated such that the plane through the petioles of the cotyledons was perpendicular to the directional blue light vector. Curvature was measured as the angle between tangents drawn through the hypocotyl at the position of the markers; positive curvatures relate to movement towards the light source, negative curvatures, away. Growth rate was measured as the percentage elongation of the hypocotyl between the markers, standardised to the time at which treatment began. Best fit lines were applied to the prestimulation growth rates. Care was taken in all cases to eliminate stray light.

Background SOX light was obtained from a 135W Thorn low pressure sodium lamp filtered through 1 layer of no. 5 orange 'Professional Cinemoid' (Rank Strand, Brentford, Middx.). Unfiltered SOX light is contaminated with c.a. 0.13% blue (350-530 nm) light which is sufficient to cause a phototropic response at the fluence rates used (see Figure 45). Low fluence rate blue light was obtained from 40W Phillips TLAK Deluxe fluorescent tubes covered with 1 layer of primary blue Cinemoid. Light sources were measured with a LI-COR 1800 spectroradiometer (Li-Cor Inc., Lincoln, Nebraska, U.S.A.) calibrated against a standard spectral source, and regularly checked with a LI-COR 185 quantum sensor. Relative spectral photon distribution scans of light sources are given in Figure 1.

Figure 1. Spectral photon fluence rate distributions A Blue light source B Low pressure sodium lamp (SOX)


A
Relative spectral photon fluence rate (μmol m-2 s-1 nm-1)
300
400
500
Wavelength (nm)


B
Relative spectral photon fluence rate (μmol m-2 s-1 nm-1)
300
400
500
600
700
800
Wavelength (nm)

Phytochrome photostationary states established by the experimental light conditions were measured using Cucurbita pepo cv 'Mammoth' hypocotyl hooks on ice at 0°C. Photostationary states were calculated assuming ϕ_{max} = 0.86 (Vierstra & Quail, 1983) and these are assumed to reflect the physiological situation.

RESULTS

The SOX technique (Thomas & Dickinson, 1979) excludes the involvement of phytochrome in the responses as addition of low fluence rate light to the SOX background does not alter the photoequilibrium (Table 1), and only increases the total fluence rate by a maximum of 1.5%. Data for the blue light source alone are given for comparison; the discrepancy between measured and calculated photostationary states suggests blue light alone was unable to establish photoequilibrium at the fluence rate and temperature used for the measurement.

The effect of blue light treatment on hypocotyl extension growth is shown in Figure 2. Control plants with no blue treatment grew at a constant rate and did not curve. The results show that addition of unilateral or bilateral blue light causes no inhibition of net extension growth, confirming the results of Thomas (1980).

As mustard hypocotyl extension growth is unaffected by blue light, the Blaauw theory of phototropism predicts that the seedlings should show no curvature in response to a unilateral light stimulus. Figure 3, a time course for the development of curvature in response to unilateral blue light, shows the plants do respond. There is an initial high rate of curvature (the lag time is not included) followed by a marked slowing of the response at about 2 hours. This is not due to the onset of a response to the SOX light (c.f. Appendix I) because the same pattern of curvature development also occurs in unilateral blue light alone (data not presented). The kinetics of curvature are investigated further in Chapter 5.

Table 1. Phytochrome photoequilibria established under experimental light conditions.

TREATMENT	ϕ measured	ϕ calculated
SOX (118 μmol $m^{-2}s^{-1}$)	0.86	0.86
SOX + Blue (119.72 μmol $m^{-2}s^{-1}$)	0.86	0.86
Blue (1.72 μmol m^{-2} s^{-1})	(0.18)	0.34

Figure 2. Net increase in length of mustard hypocotyls in response to bilateral(means of 30 plants) and unilateral (means of 37 plants) blue light.

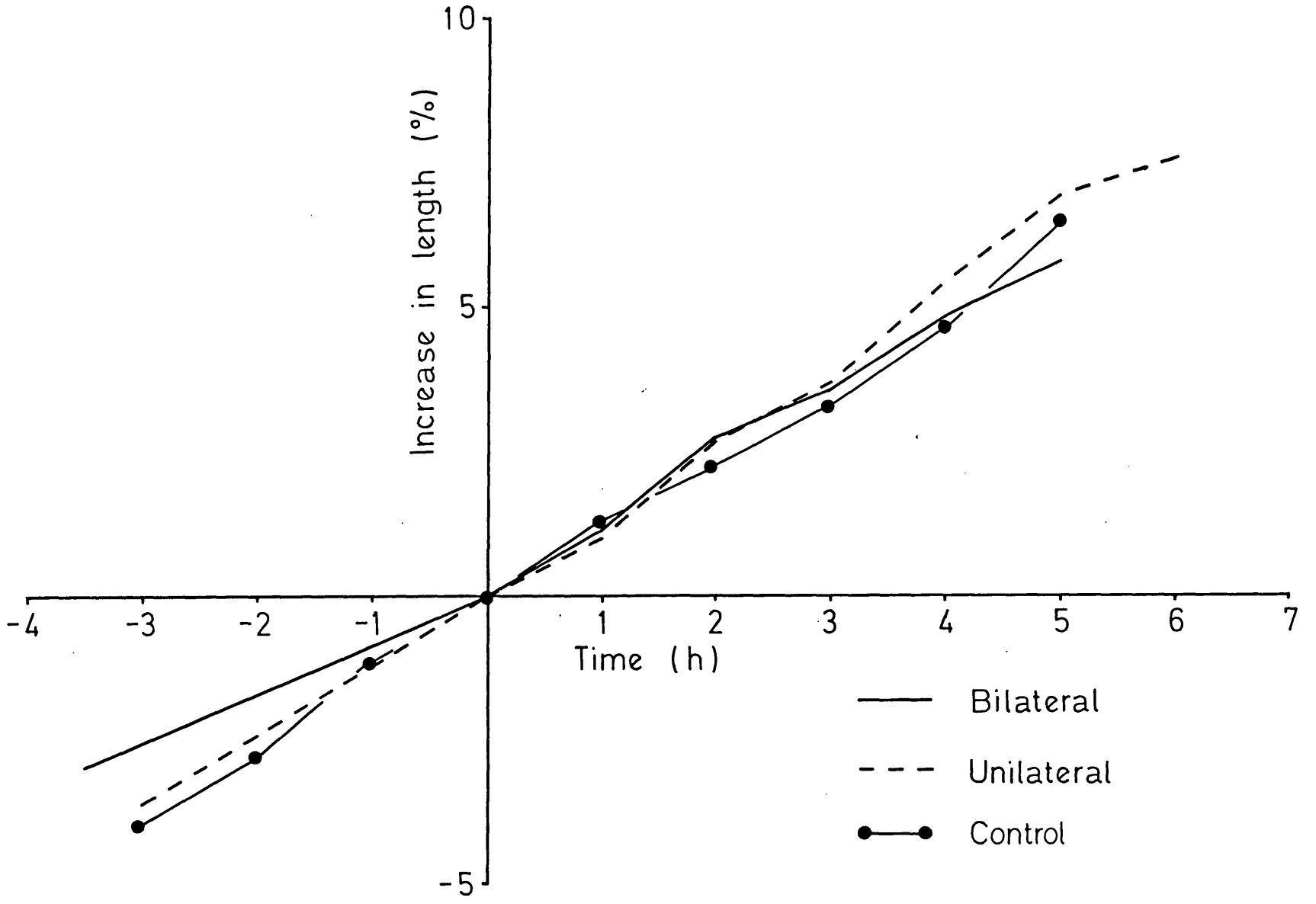
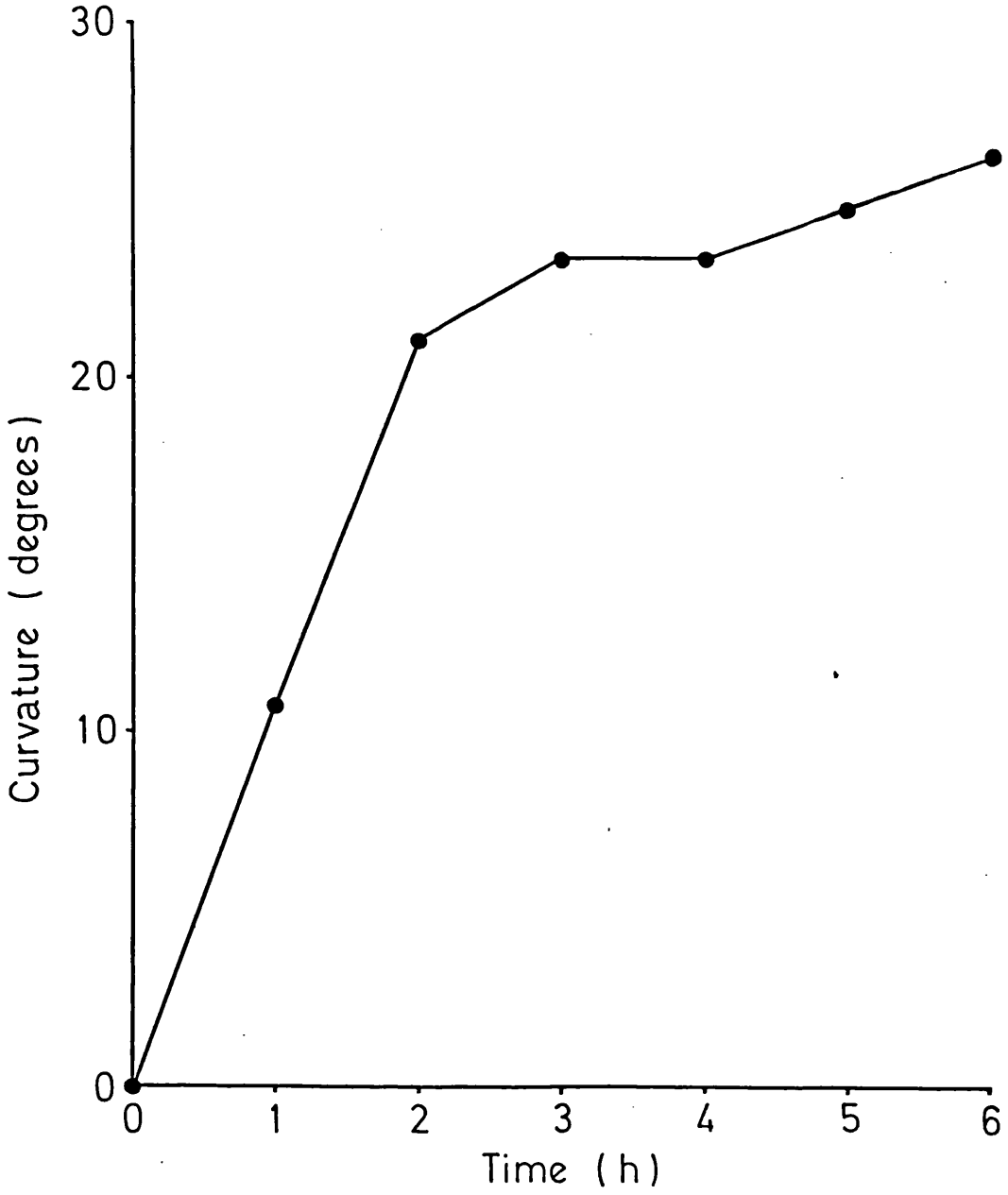

10
5
-5
Increase in length (%)
-4
-3
-2
-1
1
2
3
4
5
6
7
Time (h)
Bilateral
Unilateral
Control

Figure 3. Time course for development of phototropic curvature of mustard hypocotyls in response to unilateral blue light (means of 37 plants).


30
20
10
0
Curvature (degrees)
0
1
2
3
4
5
6
Time (h)

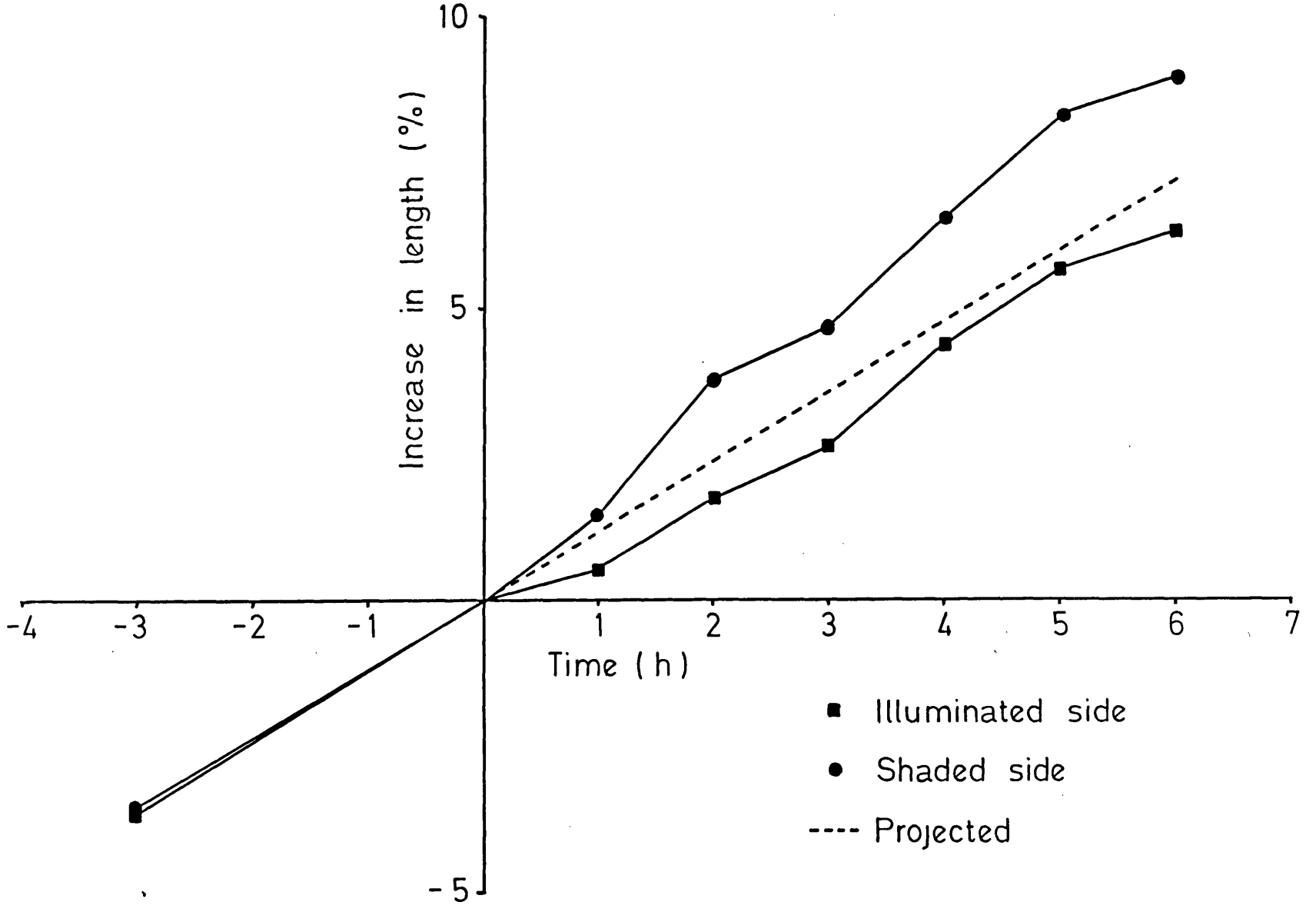
Measurement of the pattern of differential growth (Figure 4) shows that curvature is established by an inhibition of growth on the illuminated side of the hypocotyl accompanied by an acceleration on the shaded side, with little or no change in net extension growth rate (c.f. Figure 2). These results are inconsistent with the Blaauw theory of phototropism.

DISCUSSION

The SOX technique demonstrates that phototropism is mediated by a distinct, specific blue-light-absorbing photoreceptor. The fact that the response is expressed under conditions independent of phytochrome indicates that phytochrome is not directly involved in phototropism, but as it is well known to influence expression of the response (e.g. Curry, 1969; Dennison, 1979) phytochrome must be able to modify some steps in the transduction chain.

The results demonstrate that the Blaauw theory cannot account for phototropism in light-grown mustard seedlings under these experimental conditions, and therefore some other explanation must be found. The results are consistent with some predictions of the Cholodny-Went theory, but do not prove it is correct. The fact that phototropism occurs independently of inhibition of straight extension growth in mustard suggests they are not causally related, and therefore must be considered separate responses. Drumm-Herrel & Mohr (1985) also reached this conclusion for mustard, but did not support their conclusion with direct experimental data. Two other independent studies also concluded the Blaauw theory of phototropism is invalid. Macleod et al. (1985) used unequal bilateral light treatments and found the growth rate changes in Avena were inconsistent with predictions from the Blaauw theory, and Cosgrove (1985b) found a temporal separation of the kinetics of blue-light-mediated inhibition

Figure 4. Growth of both sides of mustard hypocotyls before and after phototropic stimulation (means of 37 plants).


10
5
-5
Increase in length (%)
-4
-3
-2
-1
1
2
3
4
5
6
7
Time (h)
Illuminated side
Shaded side
Projected

of axis extension growth and the onset of phototropic curvature in Cucumis. Thus it seems the Blaauw theory must be rejected as an explanation of phototropism in other plants too.

Since interpretation of the results of experiments analysing the changes in growth rate on the illuminated and shaded sides of the hypocotyl is dependent on the assumption that the observed changes are solely those involved in the phototropic response, it is essential that other potential growth rate changes are minimised, or preferably eliminated. A number of other recent studies have analysed phototropism under conditions independent of phytochrome (Iino & Briggs, 1984; Baskin et al., 1985; Macleod et al., 1986), but there have been no other studies to date to analyse growth rates independently of the blue-light-mediated inhibition of axis extension growth. In species other than mustard, both phototropism and inhibition of axis extension growth may be induced and expressed simultaneously by the blue light stimulus, and it may not be possible to clearly distinguish between them.

In conflict with the results above, Franssen et al. (1981) reported that curvature in green mustard seedlings was established solely through an inhibition of growth on the illuminated side of the hypocotyl. An investigation of the inconsistency between these two results is the subject of the next chapter.

CHAPTER 3

PATTERNS OF DIFFERENTIAL PHOTOTROPIC GROWTH: MODIFICATIONS BY SEPARATE LIGHT-GROWTH RESPONSES

INTRODUCTION

Analysis of the changes in growth rate that give rise to curvature should help indicate the mechanisms involved in phototropism (Firn & Digby, 1980). In the literature, as in mustard (Chapter 2), two main types of differential growth are generally reported. In the first type, differential growth is established by an inhibition of growth on the illuminated side of the organ with little or no change on the shaded side (du Buy & Nuernbergk, 1929; Franssen et al. 1981; Macleod et al. 1984, 1985, 1986). In the second type, an acceleration of growth on the shaded sice accompanies the inhibition on the illuminated side (Curry, 1969; Hart, Gordon & MacDonald, 1981; Iino & Briggs, 1984; Baskin et al. 1985; Rich, Whitelam & Smith, 1985; Macleod et al. 1986). Iino & Briggs (1984) suggested one explanation of the contradictory data is that there is a common basic phototropic mechanism which is accompanied in some cases by separate light-growth responses.

In this chapter, this hypothesis has been examined for mustard seedlings in order to clarify the changes in growth rate which occur during phototropism. In the first part, the potential influence of deliberately-induced phytochrome-mediated light-growth responses on the pattern of differential phototropic growth is shown. In the second part, the patterns of growth induced by different light treatments are analysed in an attempt to explain the discrepency between the results of Franssen et al. (1981) and those in Chapter 2.

MATERIALS AND METHODS

Growth conditions and light sources

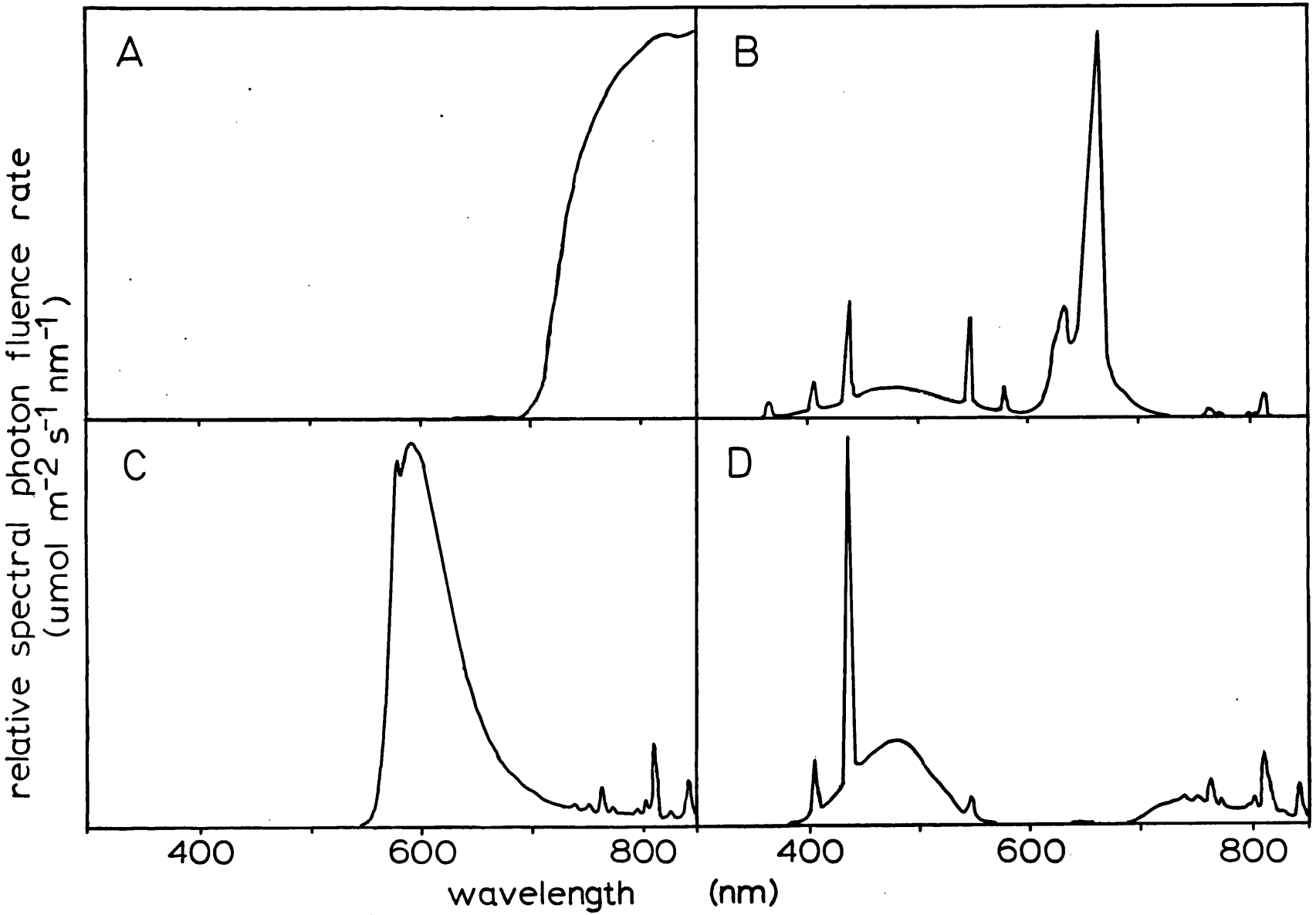
Plants were grown and treated as in Chapter 2. The light sources were modified slightly, mainly to facilitate alteration of the photoequilibrium. Background SOX (27 μmol m^{-2} s^{-1}) and low fluence rate blue light (0.46 μmol m^{-2} s^{-1}) were obtained as in Chapter 1. Far-red light was obtained from 500W Phillips tungsten-halogen lamps filtered through a cooling water bath and far-red FRF 700 plexiglass (West Lake Plastics Co., Lenn, Pensylvania, U.S.A.) and then also through the orange Cinemoid. White light was obtained from 20W Atlas Gro-lux fluorescent tubes. Broad-band orange light (25 μmol m^{-2} s^{-1}) was obtained from 40W Thorn Coolwhite fluorescent tubes filtered through 2 layers of orange Cinemoid. High-fluence-rate blue light (10 μmol m^{-2} s^{-1}) was obtained from 40W Thorn white fluorescent tubes filtered through 1 layer of no. 32 Medium blue 'Masterline Cinemoid'. Relative spectral photon distribution scans of the far-red, white, orange and high-fluence-rate blue light sources are given in Figure 5. The fluence rates used for each experiment are as stated above or given with the experimental data. Data presented, unless otherwise stated, are means $\pm$ s.e. (n=20).

Measurement of phytochrome photoequilibrium

Phytochrome photostationary states established by the experimental light sources were measured using a concentrated solution of partially-purified, native, 124 kD oat phytochrome (obtained by ammonium sulphate precipitation and hydroxyapatite chromatography) on ice.

FIGURE 5

Spectral photon fluence rate distributions of light sources. A. Far-red light. B. White light. C. Broad band orange light. D. High-fluence-rate blue light. For spectral scans of low pressure sodium (SOX) lamps and low-fluence-rate blue light, see Figure 1. For construction of light sources, see text.


A
B
C
D
relative spectral photon fluence rate
(umol m-2 s-1 nm-1)
400
600
800
400
600
800
wavelength (nm)

Measurement of relative internal light quantity

The relative light quantity inside a plant axis under different irradiation conditions was estimated using a 1mm diameter optical fibre (R.S. Components Ltd, London) attached to a LI-COR LI-185 quantum meter. The polyethylene protective sheath was stripped from the terminal 5mm of the fibre which was then covered with 2-3 layers of matt black paint; 95% of the light accepted by the resulting probe originates from angles of 40° or less to the probe. Thus, provided the long axis of the probe is always perpendicular to the direct incident light vector, it will measure scattered light from a cone of c.a. 80° arc when inserted into a plant axis. The probe could not be used on mustard hypocotyls because they are approximately the same width as the probe itself, so 3 other species with broad axes were investigated instead. Mungbean (Phaseolus aureus), gourd (Cucurbita pepo cv. 'Mammoth') and peas (Pisum sativum), all obtained from Asmer Seeds Ltd, were grown in the dark for 6-8 days and then de-etiolated for 48 hours in the constant environment room. The probe was then carefully inserted into the middle of the axis and the relative scattered light quantity measured for light from above (i.e. the incident light vector parallel to the plant axis) with or without cotyledons or primary leaves, and then also from the side (i.e. the incident light vector perpendicular to the plant axis). No attempt was made to account quantitatively for the optics of the plant tissues, and the probe was only used to obtain estimates of the relative amounts of light within the tissues as affected by geometry.

RESULTS

The influence of phytochrome-mediated straight-growth responses on the pattern of differential phototropic growth

The experiments, the results of which are shown in Figures 6 and 7, were designed to determine whether or not a light-growth response, separate from phototropism, can modify the pattern of differential growth. The experimental approach relies on the deliberate manipulation of hypocotyl growth rate by varying the phytochrome photoequilibrium by addition, or subtraction, of far-red light to, or from, the background SOX light. This can be achieved independently of the blue photoreceptor which does not absorb far-red light.

The effect of altering the photoequilibrium (ϕ) on hypocotyl growth rate is shown in Figure 6. Plants were growing at a constant rate in SOX light alone for the first 3 hours (ϕ = 0.86); far-red light was then added to the SOX light depressing the photoequilibrium to ϕ = 0.73 (Table 2), which caused a 43% increase in growth rate detectable within 1 hour. After 4 hours at this lower photoequilibrium the far-red light was removed, restoring the photoequilibrium back to 0.86, and the growth rate approximately to its prestimulation rate again within 1 hour. These data are consistent with many other data on phytochrome regulation of extension growth (e.g. Morgan, O'Brien & Smith, 1980), though more precise resolution using transducers indicates the changes in growth rate occur within 15 minutes.

The pattern of differential growth in control plants given unilateral blue light with no change in the phytochrome photoequilibrium is shown in Figure 7a. Differential elongation is established by an inhibition of growth rate on the illuminated side of the hypocotyl and an increase in rate on the shaded side, with no change in the net growth rate. After approximately two hours the growth rates tend to return to the prestimulation

FIGURE 6

Time course of hypocotyl extension growth at different phytochrome photo equilibria (ϕ). Plants were grown initially in SOX light (ϕ=0.86). At 3 hours far-red light was added, depressing the photoequilibrium (ϕ=0.73 At 7 hours the far-red light was removed, restoring the photoequilibrium to its prestimulation value (ϕ=0.86). Points are means $\pm$ s.e. (n=20).

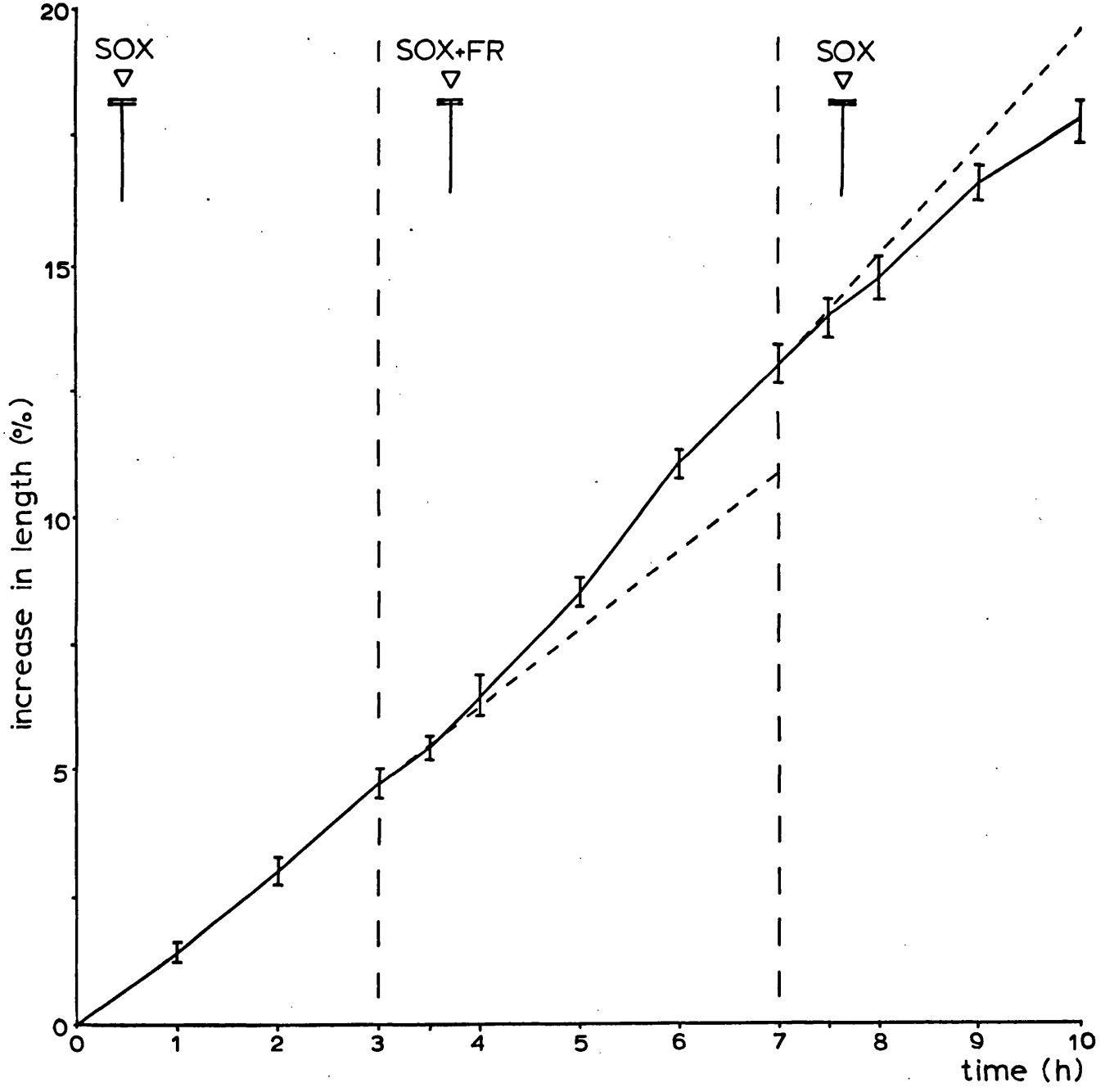

SOX
SOX+FR
SOX
increase in length (%)
time (h)
0
5
10
15
20
0
1
2
3
4
5
6
7
8
9
10

FIGURE 7

Time course for development of differential phototropic growth in respons to low-fluence-rate, unilateral, blue light (0.46 μmol m^{-2} s^{-1}), without and with simultaneous manipulation of hypocotyl growth rate through the phytochrome photoequilibrium. A. Control; no change in extension growth rate at phototropic induction. B. Increase in extension growth rate simultaneously with phototropic induction by addition of FR. C. Decrease in extension growth rate simultaneous with phototropic induction by remov of FR. ◐ shaded side, ▓ illuminated side, --- prestimulation growt rate, — net growth rate.

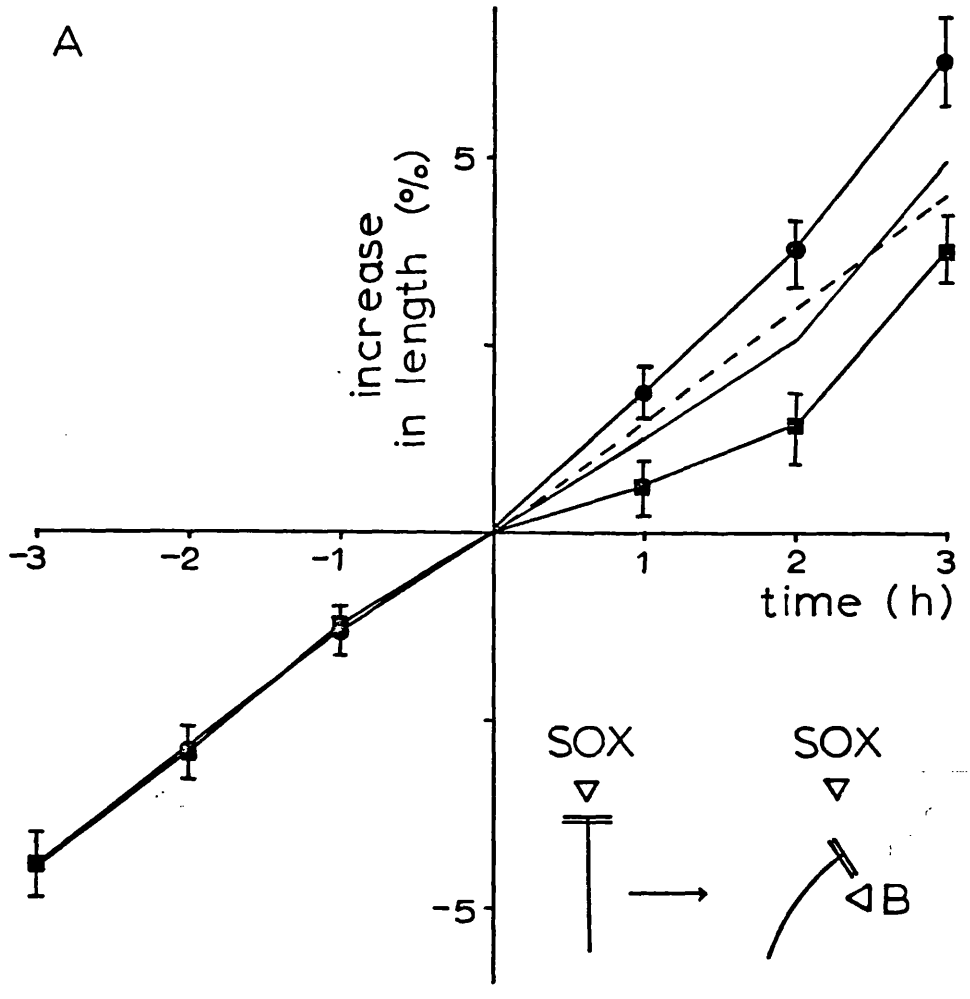

A
increase in length (%)
5
-5
-3
-2
-1
1
2
3
time (h)
SOX
SOX
B

FIGURE 7B
(SEE FIG. 7A FOR DETAILS)

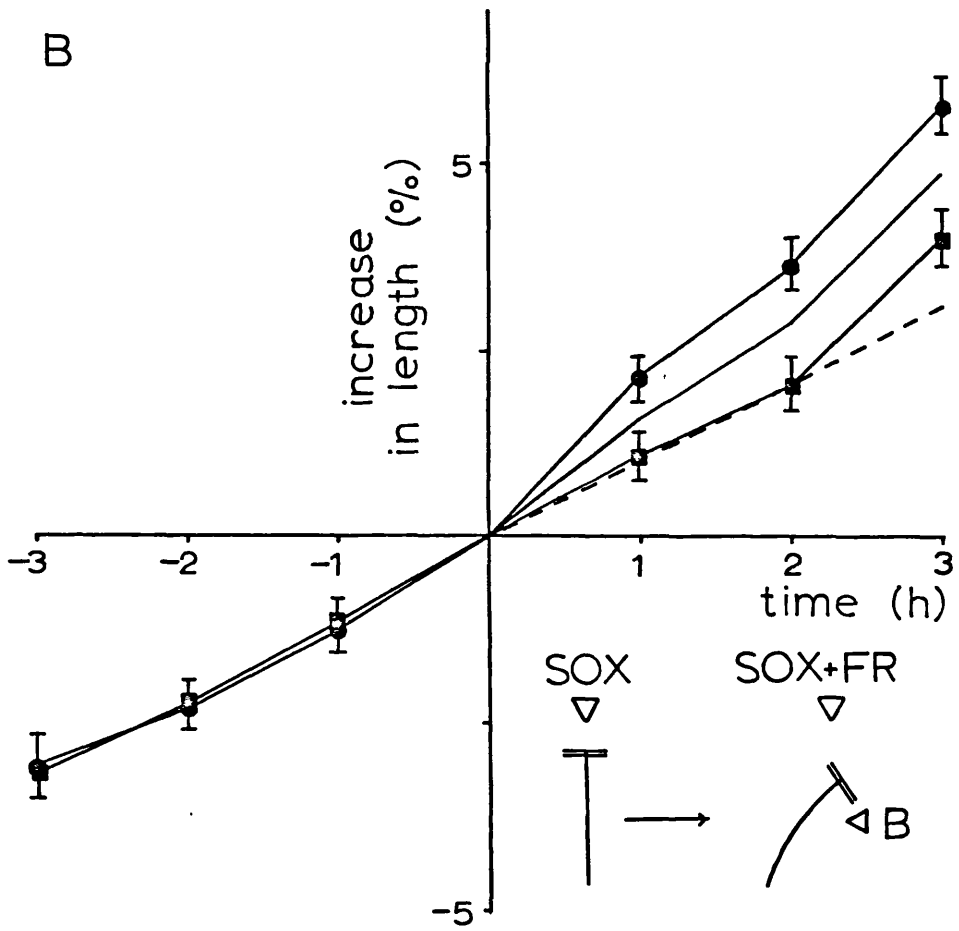


FIGURE 7C
(SEE FIGURE 7A FOR DETAILS)

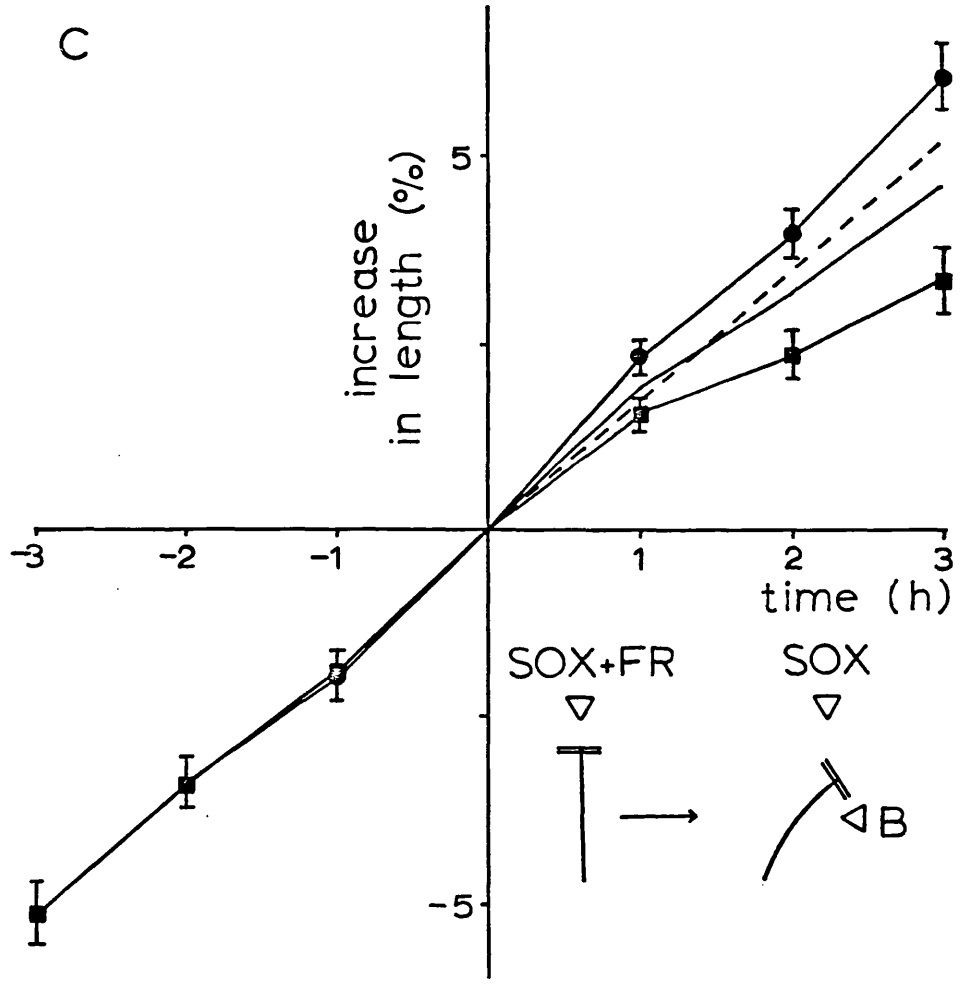


TABLE 2

Measurements of phytochrome photoequilibria (ϕ) established by the experimental light conditions. Photoequilibria were measured spectrophotometrically using partially purified oat phytochrome on ice. Photoequilibria were calculated as $\frac{[\ Pfr\ ]}{[\ Ptot\ ]}$, max. ϕ = 0.86 (Vierstra & Quail, 1983).

Light treatment	ϕ
SOX (27 µmol m^{-2} s^{-1})	0.86
SOX (27 µmol m^{-2} s^{-1}) + low fluence rate blue light (0.46 µmol m^{-2} s^{-1})	0.86
SOX (27 µmol m^{-2} s^{-1}) + far-red (15.5 µmol m^{-2} s^{-1})	0.73
SOX (27 µmol m^{-2} s^{-1}) + far-red (15.5 µmol m^{-2} s^{-1}) + low fluence rate blue light (0.46 µmol m^{-2} s^{-1})	0.74
White light (35 µmol m^{-2} s^{-1})	0.83
Orange light (25 µmol m^{-2} s^{-1})	0.86
High fluence rate blue light (10 µmol m^{-2} s^{-1})	0.43

rate due to autotropic and gravitropic compensation (Firn & Digby, 1979; see also Chapter 5). These data are consistent with those in Chapter 2.

Figures 7b and 7c show the influence of separate, phytochrome-mediated growth responses on the pattern of differential growth observed during phototropism. In Figure 7b, far-red light was added to increase the growth rate simultaneously with induction of phototropism. Differential growth is apparently caused by an acceleration of growth rate on the shaded side of the hypocotyl with no change on the illuminated side. The net growth rate increased by 37.5%, a value similar to that for plants given far-red without blue light (Figure 6). Figure 7c shows the converse experiment of decreasing the growth rate by increasing the photoequilibrium simultaneously with induction of phototropism. Curvature in this case is caused mainly by an inhibition of growth on the illuminated side with only a small acceleration on the shaded side. It is clear in both these cases that the underlying pattern of differential phototropic growth (Figure 7a) is obscured by the simultaneous expression of the separate phytochrome-mediated growth responses.

The influence of different light treatments on the pattern of differential phototropic growth

The conflicting patterns of differential phototropic growth reported in mustard (Franssen *et al*., 1981, *versus* Figure 4) were investigated by analysing the influence of different light treatments on phototropic and straight growth.

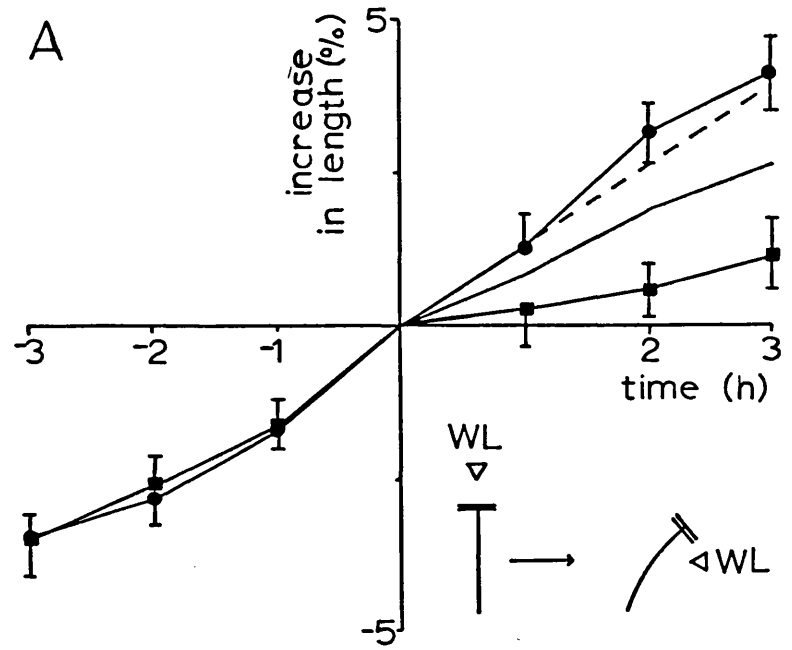
Franssen *et al* (1981) analysed differential growth following induction of phototropism by moving white light from above to one side. As they did not present their data for mustard, their experiment was repeated (though using a higher fluence rate of white light for photographic reasons)

and similar results were obtained (Figure 8a). The data show that curvature is caused largely by an inhibition of growth on the illuminated side of the hypocotyl. As light regulation of straight extension growth and phototropism are not direct cause and effect (Chapter 2), an analogous experiment was used to investigate straight growth responses. White light was moved from above to bilateral illumination keeping the total fluence rate constant (i.e. 17.5 μmol m^{-2} s^{-1} added from each side). A 32% inhibition of straight growth was found (Figure 8b), a similar figure to that in unilateral light (38%; Figure 8a). This result demonstrates that straight extension growth responses occur in the absence of phototropism when light is moved from above to bilateral illumination. As this treatment is analogous to unilateral light treatments, straight-growth responses may also be induced during phototropism if this method is used to stimulate curvature.

Due to shading of the hypocotyl by the cotyledons, and to the changed geometrical relationship between the hypocotyl and the incident light vector, it is likely that the physiologically-significant light inside the hypocotyl will increase when light is moved from above to the side. Because of the technical difficulties of measuring the absolute amount of light inside the hypocotyl, a fibre optic probe was used to measure one component of the total internal light environment, the scattered light perpendicular to the incident light vector, under different irradiation conditions (Figure 9). Assuming the scattered light is proportional to the total light inside the axis, any change in the total light under different irradiation conditions should be detectable as a change in the relative amount of scattered light by the probe. Although it was not possible to use the probe on mustard hypocotyls, the results in Table 3 for three other species under different treatments can probably be extrapolated in principle to mustard, and indeed to any plant material.

FIGURE 8

Changes in growth rate observed in white light (35 μmol m^{-2} s^{-1}).
A. Pattern of differential phototropic growth established when white ligh
was moved from above to unilateral illumination. B. Straight extension
growth response when white light was moved from above to bilateral illumi
• shaded side, ▓ illuminated side, --- prestimulation growth rate,
— net growth rate.


A
increase in length (%)
5
-5
-3
-2
-1
2
3
time (h)
WL
WL

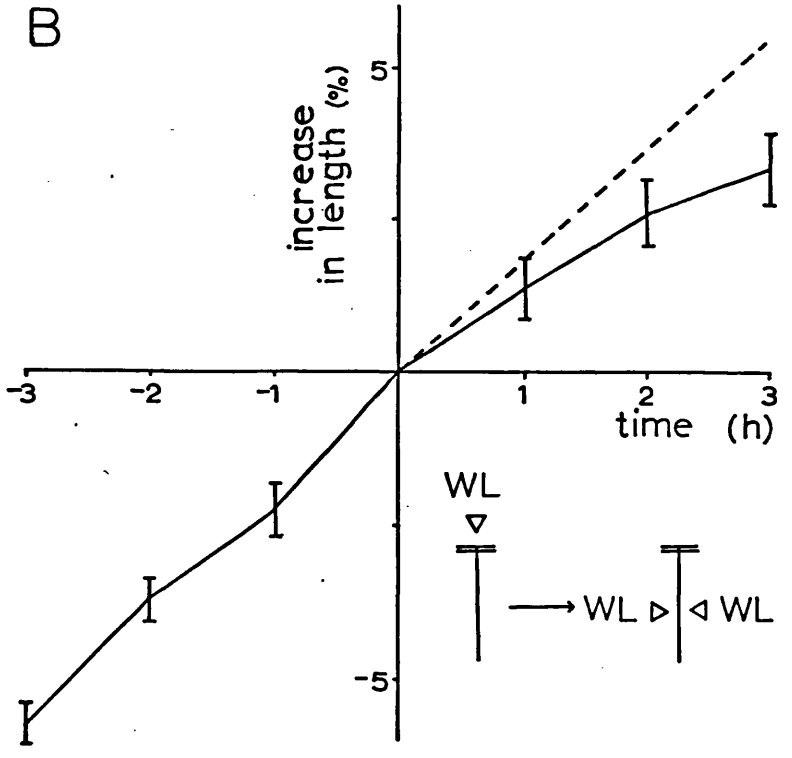

B
increase in length (%)
5
-5
-3
-2
-1
1
2
3
time (h)
WL
WL
WL

FIGURE 9

Measurement of internal light environment of plant axis under different irradiation conditions. For relative light values, see Table 3. A. Light from above, plant intact. B. Light from above, cotyledons or leaves removed. C. Light from the side.

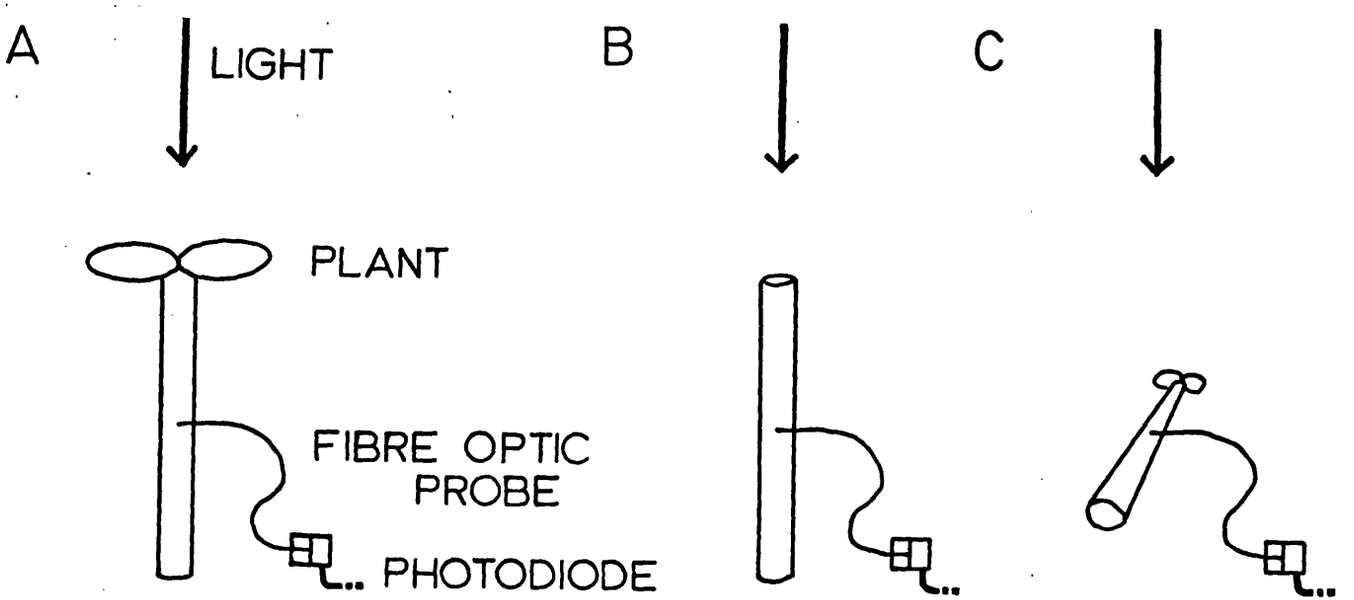

A
LIGHT
B
C
PLANT
FIBRE OPTIC
PROBE
PHOTODIODE

TABLE 3

Relative light quantity inside plant axes measured with a fibre optic probe for three different incident light conditions (Figure 9). Values are mean ± s.d. light levels relative to intact plants with light from above (n = 10).

Tissue	Light from above: intact plants.	Light from above cotyledons or leaves removed.	Light from the side.
Mungbean hypocotyls	1.0	± 1.0	5.1 ± 1.3
Gourd hypocotyls	1.0	2.3 ± 0.67	19.2 ± 11.2
Pea 1st internode	1.0	1.8 ± 0.73	8.6 ± 4.7

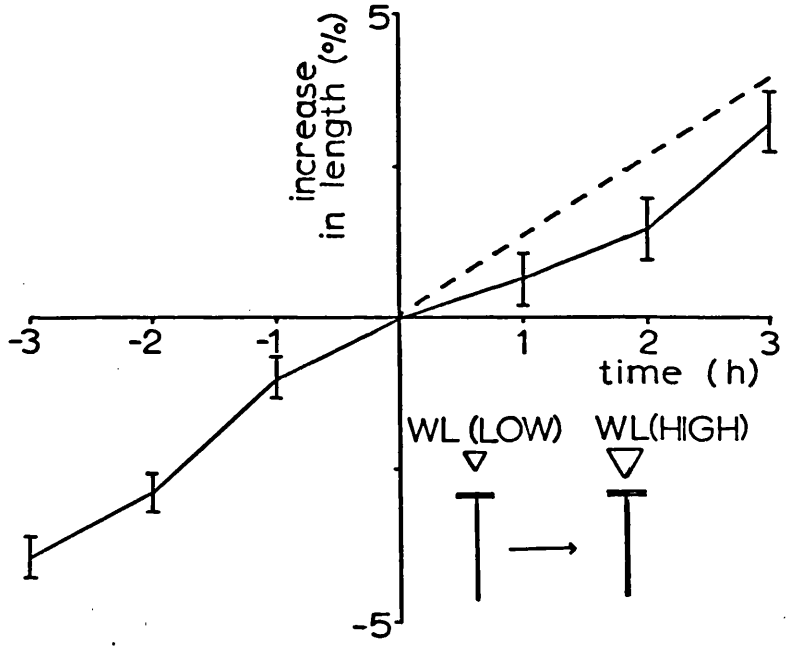
The results of this comparative assay show that both shading by the cotyledons and the geometrical relationship of the plant axis to the incident light may influence the amount of scattered light in the axis. Removal of the expanded cotyledons in gourd or the primary leaves of pea results in an approximately 2-fold increase in the scattered light quantity, but in mungbean where the cotyledons are small and undeveloped there is little or no effect. If intact plants are irradiated from above, and then from the side, there is a c.a. 5-19-fold increase in the scattered light suggesting a c.a. 4-8-fold purely geometrical effect. These results demonstrate that, in terms of the light actually inside the plant axis, light applied from the side is not equivalent to the same fluence rate light applied from above.

If a change in internal light quantity resulting from movement of the light source from above to one side is responsible for the net growth inhibition observed in white light (Figure 8), then an increase in the external fluence rate with no change in the geometry should also cause an inhibition of growth. To allow for differences between species and tissues, a $2\frac{1}{2}$-3-fold increase in fluence rate was taken as a minimum and the effect of such an increase on mustard hypocotyl straight growth analysed. Figure 10 shows there is an inhibition of growth, suggesting a fluence-rate-dependent mechanism. Further experiments were therefore carried out to investigate the photobiological basis of the response.

Under the limited conditions where blue light responses can be analysed independently of phytochrome, it was not possible to detect a straight-growth response mediated by the blue-absorbing photoreceptor (Chapter 2). However, as Drumm-Herrel & Mohr (1985) observed a "specific blue light effect" at high fluence rates of white light, hypocotyls were tested for a blue-absorbing photoreceptor response with a high initiation threshold,

FIGURE 10

Straight extension growth response observed when white light applied fht applied from above was increased from 20 to 50 μmol m^{-2} s^{-1}.


increase in length (%)
5
-5
-3
-2
-1
1
2
3
time (h)
WL (LOW)
WL(HIGH)

10 μmol m^{-2} s^{-1} blue light was added to a background of 200 μmol m^{-2} s^{-1} SOX light, but no inhibition of growth was found (Figure 11). Thomas (1980) also found no inhibition of growth in response to addition of high-fluence-rate blue light using the SOX technique. These results are consistent with those in Chapter 2 and show that blue light mediates phototropism, but does not directly regulate extension growth under these conditions.

If phytochrome were responsible for the growth inhibition observed with white light, then the experiments in Figure 8 repeated using broad band orange light would be expected to produce similar growth responses. Orange light was used in preference to the usual red light treatments because it was not possible to obtain an appropriate fluence rate with red Cinemoid, and the fluence rate used (25 μmol m^{-2} s^{-1}) represents the orange component of the white light previously used. No inhibition of growth was observed when the orange light was moved from above to bilateral (Figure 12a) or unilateral (Figure 12b) illumination. A 3-fold increase in the SOX light from above similarly causes no inhibition of growth (Figure 12c). These results indicate the white-light mediated growth inhibition is not directly mediated by phytochrome.

The experiments were repeated using the blue component of the white light spectrum (10 μmol m^{-2} s^{-1}). This treatment does not distinguish between mediation by a blue-absorbing photoreceptor, by phytochrome, or by a combination of the two photoreceptors. Surprisingly, for both bilateral and unilateral treatments (the latter inducing phototropism) there is a clear inhibition of net growth (Figure 13a & b). The growth rates are higher than in the other treatments due to the low photoequilibrium established by the blue light (Table 2). To check these results are not artifacts of the Cinemoid filters, light from the orange and blue sources was combined to produce 35 μmol m^{-2} s^{-1} "white light". The phototropic

FIGURE 11

Straight extension growth of mustard hypocotyls observed when 10 μmol m^{-2} s^{-1} high-fluence-rate blue light was added to a background of 200 μmo m^{-2} s^{-1} SOX light. (n=26).

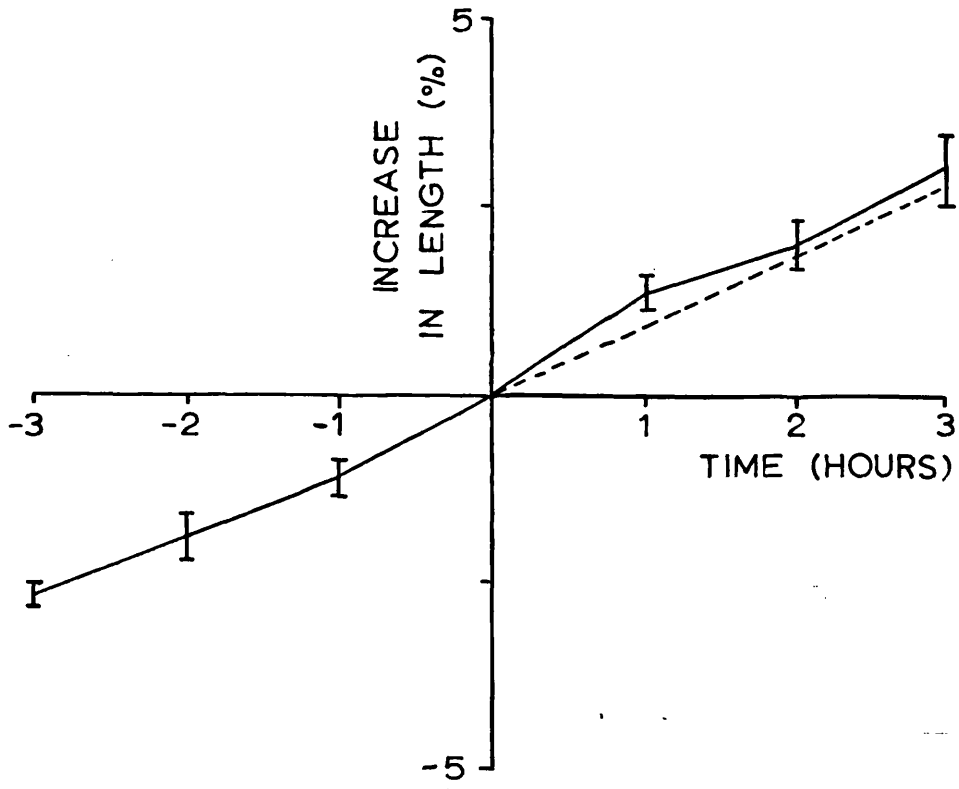

INCREASE
IN LENGTH (%)
5
-5
-3
-2
-1
1
2
3
TIME (HOURS)

FIGURE 12A

Straight extension growth of hypocotyls observed when 25 μmol m^{-2} s^{-1} broad-band orange light was moved from above to bilateral illumination.

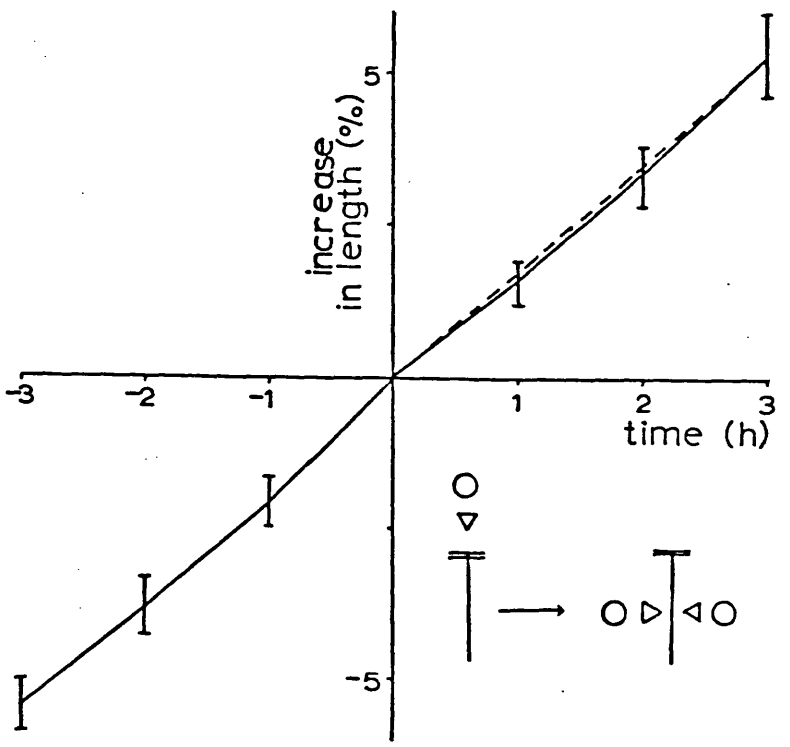

increase in length (%)
5
-5
-3
-2
-1
1
2
3
time (h)

FIGURE 12B

Straight extension growth of hypocotyls observed when 25 μmol m^{-2} s^{-1} broad-band orange light was moved from above to unilateral irradiation.

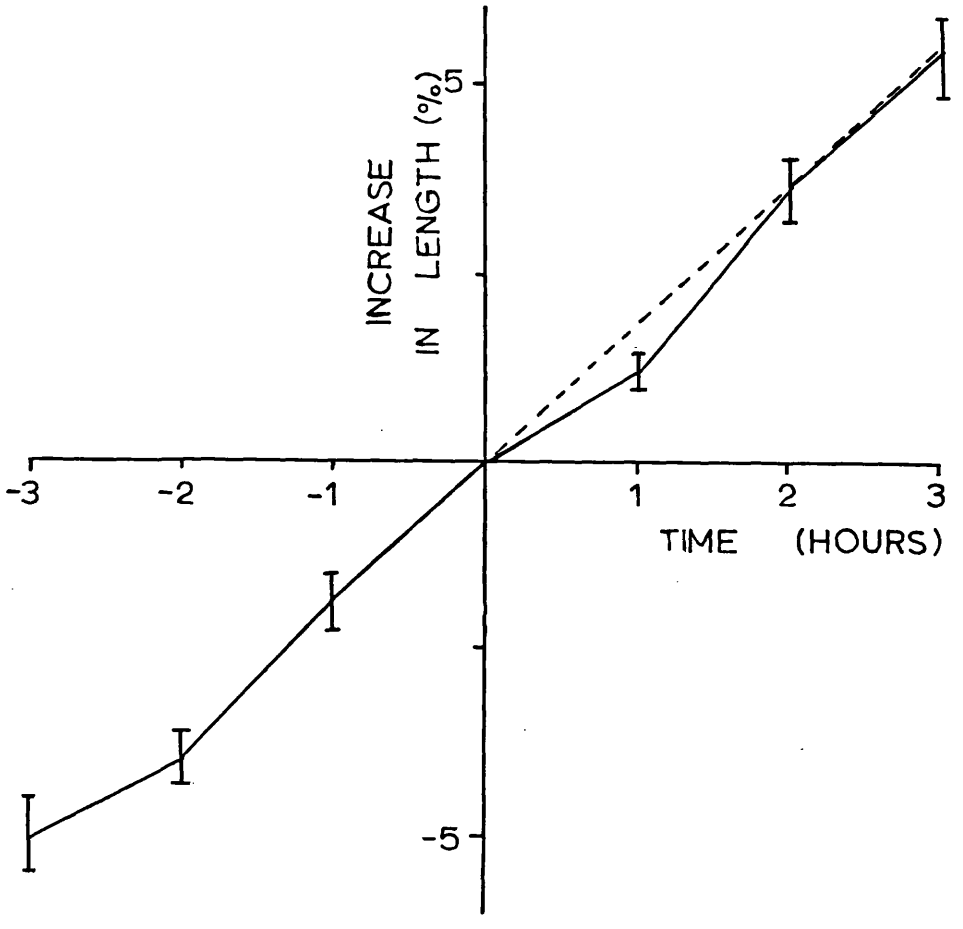

INCREASE
IN LENGTH (%)
5
-5
-3
-2
-1
1
2
3
TIME (HOURS)

FIGURE 12C

Straight extension growth of mustard hypocotyls observed when background SOX light was increased from 27 μmol m^{-2} s^{-1} to 79 μmol m^{-2} s^{-1}.

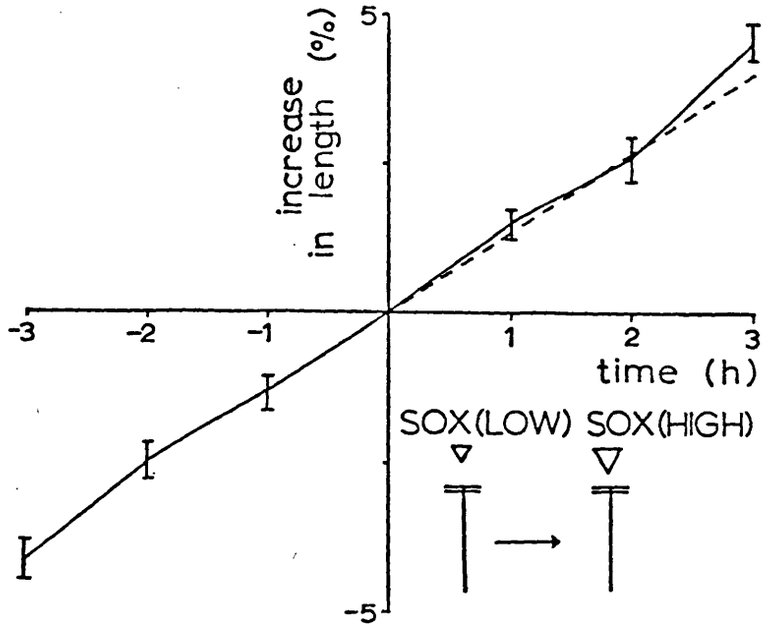
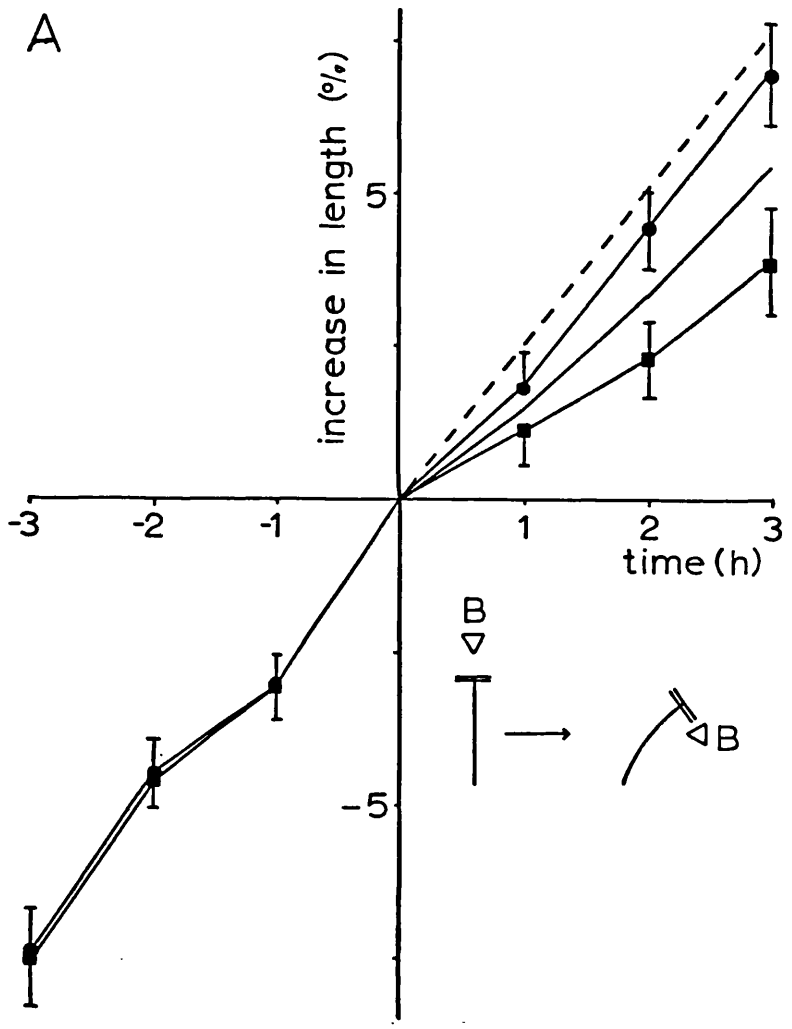
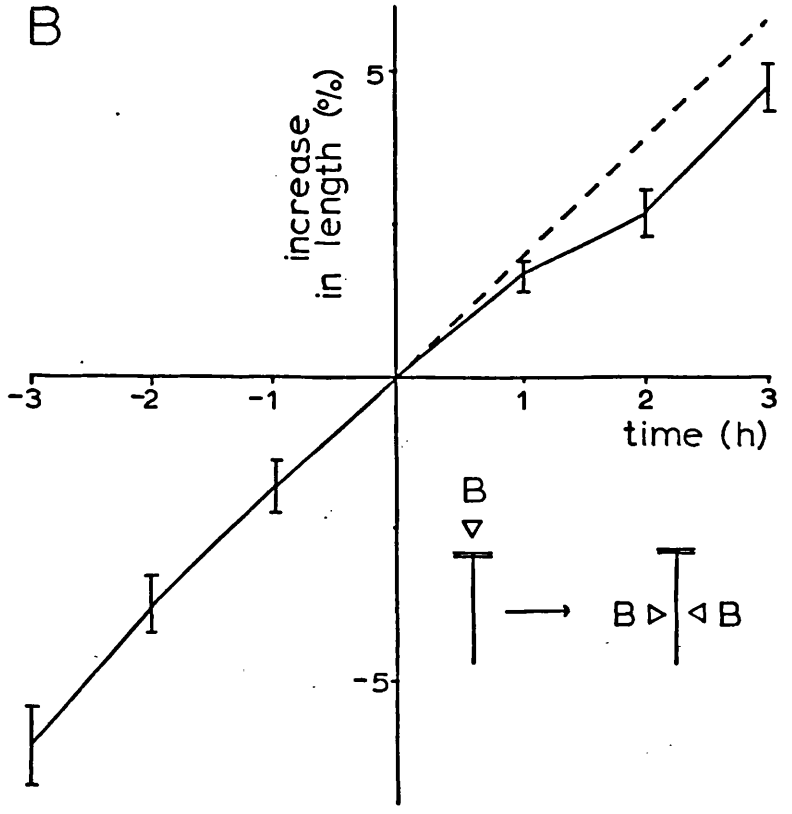

increase in length (%)
5
-5
-3
-2
-1
1
2
3
time (h)
SOX(LOW)
SOX(HIGH)

FIGURE 13

Changes in growth rate observed in high-fluence-rate blue light (10 μmol m^{-2} s^{-1}). A. Pattern of differential phototropic growth estab when blue light was moved from above to unilateral illumination (n=15). B. Straight extension growth response when blue light was moved from ab to bilateral illumination (n=15).

● shaded side, ■ illuminated side, --- prestimulation growth rate, —— net growth rate.


A
increase in length (%)
5
-5
-3
-2
-1
1
2
3
time (h)
B
B


B
increase in length (%)
5
-5
-3
-2
-1
1
2
3
time (h)
B
B
B

and straight growth responses observed using this combined source (Figures 14a & b) approximately match those observed with the fluorescent white light (Figure 8a & b).

Mohr (1980) and Drumm-Herrel & Mohr (1984) reported an interaction between phytochrome and blue light in Sesame hypocotyl growth. Because neither the blue-light-absorbing photoreceptor nor phytochrome apparently directly regulate extension growth (except through changes in the photo-equilibrium in the latter), the possibility that both blue-absorbing photo-receptor and phytochrome events are necessary for the expression of the white light growth inhibition was investigated. Experiments where there is no blue-photoreceptor inhibition of growth (Figure 7a) and no phytochrome irradiance-dependent inhibition of growth (Figure 12c) were taken and combined. Low-fluence-rate blue light was added simultaneously with a 3-fold increase in background SOX light. Both phototropic and straight growth experiments show a small inhibition of net growth (Figure 15a & b). The inhibition is not as marked as in the white and blue light experiments (Figures 8 and 13).

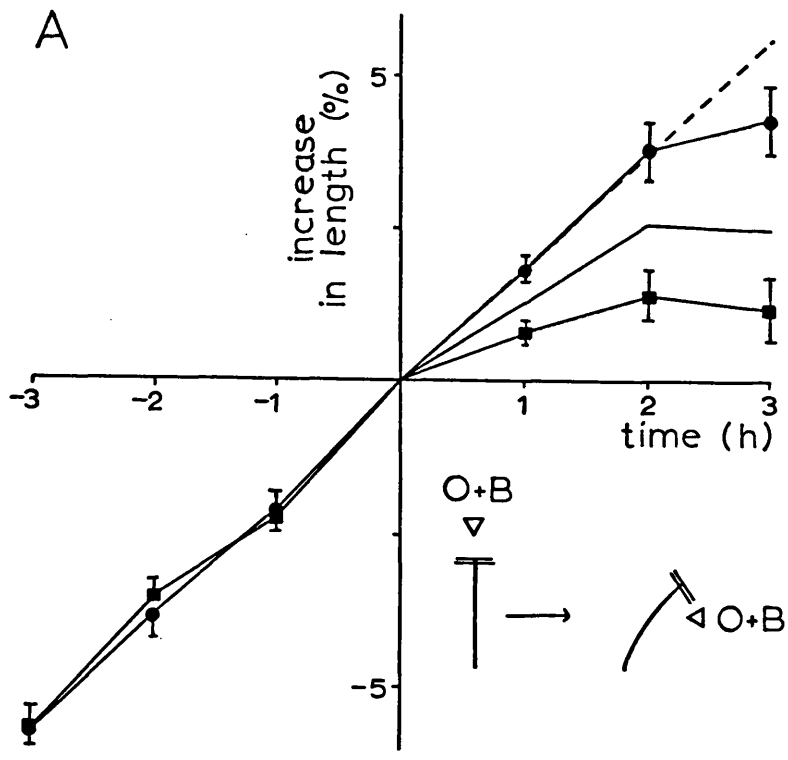
DISCUSSION

Figure 7 demonstrates that controlled phytochrome-mediated light-growth responses can modify the pattern of differential phototropic growth. Appropriate manipulation of the hypocotyl growth rate will yield all 5 types of differential growth listed by Firn & Digby (1980). Firn et al (1983) have argued however, that before "composite responses" can be accepted as an explanation for the conflicting patterns of differential growth (c.f. Iino & Briggs, 1984), they must be shown to exist.

FIGURE 14

Changes in growth rate observed in a mixture of 25 μmol m^{-2} s^{-1} broad-band orange light and 10 μmol m^{-2} s^{-1} high fluence rate blue light. A. Pattern of differential phototropic growth established when the mixed light sources were moved from above to unilateral illumination. B. Strai extension growth response when the mixed light sources were moved from above to bilateral illumination.

● shaded side, ▓ illuminated side, --- prestimulation growth rate, —— net growth rate.


A
increase in length (%)
5
-5
-3
-2
-1
1
2
3
time (h)
O+B
O+B

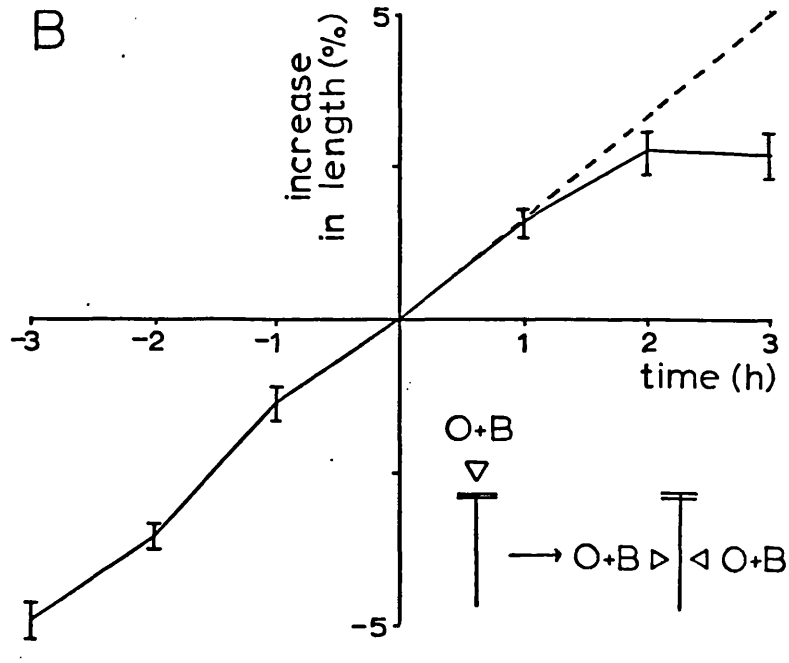
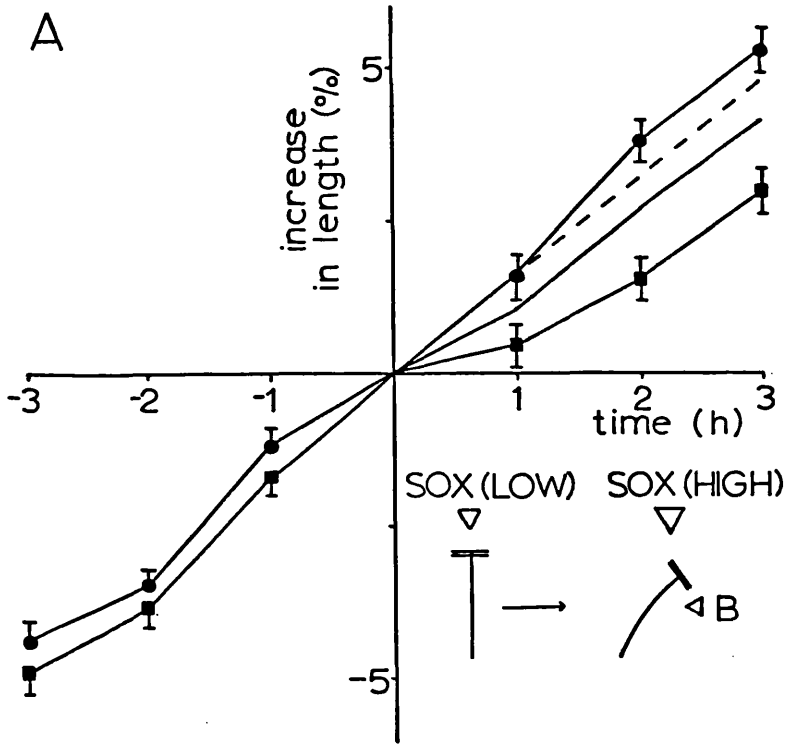
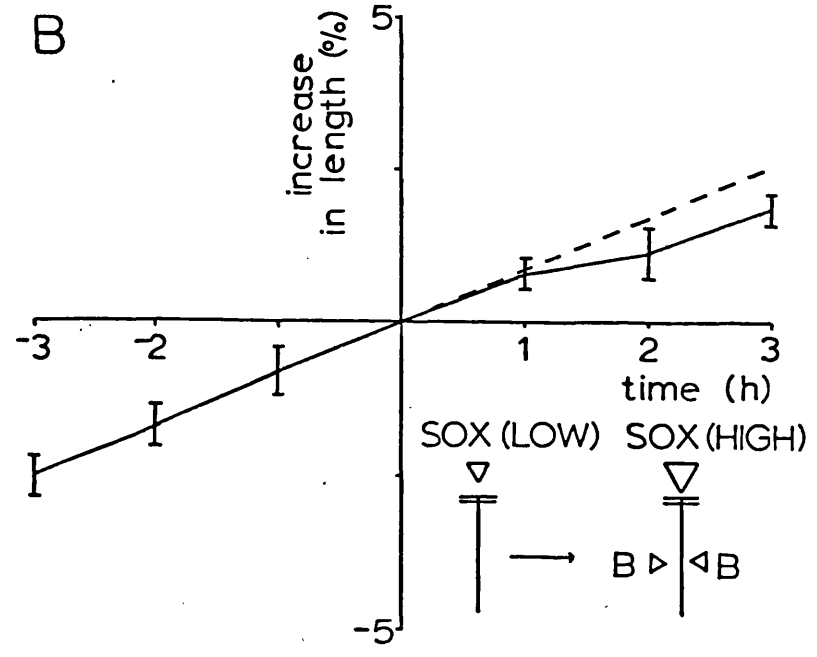

B
increase in length (%)
5
-5
-3
-2
-1
1
2
3
time (h)
O+B
O+B
O+B

FIGURE 15

Changes in growth rate observed with a simultaneous addition of low-fluer rate blue light (0.46 μmol m^{-2} s^{-1}) and an increase in background SOX light from 27 to 79 μmol m^{-2} s^{-1}. A. Pattern of differential phototropic growth with unilateral blue light and an increase in the SOX light from above. B. Straight extension growth response with addition of bilateral blue light and an increase in the SOX background light from above. ● shaded side, ▓ illuminated side, --- prestimulation growth rate, —— net growth rate.


A
increase in length (%)
5
-5
-3
-2
-1
1
2
3
time (h)
SOX (LOW)
SOX (HIGH)
B


B
increase in length (%)
5
-5
-3
-2
1
2
3
time (h)
SOX (LOW)
SOX (HIGH)
B
B

Analysis of the influence of different light treatments shows composite responses do indeed exist. Three lines of evidence show that separate light-growth responses may be induced when the technique of Franssen et al. (1981) is used to analyse phototropism. First, analogous experiments with white light show a net inhibition of growth during both phototropic and straight growth (Figures 8a & b). Because phototropism and light-regulated axis extension growth are distinct phenomena (Chapter 2), this growth inhibition is not directly involved in phototropism.

Second, there is a large increase in the internal light quantity of the axis when the light applied is moved from above to one side (Table 3). As increasing the fluence rate applied from above causes a growth inhibition (Figure 10), the technique of Franssen et al. (1981) would be expected to induce a similar response. The assumption by Franssen et al. (1981) that light from above is equivalent to light from the side when the fluence rate applied is the same is clearly invalid.

Third, the inhibition of growth observed in white light treatments is not directly mediated by the blue-light-absorbing photoreceptor, and must therefore again be accessory to, rather than causal in, phototropism. The results of the analysis to investigate the photobiological basis of the inhibition in mustard (Figures 12-15) indicate, but do not prove, that the mechanism may involve an interaction between phytochrome and blue light. Further characterisation of the response will, however, be required to clarify the situation.

In the absence of separate light-growth responses, phototropism in mustard is caused by an inhibition of growth on the illuminated side of the hypocotyl and an acceleration on the shaded side (Figure 7a; Chapter 2). The pattern of differential growth reported by Franssen et al. (1981)

cannot be considered as representative of the changes in growth rate that occur during phototropism. The results obtained from the comparison of the two techniques are in support of the hypothesis that separate light-growth responses can modify the pattern of differential growth observed during phototropism (Iino & Briggs, 1984). Hart et al. (1982) also attribute the different patterns of phototropic growth of cress seedlings to separate straight growth responses, but did not investigate their basis in detail. Whether or not separate light growth responses explain all the contradictory data is another question.

The influence or such separate light-growth responses on phototropism will depend on the relative magnitudes of the changes in growth rate. There is an exasperating lack in the literature of proper control experiments where straight growth responses have been analysed in addition to growth responses observed during phototropism; such experiments would greatly enhance interpretation of results. However, it is clear that separate light-growth responses can interfere with and modify the expression of differential growth, and therefore curvature. This has a very important implication: unless phototropism is analysed under conditions where straight growth responses are eliminated or accounted for, it is not possible quantitatively to relate light treatments to the kinetics of phototropism. An extrapolation of this conclusion to its extremes suggests that the vast majority of the quantitative phototropic literature involving kinetics, where separate light-growth responses have not been eliminated, is potentially invalid. Clearly, care must be taken with experimental design in order to analyse phototropism on a quantitative basis.

Separate light-growth responses may also explain two other phenomena noted in the literature. Firn et al. (1983) noted the interesting relationship between blue-light-mediated inhibition of straight growth and phototropism;

plants that show a strong straight growth response show poor phototropic responses and vice versa. This relationship would be predicted if curvature was modified by straight growth. Macleod et al. (1986) found no consistent pattern of differential growth in dark and red-light pretreated coleoptiles, and concluded that no single pattern of elongation rate changes causes phototropism even in a single species. Differential expression of separate straight-growth responses in physiologically different cells could account for such an observation, but no straight growth data were presented for comparison.

In the only two other studies to date where separate light-growth responses have been minimised or eliminated, curvature was established through an inhibition of growth on the illuminated side accompanied by an acceleration on the shaded side (Iino & Briggs, 1984; Baskin et al., 1985). These reports are consistent with the results above. The fact that net growth remains largely unaltered indicates that changes in growth rate are probably equal and opposite. This relationship in itself suggests a dependence of growth rate changes on one another, and that some form of "communication" is involved (c.f. Macleod et al., 1985). If there is a single basic phototropic mechanism, these three studies suggest phototropism is caused by a redistribution of growth within the plant axis.

Any phototropic theory must explain by what mechanism blue light causes phototropism but not changes in net growth rate, and how the apparently equal and opposite changes in growth rate on the opposing sides of the hypocotyl are controlled.

CHAPTER 4

BIOPHYSICAL ANALYSIS OF CELL GROWTH DURING PHOTOTROPISM

INTRODUCTION

Phototropic curvature is caused by the establishment of different rates of cell extension growth within the hypocotyl in response to a suitable light stimulus. Growth of cells is generally accepted to be due to yielding of the cell wall in response to turgor pressure (Ray, Green & Cleland, 1972), and hence simultaneous uptake of water and irreversible wall expansion are needed to maintain extension rate. In theory, as either or both of these processes may limit the growth rate (Cosgrove, 1981a), any dynamic growth response must be caused by a change in the biophysical parameters describing them. As yet, which of these parameters change to cause differential phototropic growth has not been investigated.

The most widely used model of irreversible cell enlargement is that of Lockhart (1965), who combined two equations relating water transport and wall rheological properties to growth. The basic derivation of the equation is shown below; more critical and detailed discussion can be found in Lockhart (1965), Ray, Green & Cleland (1972), Cosgrove (1981a, 1983), Tomos (1985) and Cosgrove (1986).

Water transport is a passive process and occurs in response to a gradient of water potential ($\Delta\psi$). The water potential gradient between a growing cell and its water source can be described under non-transpiring conditions as (annotation following Cosgrove, 1981a):

$$\Delta\psi = \sigma\Delta\overline{\Pi} - P \qquad \text{(Equation 1)}$$

where $\Delta\overline{\Pi}$ = difference in osmotic potential between cell and source, σ = solute reflection coefficient (a parameter describing the solute

movement in the tissue: usually assumed to be 1, implying solutes are not permeable to membranes), and P=cell turgor pressure. Under transpiring conditions this is modified to account for hydrostatic tension in the cell wall (P_{wall}):

$$\Delta\psi = \sigma\Delta\overline{\Pi}-P-P_{wall} \qquad \text{(Equation 2)}$$

The relative rate of water absorption by a growing cell (or the increase in volume, V; hence relative growth rate) will be determined by and a pathway resistance term, or relative hydraulic conductance (L), such that

$$\frac{I}{V}\cdot\frac{dV}{dt} = L\,(\Delta\psi) \qquad \text{(Equation 3)}$$

hence:

$$\frac{I}{V}\cdot\frac{dV}{dt} = L\,(\sigma\Delta\overline{\Pi}-P-P_{wall}) \qquad \text{(Equation 4)}$$

The equation relating wall rheological properties to growth has an empirical basis; expansion has been experimentally observed many times (Tomos, 1985) to be a linear function of turgor pressure above a critical 'yield' threshold (Y):

$$\frac{I}{V_o}\cdot\frac{dV_o}{dt} = \phi\,(P-Y) \qquad \text{(Equation 5)}$$

where ϕ = wall extensibility.

Under steady state conditions, because growth requires simultaneous water uptake and wall loosening, Equations 4 and 5 can be equated and rearranged to give a general growth equation (Lockhart, 1965):

$$\frac{I}{V}\cdot\frac{dV}{dt} = \frac{\phi.L}{\phi+L}\,(\sigma\Delta\overline{\Pi} - Y + P_{wall}) \qquad \text{(Equation 6)}$$

Growth can thus be described in terms of 5 biophysical parameters; two relate to wall properties (ϕ, Y) and three to water transport (L, $\sigma\Delta\Pi$, P_{wall}).

TABLE 4. Predicted short term changes in turgor pressure (P) and osmotic potential of cell ($\overline{II}_{cell}$) during dynamic growth responses (adapted from Cosgrove, 1981a; Tomos, 1985). Changes in water transport properties accompanied by osmoregulation cannot account for changes in growth rate.

Change in growth rate	Osmoregulation?	Parameters limiting growth	P	$\overline{II}_{cell}$
Increase	No osmoregulation	Wall	Decrease	Constant
	No osmoregulation	Water	Increase	Constant
	Osmoregulation	Wall	Constant	Decrease
Decrease	No osmoregulation	Wall	Decrease	Constant
	No osmoregulation	Water	Increase	Constant
	Osmoregulation	Wall	Constant	Increase

Cosgrove (1981a) extended the analysis to dynamic responses, and showed the coupling of turgor pressure to growth rate. He showed that (assuming no osmoregulation) the direction of turgor pressure changes during dynamic responses can be used to determine whether changes in wall or water properties are responsible for the changes in growth rate (Table 4). If cells osmoregulate, however, the turgor pressures may not change (Table 4; Tomos, 1985). Measurement of turgor pressure can therefore be used to investigate the biophysics of cell growth during phototropism.

The most suitable technique for measuring turgor pressure during phototropism is the micropressure probe (Husken, Steudle & Zimmermann, 1978). Other techniques such as tissue rigidity measurements (Falk, Hertz & Virgin, 1957) or calculation from water and osmotic potentials (e.g. Molz & Boyer, 1978) only provide averages for the tissue and do not give the necessary resolution for the two sides of the hypocotyl. Turgor pressures of individual cells are measured by means of an oil-filled microcapillary introduced to the cell which transmits the turgor pressure to a pressure transducer. When inserted into the cell (i.e. into the vacuole) either the cell is ruptered (i.e. leaks) and the turgor rapidly drops, in which case no measurements can be made, or a pressure-tight seal may be formed which is surprisingly stable, though very sensitive to small vibrations. Cells appear to be little affected by the probe in the latter case, and turgor can be measured for long periods of time. In some giant algae turgor can easily be followed for 6 hours or longer, and under a microscope the cytoplasm can actually be observed to grow around and over the tip of the probe (P.B. Green, pers. comm. to A.D. Tomos). The cell containing the probe acts as an in situ mini-osmometer which reflects the turgor pressure of the tissue; turgor changes induced by changing the external

water potential or the transpiration rate (c.f. below) can be rapidly detected. It is therefore assumed that the turgor measured in single cells can be extrapolated to the tissue. Further discussion of the technique and its advantages and disadvantages can be found in Husken et al. (1978) and Zimmermann (1978).

MATERIALS AND METHODS

Plants were grown and treated essentially as described in Chapter 2, but with the following modifications due to the different facilities available in Bangor; plants are assumed to be physiologically comparable. Plants were grown in a Fisons 600 G3/THTL growth cabinet under 50 μmol m^{-2} s^{-1} white light with a 16h light/8h dark photoperiod. Background SOX light was 45 μmol m^{-2} s^{-1}. Blue light fluence rate was 0.27 μmol m^{-2} s^{-1}, which was added from an angle of c.a. 25° above horizontal to provide working space for the pressure probe underneath. Light sources were measured with a 550 Crump Quantum Photometer (T. + J. Crump, Rayleigh, Essex).

Turgor pressures were measured using a micropressure probe mounted on a Leitz micromanipulator. The probe was introduced to the tissue under a high-powered microscope, and the position of the oil:cell sap maniscus monitered by eye. Constant volume was maintained in the cell by means of a motor-driven plunger in the chamber of the pressure probe. During turgor pressure measurements it was necessary to hold seedlings steady against the tip of a Pasteur pipette by a loop of cotton tensioned with a small weight (10g); no bruising of the hypocotyl was observed. This lasso was positioned near the middle of the hypocotyl, and although restricting movement of the plant did not stop growth. Turgor pressures

were measured in cells close above the lasso. Plants were placed in a shallow dish of water to ensure an adequate water supply. No data from leaking cells are included.

Transpiration rates and osmotic potentials of plants were measured in the SOX background without and with blue light. Transpiration rates were determined from the weight loss of plants whose pots were carefully wrapped in aluminium foil to avoid water loss from the vermiculite. Bulk osmotic potentials were measured in a Wescor Inc. S100B Vapour pressure osmometer using 8 µl sap samples. Sap samples were obtained by centrifuging 10-15 freeze-thawed hypocotyls.

Yield thresholds were determined by the *in vivo* stress relaxation technique (Cosgrove, Volkenburgh + Cleland, 1984). Seedlings were attached to small wire stakes by a thin strip of insulating tape at the base, and tied at the top with cotton; this held the plants steady after excision. Plants were placed in a small, clear plastic box and turgor pressures determined for transpiring plants. Damp tissue paper was then placed in the box and the lid closed to stop transpiration. Turgor pressures were then measured by the probe inserted through a narrow slit in the side of the box. The box was thus not completely sealed but the air was probably saturated as small drops of water placed on the outside of the box caused localised condensation inside. Plants were then excised below the insulating tape with a scalpel inserted through the slit, and the turgor pressure decline followed with the pressure probe until a steady state was obtained.

RESULTS -

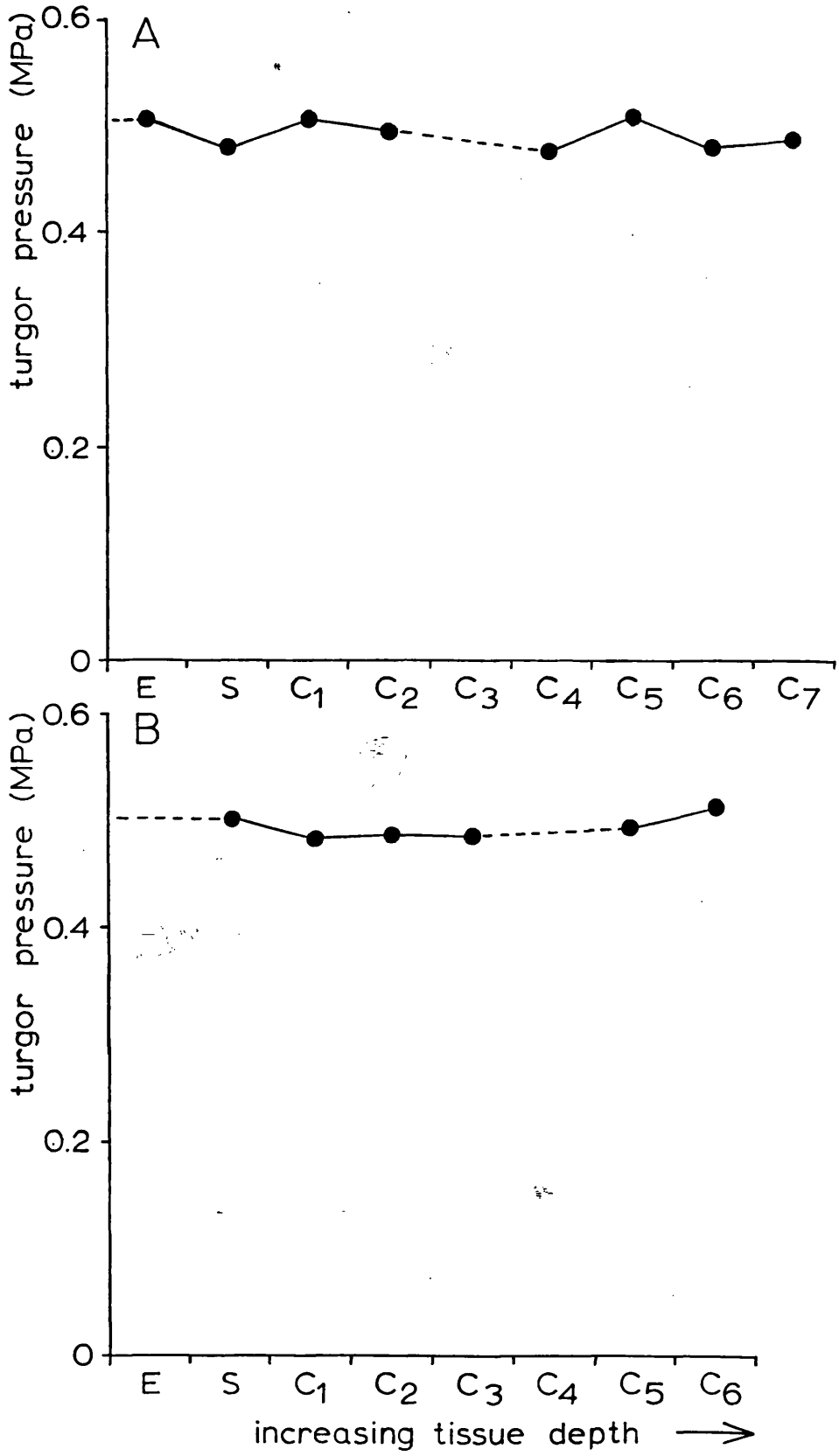
It was not possible to determine the onset of phototropic curvature accurately whilst turgor pressures were being measured with the probe due to the lasso holding the plants steady. A time course for development of curvature of free-standing plants was therefore determined separately (Figure 16), and it is assumed the probed plants follow a similar time course. The time course shows a 'first detectable response' (sensu Appendix II) at 5 min and a true mean lag time (derived by extrapolating the linear portion of the curve to zero curvature) of about 18 minutes. The curvature obtained at one hour was 60-70% smaller than expected from a comparison with Figure 29, possibly due to modification of the light gradient by the different angle of the light stimulus in relation to the plant (e.g. Chapter 6).

The probe itself probably has little effect on the growth of plants and development of the response. Unilateral light caused curvature in plants (as judged by eye) which were being probed. Control plants which had been probed without phototropic stimulation were subsequently given unilateral light and these also responded. Growth rates of plants (c.a. 3 μm/min) measured using a graduated eyepiece in the microscope were similar to those calculated from other data (c.f. Chapters 2, 3 & 5). Cosgrove & Cleland (1983b) also found no influence of the probe on the growth of pea epicotyls.

The turgor pressures of different cells within a plant were found to be surprisingly constant to within 0.02 MPa. No difference in turgor was observed for cells at the top and base of the hypocotyl, and no gradient of turgor was found through the hypocotyl (Figure 17), hence cortical and epidermal cells are assumed to have the same turgor; measurements

FIGURE 17

Consistency of turgor pressure measurements within the hypocotyl of a single plant: A base, B apex. The turgor pressures were measured across the hypocotyl starting with the epidermal (E) and subepidermal (S) cells followed by a series of cortical cells (C). Leaking cells have not been included.


A
B
turgor pressure (MPa)
0.6
0.4
0.2
0
E
S
C1
C2
C3
C4
C5
C6
C7
increasing tissue depth

of both were therefore included in the analysis. Cosgrove & Cleland (1983b) also found no gradient of turgor across pea epicotyls. The turgor pressures of different plants were also very similar, usually in the range 0.4-0.55 MPa. These observations suggest that mustard has an efficient osmoregulatory system. It was not possible to follow the turgor pressure of individual cells for long periods because growth of the plant often caused deformation of the tip giving apparent turgor variations of 0.05 MPa or more (Figure 18). Figure 18 also indicates the length of time over which it is possible to obtain a reliable reading. Measurements were therefore made on a series of cells in the plant during the course of the experiments. Gaps in the time course data are a result of this changing between cells; it was rarely possible to obtain reliable (i.e. non-leaking) cells continuously.

Transpiration rate and solute potential were unaffected by unilateral blue light. The transpiration rate (0.5 ml/min/plant) was identical with and without blue light (Figure 19), indicating changes in P_{wall} do not contribute to the changes in growth rate. Similarly, the osmotic potentials (Table 5) are not significantly different following blue light. The osmotic potentials are an average figure for the tissue and do not indicate the distribution of solutes within the hypocotyl. The difference between osmotic potential and the turgor pressure of non-transpiring plants (Figures 21 & 22) is probably attributable to solutes in the cell walls (Cosgrove & Cleland, 1983a).

Figure 20 shows the turgor pressure measurements during phototropism for the illuminated and shaded sides of the hypocotyls. The two most complete sets of measurements obtained for each side are presented. Similar, less complete, data were also obtained in other plants. No change of

TABLE 5

Osmotic potentials of bulk hypocotyl sap samples before and after phototropic stimulation.

Treatment	mOsmol/kg	MPa
SOX light alone (n=2)	282	0.701
SOX light + 60 min unilateral blue light (n=1)	285	0.705

FIGURE 18

Variation of turgor pressure in a single cell during a long period of measurement.

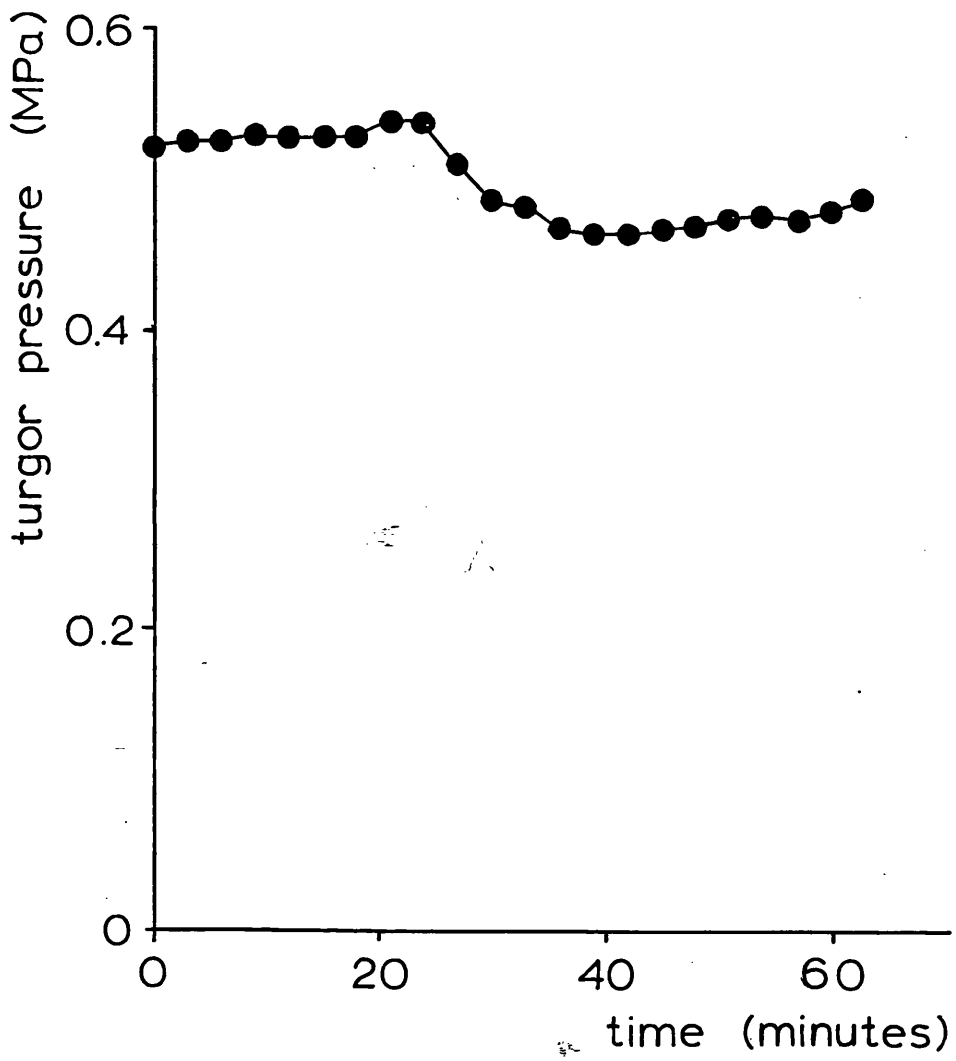

turgor pressure (MPa)
0.6
0.4
0.2
0
0
20
40
60
time (minutes)

FIGURE 19

Weight loss by transpiration of 10 mustard hypocotyls under the experime conditions used to measure turgor pressures. At 40 min, 0.27 μmol m^{-2} s unilateral blue light was added to the SOX background.

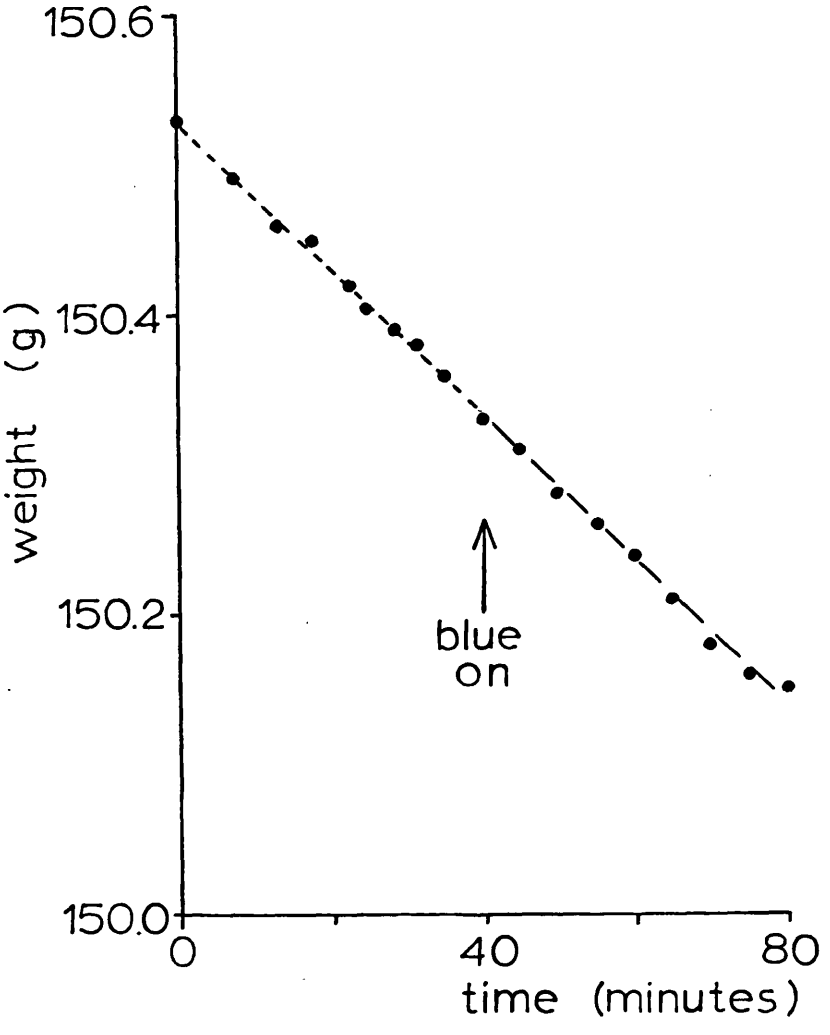
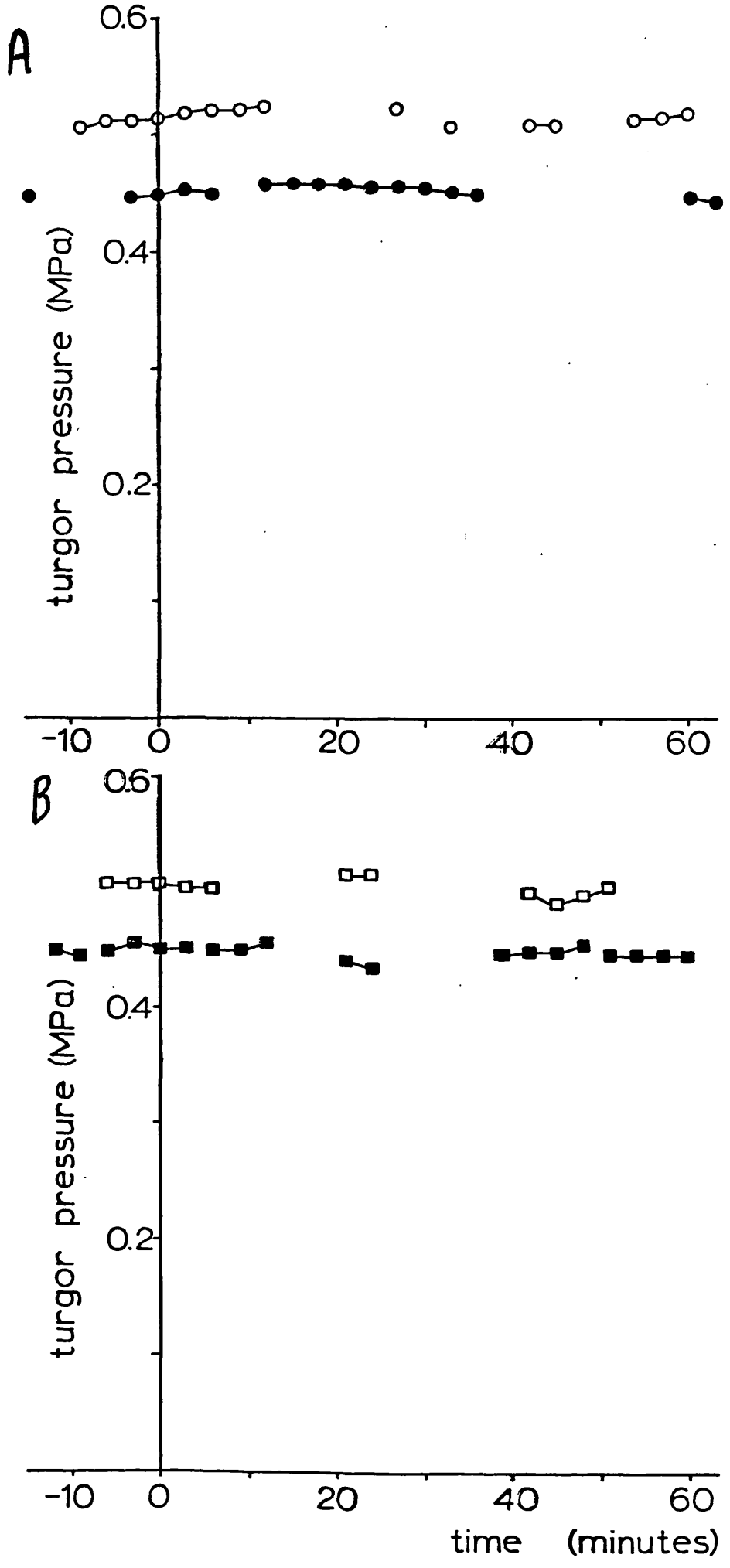

150.6
150.4
150.2
150.0
weight (g)
blue
on
0
40
80
time (minutes)

FIGURE 20

Turgor pressures (data plotted at 3 minute intervals) measured during phototropism of mustard hypocotyls: A illuminated side; B shaded side. Two plants are shown for each side. Readings in the same cell are joine All data obtained by Dr A.D. Tomos.


A
B
0.6
0.4
0.2
turgor pressure (MPa)
-10
0
20
40
60
time (minutes)

turgor pressure to within 0.02 MPa was observed even after 60 minutes stimulation. Control plants without blue light similarly showed no change in turgor pressure with time (data not presented).

Preliminary results for the yield threshold measurements are shown in Figures 21 and 22. The values of the yield threshold obtained assume that no net exchange of water occurs between the plant and the atmosphere of the box, and assume that the yield threshold is constant following excision (Cosgrove, 1985a). When transpiration is stopped by closing the lid of the box, the turgor pressure rapidly increases to over 0.6MPa, indicating a hydrostatic tension in the wall (P_{wall}) of 0.08-0.15MPa. Following excision, the turgor pressures drop with an exponential time course (as expected, Cosgrove 1985a) until a constant turgor pressure of about 0.1 MPa is reached; this value is taken to be the yield threshold (Cosgrove, Volkenburgh & Cleland, 1984). The plants were still turgid at this pressure, indicating water was not lost to the atmosphere of the box. A similar value was observed in one other plant. Further replication was not possible within the time available at Bangor.

In a final experiment carried out at the 11th hour, unilateral blue light was added after the yield threshold had been reached (Figure 22), and the turgor pressure followed on the shaded side of the hypocotyl for a further 30 minutes. No further change in turgor was observed indicating that a decrease in yield threshold may not contribute to the increase in growth rate on the shaded side.

FIGURE 21

Determination of yield threshold using the *in vivo* stress relaxation technique. Turgor pressures were first determined for plants under norm transpiring conditions and then the box covered with the lid to stop transpiration. Plants were then excised to isolate them from their wate supply, and the decline in turgor followed to the yield threshold. See text for further details. Data obtained by Dr A.D. Tomos and T.C.G. Ric

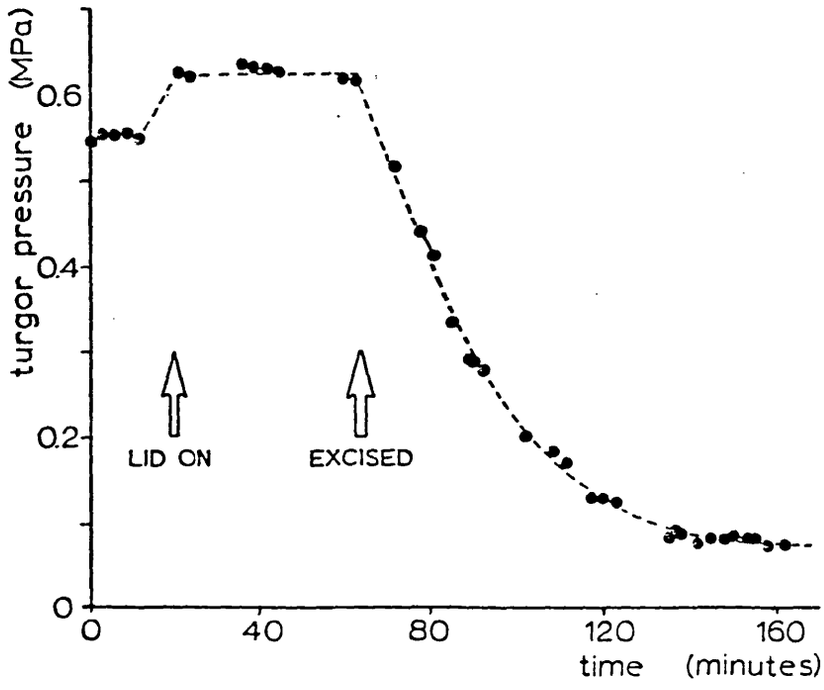
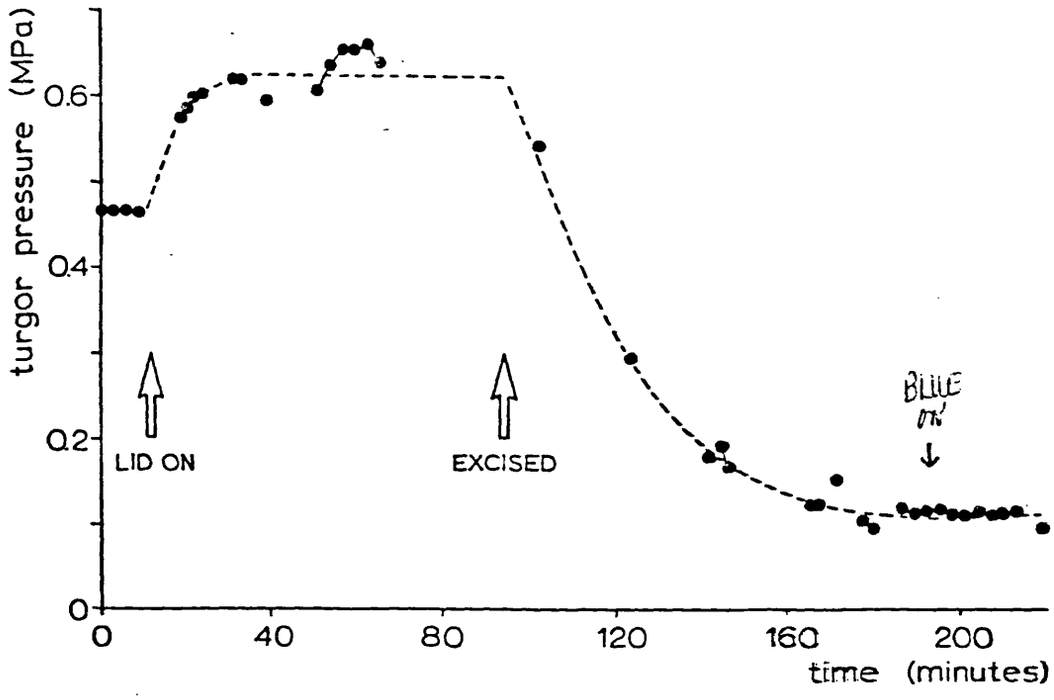

turgor pressure (MPa)
0.6
0.4
0.2
0
LID ON
EXCISED
0
40
80
120
160
time (minutes)

FIGURE 22

Determination of yield threshold as in Figure 20 for a different plant. Once the yield turgor pressure had been reached, unilateral blue light was added and the turgor followed for a further 30 minutes. Data obtain by Dr A.D. Tomos and T.C.G. Rich.


turgor pressure (MPa)
0.6
0.4
0.2
0
LID ON
EXCISED
BLUE ON
0
40
80
120
160
200
time (minutes)

DISCUSSION

Any changes in biophysical parameters can be attributed to phototropic growth responses because separate light-growth responses are eliminated (Chapters 2 & 3), the transpiration rate is constant (Figure 19) and the osmotic potential is constant (Table 5).

Three possibilities can account for the failure to detect any changes in turgor pressure during phototropism (Figure 19). First, if water transport was completely limiting growth rate, the yield threshold would be very close to the growth turgor pressure and the changes in P needed to account for the growth rate changes would be small and undetectable within the 0.02 MPa resolution. Second, if wall properties were completely limiting growth, the changes in turgor accompanying growth rate changes may again be small (c.f. Cosgrove, 1981b) and undetectable. Third, cells may osmoregulate to maintain a constant turgor pressure.

An estimate of the yield threshold is needed to assess the first possibility. The theory behind the stress relaxation technique used to measure yield thresholds is very simple: if no water is available for uptake by cells (for instance by excising hypocotyls to isolate them from their roots), stress relaxation of cell walls should result in a drop in turgor until the yield threshold is reached; at this point further stress relaxation, and hence turgor relaxation should stop (Cosgrove, 1981a; Cosgrove, Volkenburgh & Cleland, 1984). This technique is again the most suitable for phototropism because yield thresholds could be measured for both sides of the hypocotyl (although this has not been attempted here), avoiding the tissue average involved with other (e.g. psychrometric) methods. The influence of solute release and absorption upon excision has not been critically assessed, so the values obtained (Figures 21 and 22) are rough

estimates. They are sufficient, however, to enable calculation of the changes in turgor needed to cause phototropism if water parameters limit growth. Calculations from the growth rate data in Chapter 5, assuming 60-70% maximum curvature rate and true mean lag time of 18 minutes (Figure 16), indicate the growth rate of the illuminated side should decrease to c.a. 40-50% of its prestimulation value within the first hour and that of the shaded side increase to 150-160%. The 0.1 MPa yield threshold (Figures 21 and 22) is approximately 1/4-1/5th of the turgor of growing cells indicating that, given the appropriate coupling between growth rate and turgor (Cosgrove, 1981a), turgor changes of at least 0.2 MPa would be necessary to account for the observed curvatures, hence water transport parameters are not limiting growth rate changes during phototropism. Three additional pieces of circumstantial evidence also indicate water transport is not limiting growth; the lack of a turgor pressure gradient across the hypocotyl (Figure 16), the rapid rise in turgor when transpiration is stopped (Figure 20 and 21), and the large difference between the growth turgor pressure and the yield threshold. These observations also suggest that a fourth possibility to account for no change in turgor, that of wall and water properties changing equally (Cosgrove, 1981a), is unlikely.

It is not possible to distinguish clearly between the second and third explanations. Cosgrove (1981b) found only a small (less than 0.02 MPa) increase in turgor when growth rate decreased by more than 50%. This is similar to the accuracy of resolution here. The consistency of turgor pressures between plants suggests that mustard is certainly capable of long term osmoregulation, but the rapid rise in turgor when transpiration stops indicates that under some conditions short term osmoregulation does

not occur. It is possible both explanations contribute to the consistency of turgor measurements.

The results suggest that phototropism is not caused by changes in water transport parameters since large changes in turgor would be expected in order to control growth osmotically. Hence phototropic growth must be caused by changes in wall rheological properties. Further experiments are required to clarify whether the wall extensibility or yield threshold, or both are responsible for the growth rate changes, though changes in the yield threshold alone cannot account for the differential growth. The result of a preliminary experiment (Figure 22) indicates that the yield threshold may not change in response to a unilateral light stimulus, hence phototropic growth may be attributable to changes in wall extensibility alone. Clearly, however, the changes in wall properties must be in opposite directions and equal in magnitude on the shaded and illuminated sides of the hypocotyl in order to account for the pattern of differential growth (Chapter 3), and further work must identify what causes the changes and if they are controlled by the same mechanism on the two sides.

These results are preliminary and require further quantification, particularly in the cells which limit the growth rate of the hypocotyl, but they represent the first attempt to analyse the biophysical parameters of growth during phototropism.

CHAPTER 5

THE RELATIONSHIP BETWEEN LIGHT DOSE AND PHOTOTROPIC RESPONSE

INTRODUCTION

The relationship between light dose and response is a standard physiological investigation used to provide information on the nature of the photomorphogenetic system. Two main features of the relationship are usually investigated: first, the gross features such as threshold and saturation doses and the nature of the relationship between these two limits; these can be used to interpret the function of the photoreceptor system in the plant and also as important indicators for selection of experimental conditions. Second, reciprocity; this can sometimes be used to establish whether a single photoreceptor is operating. The Bunsen-Roscoe reciprocity law states for any photochemical reaction where a single photoreceptor is operating, that if the quantity or dose of light (fluence rate x duration) is constant, then the photochemical effect remains the same, irrespective of how the dose is achieved. If reciprocity holds over a range of doses, there is good evidence that a single photoreceptor is operating and that light is the limiting step in the transduction chain under those conditions. Failure of reciprocity does not, however, prove that more than one photoreceptor is operating as other steps in the transduction chain may be limiting.

The complex nature of the dose-response relationship found in phototropism of higher plants is well-known (for detailed review, see Dennison, 1979). In etiolated Avena coleoptiles, there are first-positive, first-negative and second-positive (and sometimes third-positive) phases of the response curve (e.g. du Buy & Nuernbergk, 1934; Zimmerman & Briggs,

1963; Blaauw & Blaauw-Janse, 1970a,b). Other species lack the negative phase and may either show distinct first and second positive responses (e.g. Steyer, 1967; Iino & Briggs, 1984) or a more gradual transition between the two phases (e.g. Everett, 1974; Ellis, 1984). The characteristics of the response curve vary with species, pretreatment, physiological state, etc., and also depend on how the phototropic stimulus is given. Curry (1969) suggested the observed relationship may be a consequence of the way the basic system is experimentally probed.

Part of the problem lies in the fact that there is little agreement as to what actually constitutes the best measure of a phototropic response (Dennison, 1979) or when to measure it. Two parameters which have been used to quantify phototropism are the rate of curvature and the final angle obtained. More frequently an intermediate measure is used, the curvature obtained after a specific time period (usually 100 minutes in *Avena* or *Zea*). Firn *et al* (1983) pointed out that measurement of the changes in growth rate provides more useful information about the mechanisms of phototropism, and Badham (1984) pointed out that % differential growth should be used if there is a high variance amongst growth rates or organ diameters.

There are also a number of other factors which may add to the complexity. Both gravitropic compensation and autotropic straightening may obscure the development of phototropic curvature (Shen-Miller & Gordon, 1967; Pickard *et al*.,1969; Dennison, 1979; Firn & Digby, 1979; Franssen, Firn & Digby, 1982); furthermore, separate light-growth responses have been shown to modify differential growth (see Chapter 3). Pickard *et al* (1969) pointed out that the light gradient within the tissue changes as plants curve, and this would also be expected to modify curvature (see Chapter 6). Furthermore, there are several phases of the phototropic response which

may also vary in their relationship to the stimulus dose. Although the careful investigations of Pickard et al (1969) and Iino & Briggs (1984) indicate that many of the previously reported (c.f. above) features of the dose-response relationships in monocots are real, there have been no thorough investigations of the relationship in dicots. Hence, in order to clarify and simplify the situation, the individual components of the phototropic response in light-grown mustard seedlings have been analysed for fluence rate dependency. As these plants are light-grown, changes in sensitivity concurrent with de-etiolation induced by the experimental light treatments are also avoided (e.g. Blaauw & Blaauw-Jansen 1970b).

MATERIALS AND METHODS

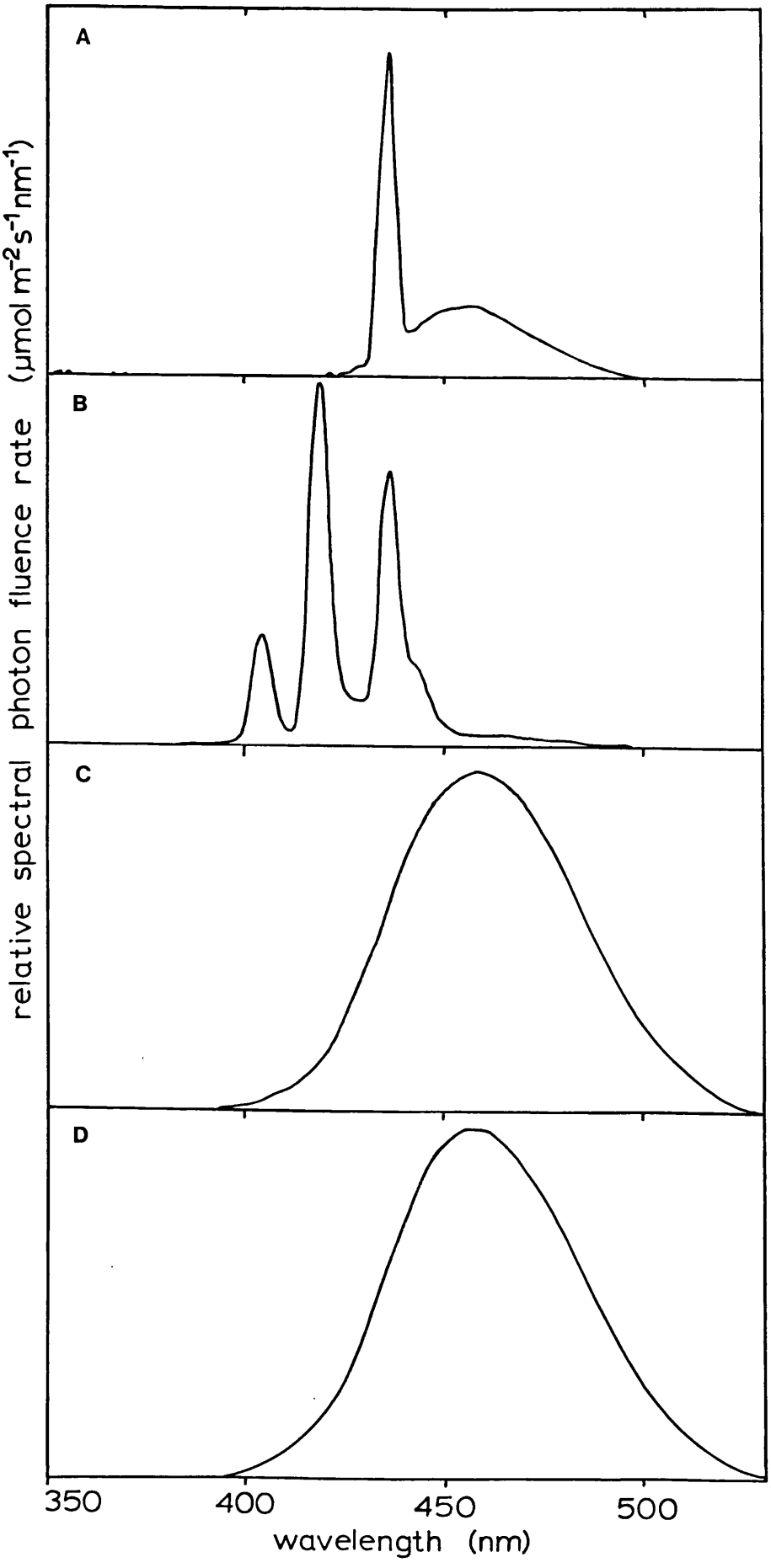
For the general experiments, mustard seedlings were grown and treated as previously described (Chapter 2) and with one exception, the background SOX light was 45 μmol m^{-2} s^{-1}. For each fluence rate, 10 plants were used in each experiment, and each experiment repeated three times on different days.

Light sources

Continuous broad-band blue light of varying fluence rates was obtained for the general experiments from a variety of sources for technical reasons. Blue light of low fluence rates 0.0016 and 0.005 μmol m^{-2} s^{-1} was obtained from a 40W Phillips TLAK Deluxe fluorescent tube covered with 2 layers of Primary blue Cinemoid (s.p.d.s. Figure 23a). Blue light of medium fluence rates 0.046, 0.1, 0.21, 0.34, 0.46 and 0.866 μmol m^{-2} s^{-1} was obtained as above but with only 1 layer of cinemoid (s.p.d.s. Figure 1a). Blue light of high fluence rates 2.16 and 4.66 μmol m^{-2} s^{-1} was obtained

FIGURE 23

Spectral photon fluence rate distributions. A:Low-fluence-rate blue l for general phototropism experiments. B: High-fluence-rate blue light for general phototropism experiments. C: Low-fluence-rate blue light for lag time experiments. D: Blue light source used in clinostat experiments.


A
B
C
D
relative spectral photon fluence rate (μmol m⁻² s⁻¹ nm⁻¹)
350
400
450
500
wavelength (nm)

from a 150W Thorn Graph-X lamp filtered through 1 layer of primary blue Cinemoid (s.p.d.s. Figure 23b). Fluence rates were varied either by altering the light-plant distance or with tracing paper neutral density filters.

For the lag time experiments, low-fluence-rate 0.033 μmol m^{-2} s^{-1} blue light was obtained from a 60W incandescent bulb filtered through 1 layer of primary blue Cinemoid (s.p.d.s. Figure 23c). Medium-fluence-rate 0.64 μmol m^{-2} s^{-1} blue light was obtained from 40W fluorescent tubes with 1 layer of Cinemoid as above. High-fluence-rate 9.75 μmol m^{-2} s^{-1} blue light was obtained from 40W tubes covered with 1 layer of No.32 Masterline medium blue Cinemoid (s.p.d.s. Figure 5d).

For the clinostat experiments, the blue light source was specially constructed to fit into the centre of the clinostat wheel and radiate light as evenly as possible towards the plants located on the perimeter. A 500W Phillip tungsten-halogen lamp controlled by a variable resistor was inserted into the core of a circular water distillation vessel. The outside of the vessel was covered with 1 layer of primary blue Cinemoid. Water was passed through the vessel jacket to cool the lamp and remove heat from the light. The light spectrum (s.p.d.s. Figure 23d) did not change significantly at the different fluence rates.

Measurement of the lag

Seedlings were grown as above, and the lag time between induction of phototropism and the first detectable phototropic movement of the plant measured using an "electronic arm" angular transducer (Phillip Harris Ltd, Shenstone, Staffs., UK). The arm was orientated horizontally and carefully balanced so as to offer as little resistance to movement as possible. Plants were attached to the arm by a fine Nickel-Chrome wire (s.w.g. 40) 2cm long, weighing c. 2.5 mg. The wire was gently wedged

between the two petioles of the cotyledons and held in place with a small drop of lanolin. The wire was attached to, and insulated from, the transducer arm with BLU-TACK (Bostik Ltd., Leicester). This mode of attachment was as unintrusive as possible and flexible enough at the joints to allow for growth of the seedlings and the changes in the geometry of orentation, but rigid enough to transmit the smallest lateral movement of the plant (e.g. the plants and transducer were sensitive to small air currents). After handling, the plants were left either overnight or for a minimum of four hours before recording began.

The transducer was used on its 10° sensitivity range. It works in the following manner: the transducer arm, which is delicately pivoted at its centre, has a capacitor blade attached which lies between two fixed plates. A high-frequency alternating voltage applied to the capacitor blade induces a current through the fixed plates dependent on their relative positions. This current is then amplified and output to a chart recorder. Hence any movement of the transducer arm changes the current through the plates which is then registered on the chart recorder. The transducer arm was used within the limits between which output voltage is directly related to position of the arm. The angular movement of the arm is not directly proportional to the angular curvature of the plant, but calculations of the geometrical errors involved over the limited ranges recorded indicate the errors are of the order of 1% maximum and are thus considered insignificant. Careful calibration of the transducer indicates the chart recorder gives a reasonably accurate representation of the linear lateral displacement, or phototropic response, of the plant. The lag time was taken as the first detectable movement or change in rate of movement after phototropic stimulation. For each of the three fluence rates investigated, data from 15 plants were used.

Gravitropism experiments

To determine gravitropic sensitivity of plants to small angles of curvature, plants were grown as above and then tilted at an angle of 15° to the vertical, and the time course for gravitropic compensation followed photographically.

Clinostat experiments

As the growth conditions used in the previous experiments were unsuitable for plants to be used on the clinostat, they were modified as follows. Seeds were soaked in tap water for 30 minutes and then sown in moist perlite in small, glass scintillation vials. They were then grown in clear plastic sandwich boxes in the constant environment room to prevent desiccation of the perlite, and the seedlings used at 5½ days when of equivalent size to the vermiculite-grown seedlings (even though not of comparable age). The moist perlite was sufficiently cohesive to hold the seedlings steady when they were orientated horizontally on the clinostat. Seedlings were equilibrated overnight on the clinostat in a background of 20 μmol m^{-2} s^{-1} SOX light. This SOX light was of lower fluence rate than other experiments due to the technical limitations of applying a uniform irradiation field over the clinostat wheel. Treatment was started the following day and the curvature followed photographically (without stopping the clinostat) using a camera with autowind. Although this procedure allowed accurate estimation of the rate of curvature, it was not possible to obtain the resolution necessary for analysis of the changes in growth rate during phototropism.

The clinostat was a large (29 cm radius), vertically orientated, wooden wheel which rotated at 1 r.p.m. giving the plants a centripetal acceleration of 0.0032 ms^{-2} (3.3×10^{-4}g). Vials containing the seedlings were held in place on the perimeter of the wheel by spring clips. Ten plants were used in each experiment.

RESULTS

A detailed time course for the development of curvature in plants not treated on the clinostat in response to 2.16 μmol m^{-2} s^{-1} is shown in Figure 24. The response can be divided into 4 phases. First, a lag or latent period during which no curvature occurs. Second, an initial response phase with a high rate of curvature which continues until c. 90-120 minutes after stimulation. Third, a phase with a slower rate of curvature, and finally a fourth phase where a constant angle of 35-40° is maintained. This time course is qualitatively similar to that in many other plants.

During analysis of growth rates, the general impression of the development of curvature was that it occurred more or less simultaneously down the length of the hypocotyl. A pictorial representation of development of curvature within the hypocotyl for the time course in Figure 24 is shown in Figure 25. Interpretation is quite simple; the relative lengths of the arms give a measure of the distribution of curvature within the axis. If curvature occurs uniformly down the length of the hypocotyl the arms should be equal in length. If more curvature occurs in the upper half of the hypocotyl the lower arm will be longer, and vice versa. Initially, there is more curvature in the upper half of the hypocotyl as expected as the extension rate of the upper half is approximately twice that of the lower half (data not presented). This curvature distribution is maintained for approximately 120 minutes before the relative lengths of the arms change, the upper arm becoming progressively longer despite the increase in angle of curvature. A similar pattern was observed consistently in other experiments including the clinostat treatments, and the phenomenon appears to be independent of the light stimulus.

FIGURE 24

Detailed time course for development of phototropic curvature in respons to 2.16 μmol m^{-2} s^{-1} continuous, unilateral blue light. Points are mean ± s.e. (n=15).

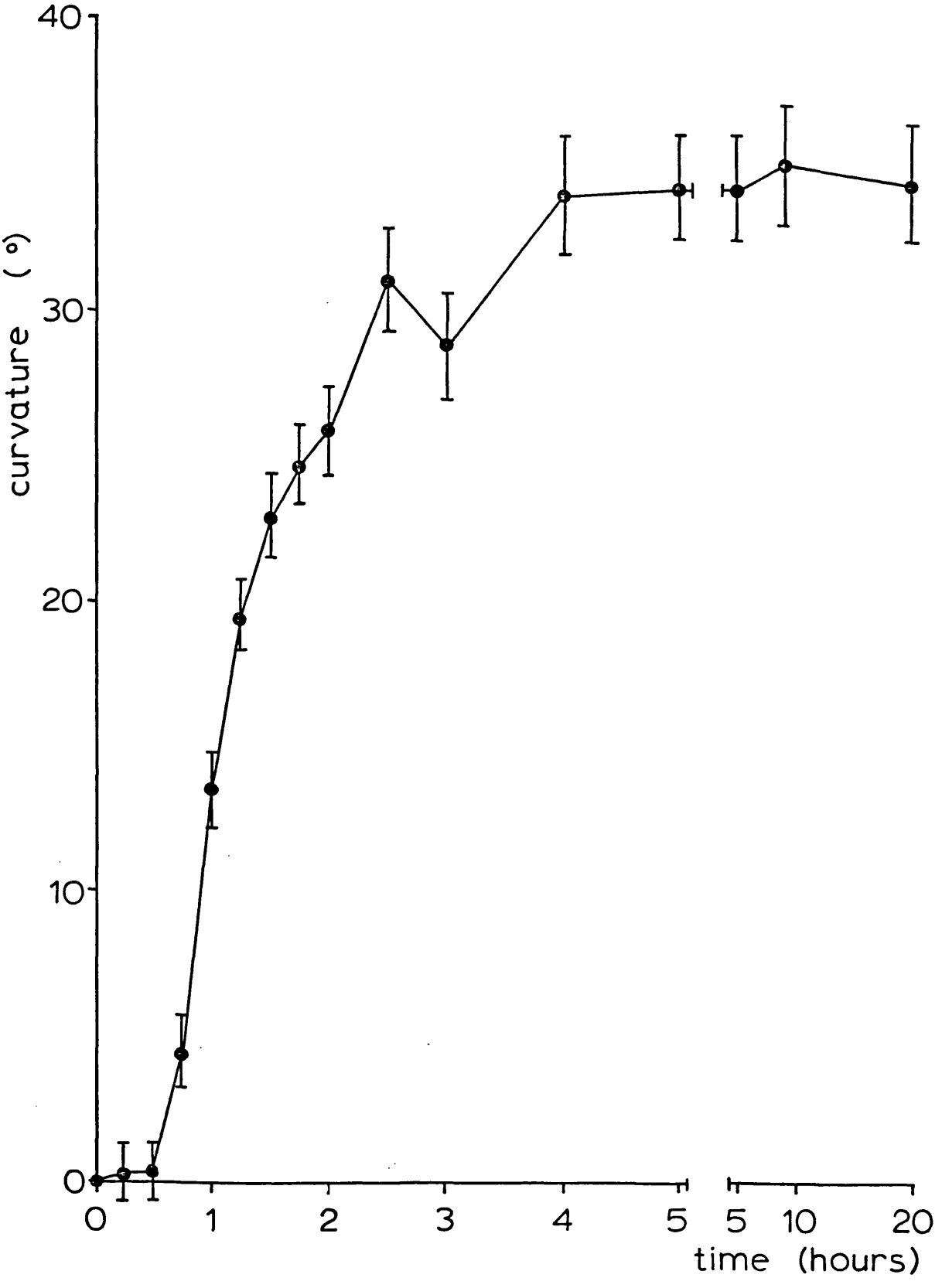
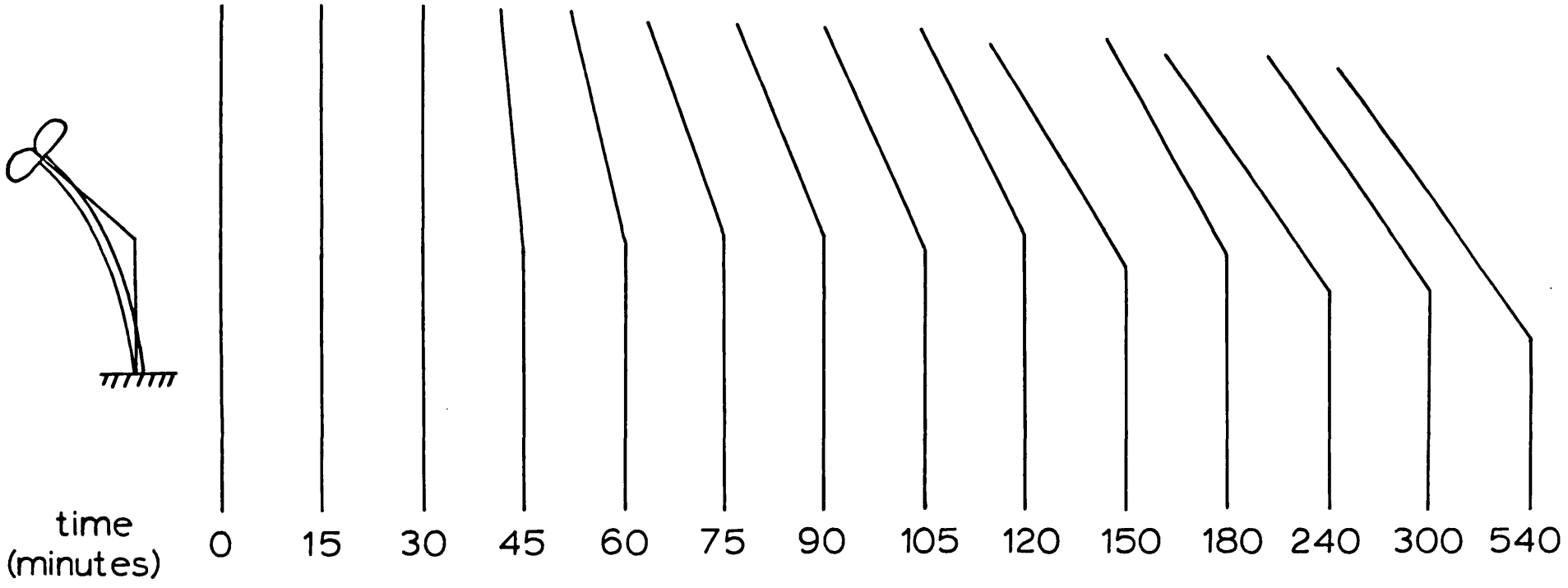

40
30
20
10
0
curvature (°)
0
1
2
3
4
5
5
10
20
time (hours)

FIGURE 25

Pictorial representation of development of phototropic curvature within mustard hypocotyls (data from plants in Figure 24). Figures were constructed by taking the mean relative lengths of the tangents used to determine curvature.


time
(minutes)
0
15
30
45
60
75
90
105
120
150
180
240
300
540

Cosgrove (1985b) also found that curvature began simultaneously along the stem.

The first three phases of the response curve were analysed for fluence rate dependency. Under conditions of continuous illumination, the final angle of curvature obtained (35-55°) was found to be related to the geometry of the light source in relation to the plant, and is not dependent on the fluence rate (data not presented).

Because the blue light sources used to obtain the different fluence rates differ in their relative spectral photon distributions (Figures 1 and 23), they may also differ in their relative efficiencies at inducing phototropism due to the absorption spectrum of the photoreceptor. To compare relative efficiencies, spectral scans were recalibrated against an action spectrum taken from Curry (1969) (a procedure also used by Pickard et al., 1969). The light sources were only found to differ slightly in their relative efficiencies and when replotted, data showed the same features. The fluence rates were therefore left in their original form.

The lag phase

The transducer was selected to investigate fluence rate dependency of the lag because it gives a more precise resolution of the kinetics than time-lapse photography. The two techniques do not give the same result (compare Table 6 with the lags in Chapter 4 and Appendix II), but the transducer is consistent within the confines of the experiment and thus directly comparable between fluence rates.

Circumnutation, here considered to be an autonomous growth movement, can obscure the onset of curvature. To minimise this problem, plants showing more than 0.5° of circumnutation in the period before treatment commenced were rejected (c. 50% of the plants). This may introduce bias to the results for comparison with other techniques, even though phototropism

and circumnutation are independent phenomena (Britz and Galston, 1983). Examples of traces showing circumnutation are shown in Figure 26.

All 15 transducer traces for the 0.64 μmol m^{-2} s^{-1} blue light treatment are shown in Figure 27 to give an estimate of the variability. The traces show a relatively long lag period during which time no curvature occurred, followed by a fairly rapid phase of onset of curvature which makes estimation of the lag time relatively precise. A surprisingly constant rate of curvature is then achieved after a mean of 10.3 $\pm$ 3.2 SD minutes, and then maintained, at least for the first 30 minutes after onset of curvature. The kinetics of curvature observed are similar to those predicted from the high-resolution growth data of Baskin et al., (1985).

The effect of the three different fluence rates on the duration of the lag is shown in Table 6. The data show the lag is independent of the fluence rate, at least over the 250-fold range examined.

The curvature phases

To confirm that separate light-growth responses unrelated to phototropism are eliminated, the changes in growth rate that give rise to curvature were measured. For all fluence rates examined (selected examples in Figure 28), curvature during the first 3 hours at least was caused by an acceleration of growth on the shaded side of the hypocotyl accompanied by an inhibition on the illuminated side, with little or no change in net growth. The mean prestimulation growth rates differed slightly between experiments but were not significantly different. In some cases after 3 hours the net growth rate increased or decreased slightly in comparison to the pre-stimulation rate. These data are consistent with those in Chapters 2 and 3 and indicate that at least for the first half of the time course, the different curvatures are caused by the same pattern of differential

FIGURE 26

Examples of transducer traces showing circumnutation in mustard seedlings

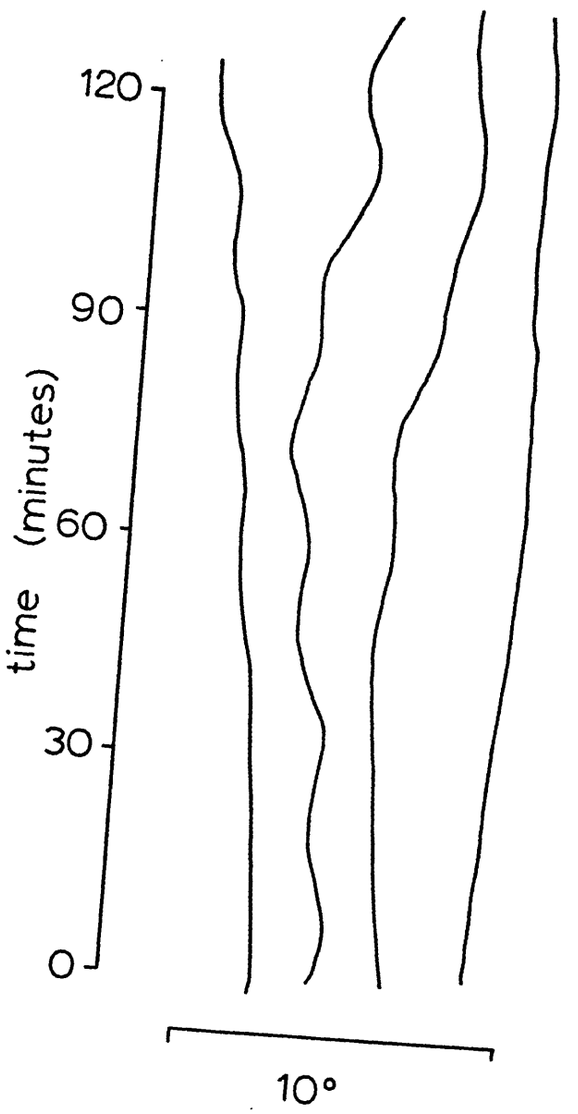

120
90
60
30
0
time (minutes)
10°

FIGURE 27

Transducer traces showing the initial kinetics of phototropism of mustard seedlings in response to 0.64 μmol m^{-2} s^{-1} continuous blue light. Movement to the left indicates curvature towards the light. Arrows indicate the time at which curvature begins.

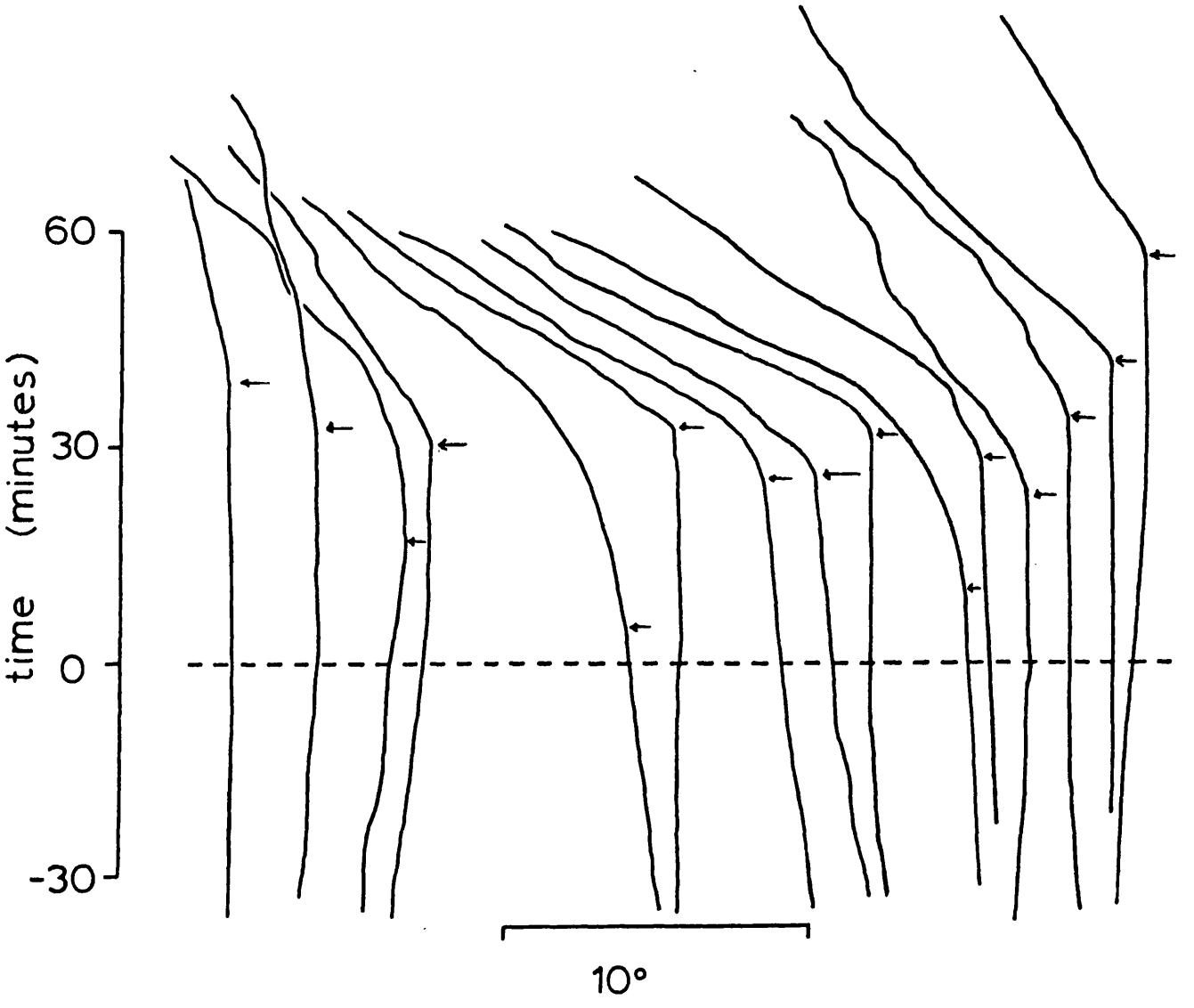

60
30
0
-30
time (minutes)
10°

FIGURE 28

Selected patterns of differential phototropic growth at 3 different flue rates of continuous, unilateral blue light. Points are means of 3 experiments, 10 plants to each experiment. A: 4.66 μmol m^{-2} s^{-1}. B: 0.21 μmol m^{-2} s^{-1}. C: 0.046 μmol m^{-2} s^{-1}.

■ illuminated side. ● shaded side. --- prestimulation rate.

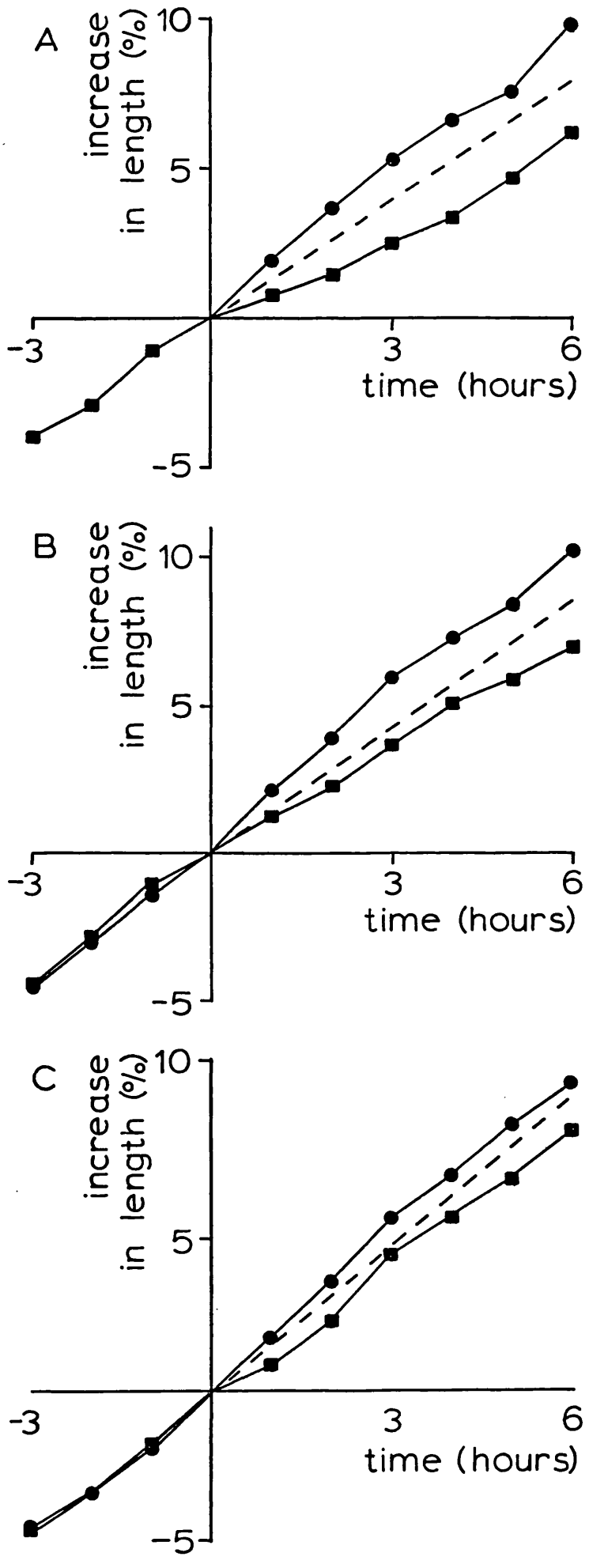

A
increase in length (%)
10
5
-5
-3
3
6
time (hours)
B
increase in length (%)
10
5
-5
-3
3
6
time (hours)
C
increase in length (%)
10
5
-5
-3
3
6
time (hours)

TABLE 6. True mean lag times for phototropism in response to different fluence rates of continuous, unilateral blue light. Figures are the mean lag time of individual plants ± s.e. (n=15).

Fluence rate (μmol m^{-2} s^{-1})	lag time (minutes)
0.0334	30.93 ± 2.3
0.64	30.2 ± 3.1
9.75	30.77 ± 3.6

growth, and presumably therefore, the same mechanism. Hence, as there is a highly significant correlation between magnitude of differential growth and magnitude of curvature ($P < 0.005$), simple measurements of curvature can be used as an unambiguous measure of the phototropic response. Because of the consistency in pattern of differential growth, it is assumed the same mechanism occurs in all other treatments too.

Time courses for development of curvature for plants not treated on a clinostat at different fluence rates of continuous, unilateral blue light are shown in Figure 29. There is a complex time-dependent relationship between curvature and fluence rate. The data show that during the initial rapid phase of curvature (i.e. after the lag and before ca. 2 hours), the rate of curvature increases with increasing fluence rate until it reaches a maximum at about 0.21 μmol m^{-2} s^{-1}. This maximum rate of curvature is also observed with fluence rates of up to 24 μmol m^{-2} s^{-1} (data not presented). After two hours the rate of curvature slows and sometimes even stops, the onset and characteristics of which are not clearly related to fluence rate or curvature already obtained.

To determine whether or not the maximum rate of curvature obtained during the initial phase of curvature is caused by saturation of the photoreceptor, plants were simultaneously irradiated with 0.866 μmol m^{-2} s^{-1} continuous blue light from one side and 0.34 μmol m^{-2} s^{-1} continuous blue light from the other. If the photoreceptor is saturated at 0.21 μmol m^{-2} s^{-1} then the plant should not be able to distinguish between the two different fluence rates and no phototropic response should occur. Figure 30 shows the plants do respond by curvature towards the brighter light, indicating the response is not light-limited. This experiment also demonstrates that the rate of curvature is determined by the relative

FIGURE 29

Time course for development of phototropic curvature of plants not treate on the clinostat in response to different fluence rates of continuous, unilateral blue light. Points are means of 3 experiments, 10 plants to each experiment. The mean standard error of each experiment was 2.16°, and 95% of the experiments showed SE's smaller than 2.32°.

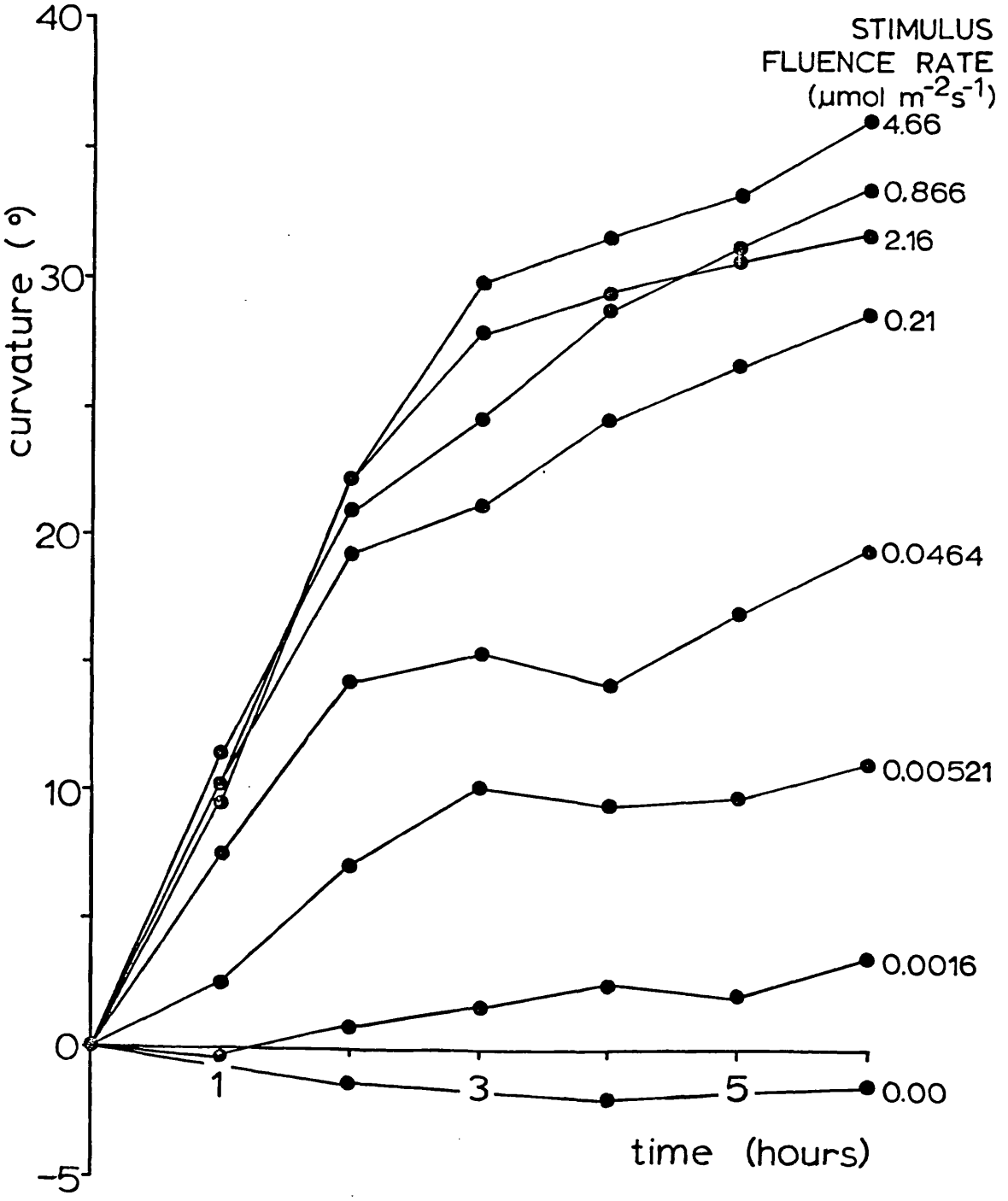
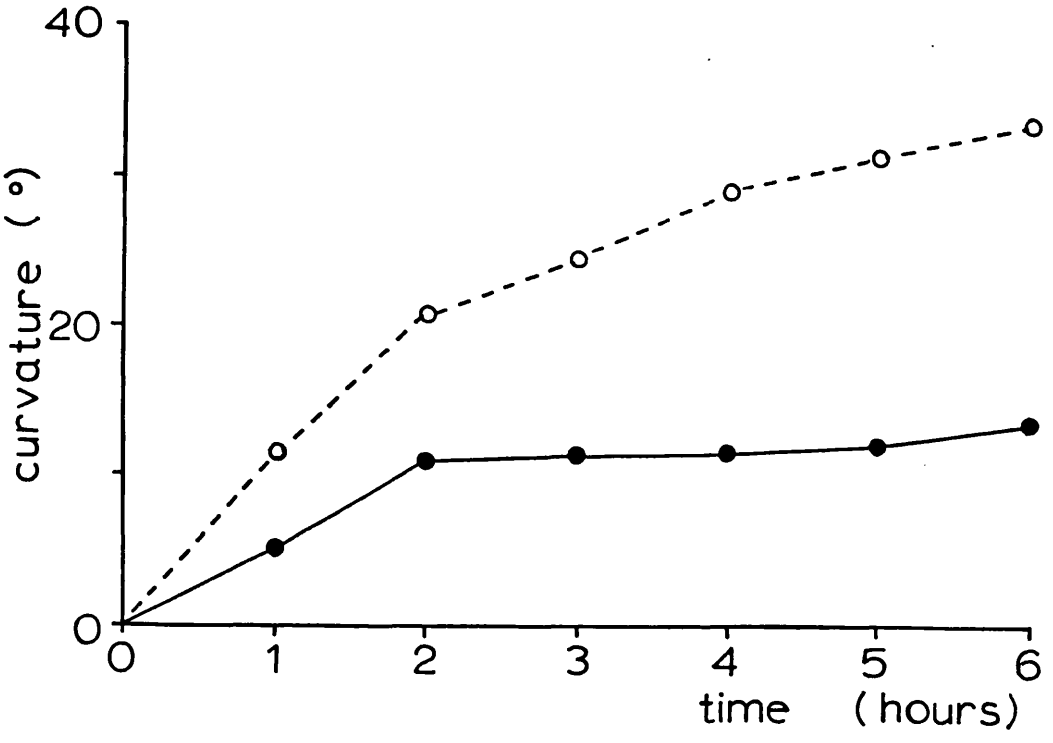

40
30
20
10
0
-5
curvature (°)
STIMULUS
FLUENCE RATE
(μmol m^{-2}s^{-1})
4.66
0.866
2.16
0.21
0.0464
0.00521
0.0016
0.00
1
3
5
time (hours)

FIGURE 30

Time course for development of phototropic curvature in response to simultaneous addition of 0.866 μmol m^{-2} s^{-1} continuous blue light from one side and 0.34 μmol m^{-2} s^{-1} from the opposite side (-●-). The plants curve toward the brighter light. The 0.866 μmol m^{-2} s^{-1} unilateral light data (Figure 28) are given again for comparison (--O--).


40
20
0
curvature (°)
0
1
2
3
4
5
6
time (hours)

difference in fluence rates on the opposite sides of the hypocotyl and not the absolute difference. If the absolute difference in fluence rates determined the rate of response (as, for instance, predicted from a Blaauw-type mechanism) the curvatures would be the same in both unilateral and unequal bilateral treatments as the absolute difference in fluence rates is above the maximum established by the saturation fluence rate (c.f. Figure 29). The decrease in curvature is not due to sensitivity adaptation (e.g. Delbrück and Reichardt, 1956 (see Dennison, 1979); Galland and Russo 1984) because fluence rates of unilateral light higher than the total applied during the unequal light treatment also give the maximum curvature rate. The relationship between light gradient and phototropism is examined further in Chapter 6.

Gravitropic compensation and autotropic straightening

To determine if a gravitropic response can be induced by the small angles of curvature obtained during the initial phototropic responses, upright plants were tilted at an angle of 15° to the vertical (Figure 31). The time course for curvature shows the plants reorientate towards the vertical after an estimated mean lag time of 30-35 minutes. The development of phototropic curvature was therefore analysed on a clinostat to eliminate potential gravitropic responses.

The data show several features in common with Figure 29, although fewer plants were analysed and curvatures more variable. The pattern of curvature at different fluence rates is very similar to that of plants not treated on a clinostat, at least for the first two hours. The rate of curvature of plants treated on a clinostat is greater than that of plants not treated on a clinostat, possibly due to the differences in

FIGURE 31

Time course for gravitropic compensation of plants tilted at an angle of 15° to the vertical. Points are mean $\pm$ s.e. (n=10).

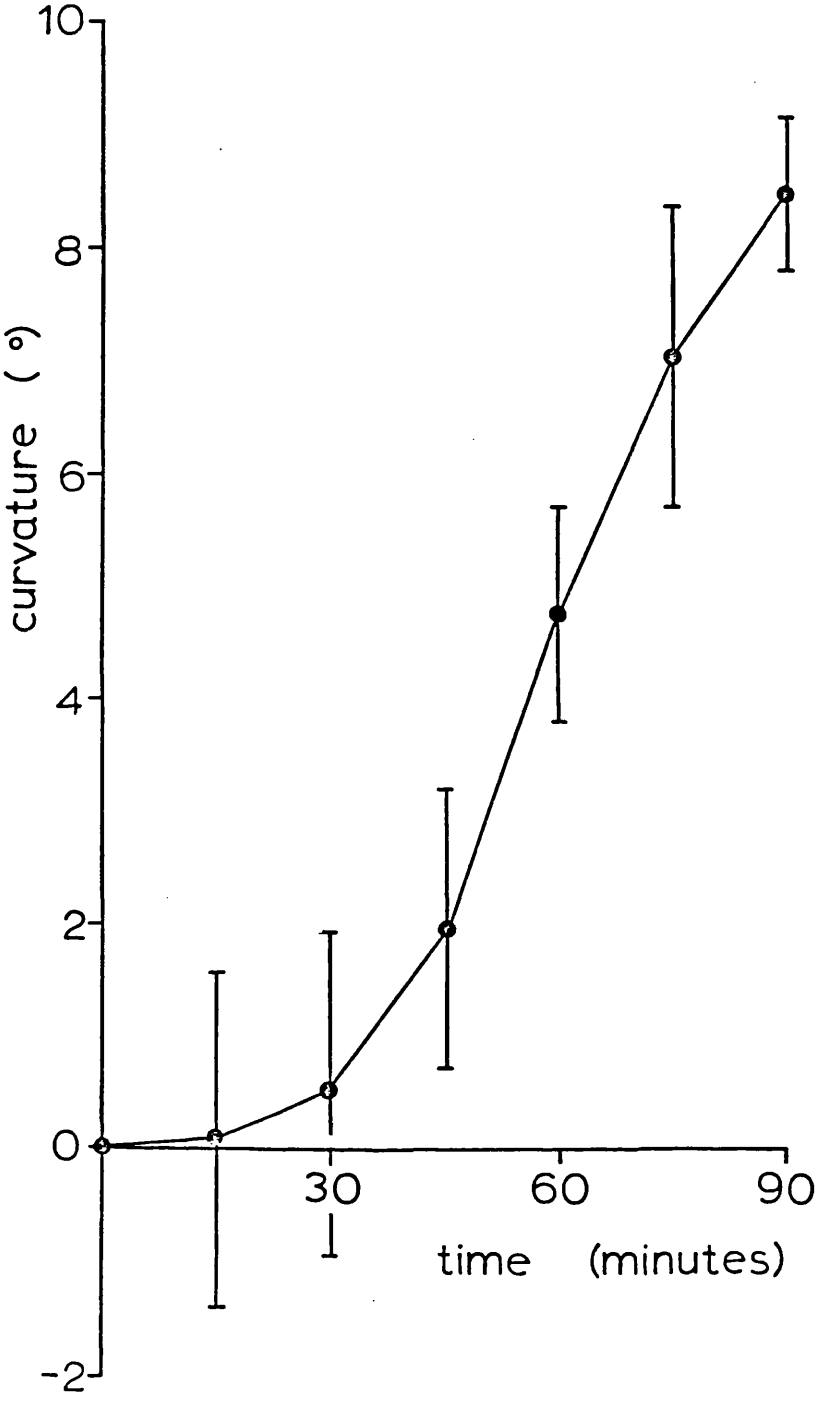
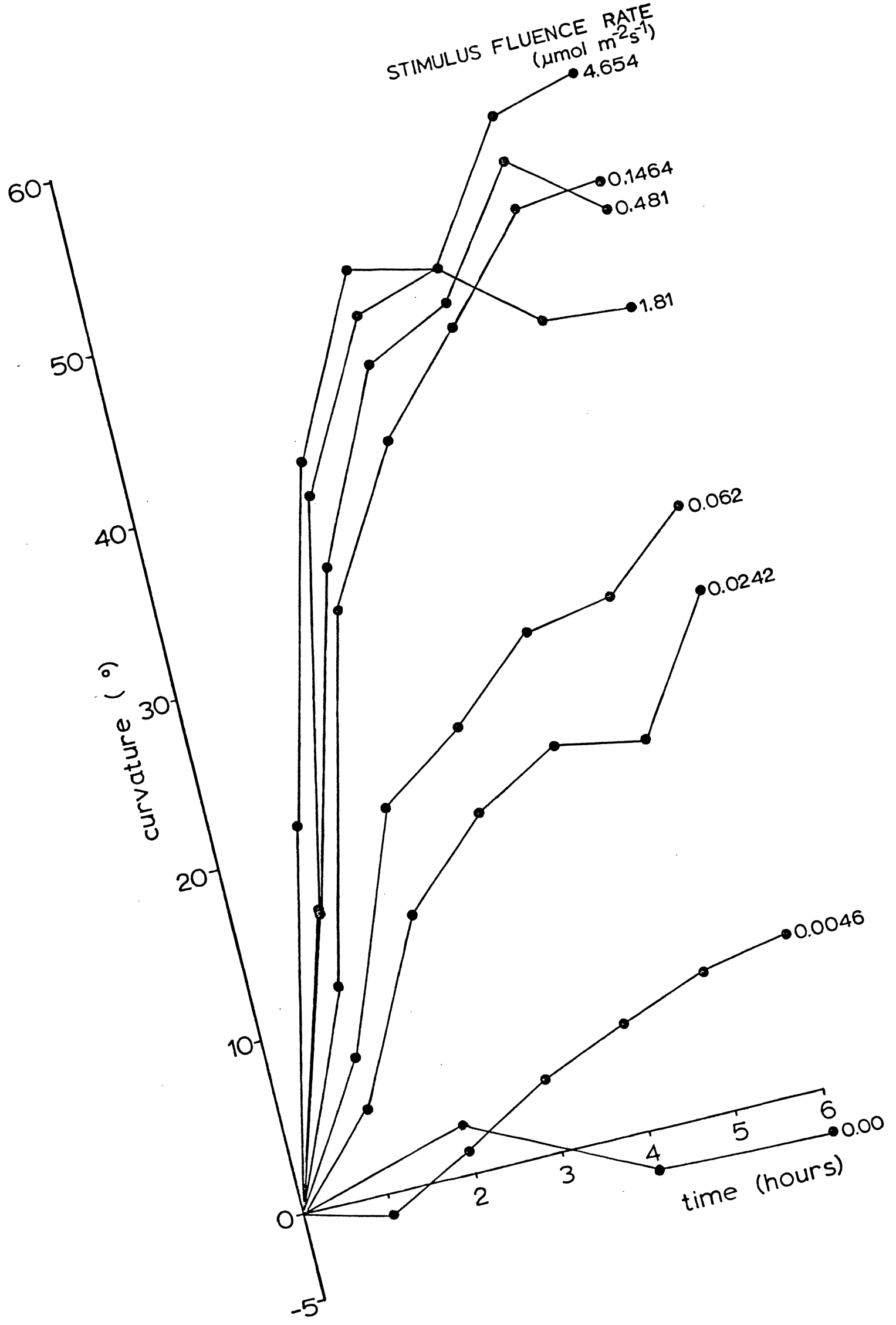

10
8
6
4
2
0
-2
curvature (°)
30
60
90
time (minutes)

FIGURE 32

Time course for development of phototropic curvature of plants treated on a clinostat in response to different fluence rates of continuous, unilateral blue light. Points are means of 10 plants. The mean standar error of each experiment was 3.78°, and 95% of the experiments showed SE's smaller than 4.21°.

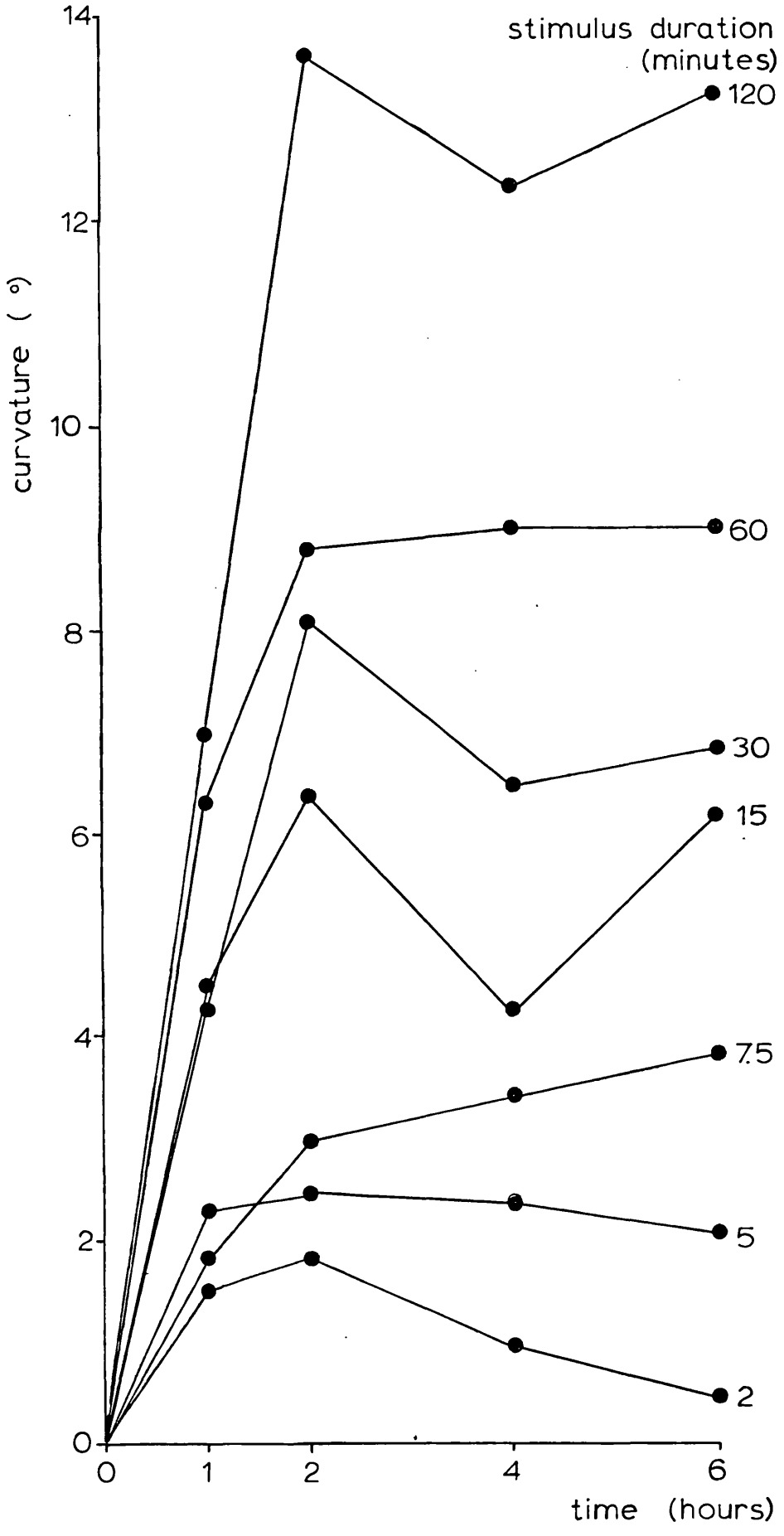

STIMULUS FLUENCE RATE
(μmol m-2s-1)
4.654
0.1464
0.481
1.81
0.062
0.0242
0.0046
0.00
60
50
40
30
20
10
0
-5
curvature (°)
2
3
4
5
6
time (hours)

net growth rate of the hypocotyl. Plants treated on the clinostat in general had longer hypocotyls at the end of the experiment than plants not treated on the clinostat, indicating the former had higher growth rates. Franssen et al. (1982) also found that plants treated on the clinostat grew faster than plants not treated on the clinostat. A maximum rate of curvature was obtained at 0.15 umol m^{-2} s^{-1}, a fluence rate very similar to that observed in plants not treated on the clinostat (Figure 29). The absolute magnitude of the curvature of plants treated on the clinostat is also greater than that of plants not treated on the clinostat, indicating the latter are subject to gravitropic compensation. Pickard et al (1969) and Franssen et al (1982) also found that curvature continued longer in the absence, than in the presence, of a directional gravitropic stimulus. Plants receiving fluence rates above the saturation level of 0.15 μmol m^{-2} s^{-1} all behaved in a similar manner, suggesting that in plants not treated on the clinostat the onset of gravitropic compensation is related to the fluence rate of the light, implying a counteractive expression of the two responses (Pickard et al., 1969; Franssen et al., 1982). Plants on the clinostat show a slowing of the rate of curvature which can be interpreted in terms of autotropic straightening.

If the fluence rate is kept constant (0.1 μmol m^{-2} s^{-1} was selected to avoid saturation) but the duration of exposure varied to give different doses, the kinetics of curvature of plants not treated on the clinostat change (Figure 33). The data show that the response is completed much more rapidly for short exposures than for long exposures. The development of the response therefore also depends on the duration of the exposure in addition to the fluence rate. When the data are replotted to show the curvature obtained after 1 and 2 hours (Figure 35) there is a non-linear relationship between dose and curvature. The shapes of the curves

FIGURE 33

Time course for the development of phototropic curvature in response to different times of exposure to 0.1 μmol m^{-2} s^{-1} unilateral blue light. Points are means of 3 experiments, 10 plants per experiment.


stimulus duration
(minutes)
120
60
30
15
7.5
5
2
curvature (°)
14
12
10
8
6
4
2
0
0
1
2
4
6
time (hours)

are similar to those reported by Everett (1974) and Hart and MacDonald (1981).

DISCUSSION

What is the best measure of a phototropic response?

There are two problems relating to measurement of a phototropic response; first, what to measure, and second, when to measure it. The extent of phototropism is usually expressed as an angle of curvature, but Firn *et al* (1983) pointed out that as measurement of the changes in growth rate provides more information about the mechanism of phototropism, they should be a better measure of the response than simple angles of curvature. In practice, as differential growth and curvature are directly geometrically related, measurement of either can be used to quantify the response if the pattern of differential growth is the same in all cases. On *a priori* grounds, the rate of development and/or the final extent of the response might be expected to be related to the light stimulus, but as the development of phototropism with time is complex (e.g. Figure 24) the time of measurement must be carefully selected.

High-resolution measurements of growth in maize coleoptiles (Baskin *et al*, 1985) show that the initial changes in growth rate are rapid (probably complete within 5 minutes), and that once established, the new growth rates are constant. The apparent slow build-up to high rates of curvature frequently observed in plots of mean curvature (such as Figure 24) are largely due to the contribution of different lag times in the

population. The transducer traces (Figure 27) also show that the growth rate changes (here expressed as curvature) in continuous light are rapid and then maintained for at least 30 minutes after the onset of curvature, and probably for at least a further 30-60 minutes (Figures 24 and 29). For peak first-positive responses in maize (Iino and Briggs, 1984), the constant differential growth rate is maintained for about 90 minutes. For short-term light treatments in mustard (Figure 33), the growth rates are probably only maintained for short lengths of time. The period during which the differential growth rates are maintained is hereafter termed the phase of constant differential growth, and is equivalent to the second phase of curvature in Figure 24. Responses measured during this phase, assuming a constant lag time (Table 6), can therefore be used to estimate the rate of development.

Subsequent to the phase of constant differential growth, the growth rates usually revert back towards their prestimulation rates. The features of this subsequent period (termed the phase of decreasing differential growth) do not appear to have been characterised in detail. It is not known whether the differential growth rates decrease gradually or abruptly, but the decrease in rate of curvature in Figure 24 is at least partly due to data averaging again. This onset of this phase could be an intrinsic part of the phototropic response, but Pickard et al (1969) found that plants treated on a clinostat continued to curve for much longer than plants not treated on a clinostat, hence gravitropic compensation is at least one factor involved. It is also likely that autotropic straightening, an intrinsic feature of tropic curvatures (Firn and Digby, 1979), may be involved. Assuming no gravitropism or autotropism, the final extent of the response will be obtained when differential growth ceases. One

feature of the final extent of the response worth noting is that it is an integral of the differential growth, and thus not completely independent of the growth rates during the development of the response. Any limitations of the magnitude of differential growth will therefore obscure the true maximum extent of the response.

When comparing the effects of different light treatments it is clearly important to measure the response in an appropriate and equivalent developmental phase, and the kinetics of curvature should therefore be examined for all treatments. If the response is measured after a specific time only (as in the "classical" 100 minutes), different phases may be being compared, e.g. curvatures measured between 1 and 2 hours in Figure 33: At the shortest duration (2 minutes), the final angle of curvature has been obtained, whilst at the longest (2 hours) the response is probably just completing the phase of constant differential growth. As the kinetics of response development have usually not been examined for all doses in many published data, caution must be taken in interpreting their significance. It should also be noted that responses measured during the phase of decreasing differential growth measure neither rate of development nor the final extent of the response and are thus liable to be ambiguous.

In this study, the best measure of the phototropic response is considered to be the initial rate of curvature measured during the phase of constant differential growth. Measurement of the changes in growth rates show the pattern of differential growth is the same for all fluences (Figure 28) hence differential growth and curvature are directly equivalent. There is also little variability between organ diameters or growth rates. The distribution of curvature within the hypocotyl is also constant during this phase (Figure 25). The final angle of curvature obtained in continuous

light was not related to fluence rate and in any case would be subject to autotropism and gravitropism (see below); this is not to say that under other circumstances the final extent of the response could not be an adequate quantitative measure of the response. The light gradient established within the tissue should also not change significantly at the small curvatures obtained during this phase (Pickard et al, 1969); the relationship between light gradient and curvature is examined in more detail in Chapter 6. Curvature is preferred here to differential growth for presentation of the results because it is conceptually simpler, more directly compared with other data and also far easier to measure!

Relationship of individual components of the response to the fluence rate

The independence of the true mean lag times from stimulus fluence rate (Table 6) indicates that complexity of dose-response relationships cannot be attributed to different latent periods. Reciprocity would not be expected to hold if both the lag time and the rate of curvature were fluence rate dependent. There have been few attempts to analyse this relationship for classical blue-light-mediated phototropism. Hart and MacDonald (1981) implied a fluence-rate-dependent lag but did not present their data. Cosgrove (1985b), as here, found the lag to be independent of fluence rate. These previous reports should, however, be treated with caution as lag times were compared for only two relatively high fluence rates using the first detectable response in the population (c.f. Appendix II). Dose-dependent lag times have been reported in other systems, e.g. phytochrome-mediated phototropism (Iino, Briggs & Schäfer, 1984), sun-tracking in Lavatera (Schwartz & Koller, 1978) and leaflet folding in Oxalis (Bjorkman and Powles, 1981). One possible interpretation of a

dose-independent lag is that the initial photoactivation is short in comparison to the remainder of the transduction chain.

The rate of curvature measured within the phase of constant differential growth is fluence-rate-dependent (Figure 34; redrawn from Figure 29). Data for curvatures at both 1 and 2 hours are presented, although strictly only the former should be included if a true mean lag time of 30 minutes is assumed for both phototropic (Table 6) and gravitropic (Figure 31) responses. Over the 250-fold range between threshold and saturation fluence rates the response is linearly related to log fluence rate: phototropism thus obeys the Weber law (Shropshire, 1979). This suggests (assuming reciprocity holds) the photoreceptor detects photons and initiates the response in a logarithmic manner. The maximum rate of curvature is attained at a surprisingly low fluence rate (0.1-0.2 μmol m^{-2} s^{-1}). Ellis (1984) points out that fluence rates of 1.0 μmol m^{-2} s^{-1} or greater are in common use and suggests that phototropism is light-saturated under such conditions. In mustard at least (Figure 30), the response is limited by dark processes in the transduction chain and not by the photoreceptor. One possible explanation is that a maximum amount of differential growth has been achieved (i.e. the potential for differential growth is saturated), and no further increase in the rate of curvature can occur. In other studies where the growth rate on the illuminated side is usually observed to stop (Iino & Briggs, 1984; Baskin et al., 1985) limitation of the magnitude of differential growth must restrict the potential for further increase curvature rate. Above the saturation fluence rate, the final angle of curvature would be expected to be a function of stimulus duration.

After 2 hours as the differential growth rates begin to decline there is no longer a clear relationship between fluence rate and curvature rate. The shape of the fluence-rate response curve varies with time (not presented)

FIGURE 34

Curvatures at 1 hour (●) and 2 hours (■) replotted from Figure 28 to show the relationship between fluence rate and rate of curvature.

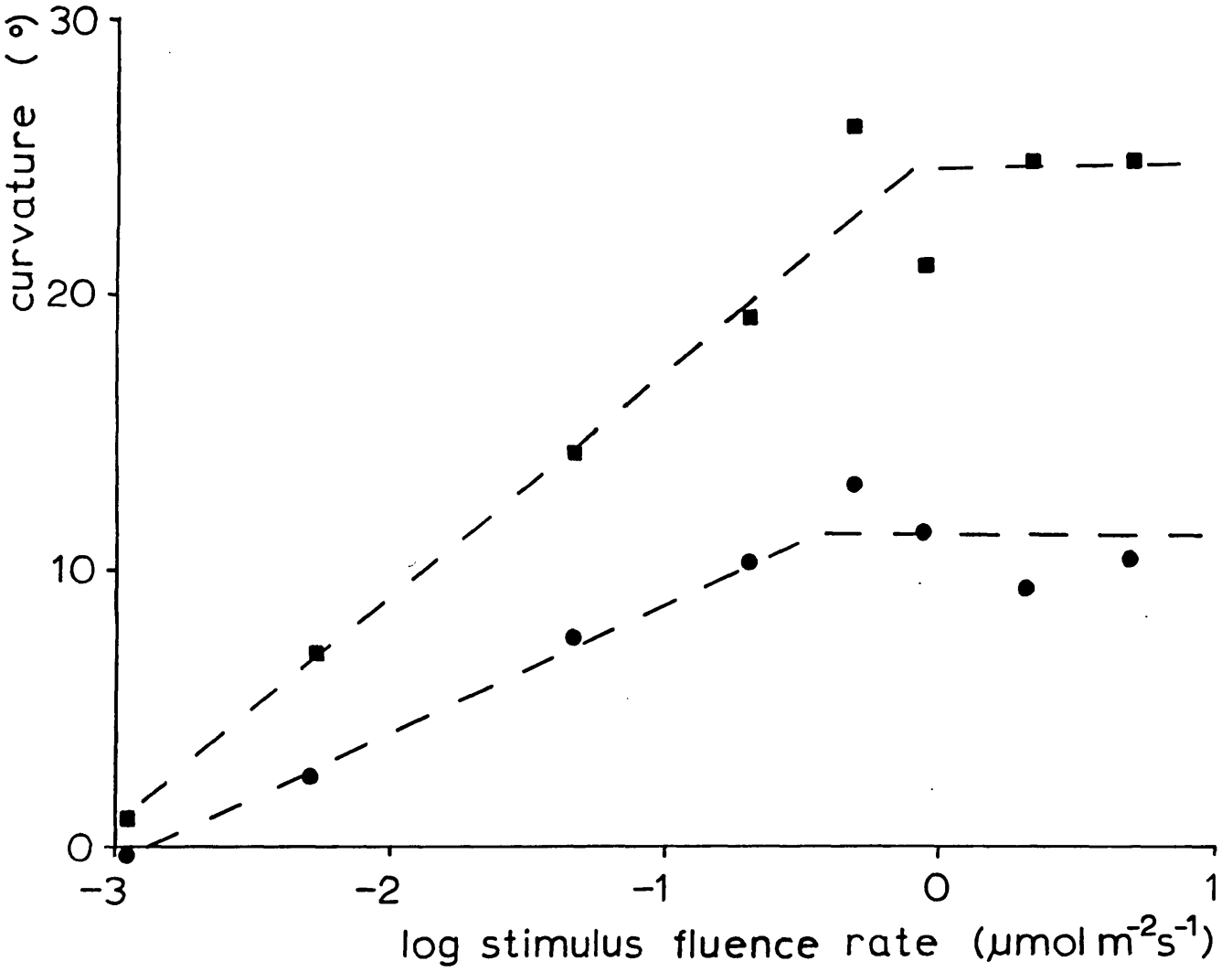
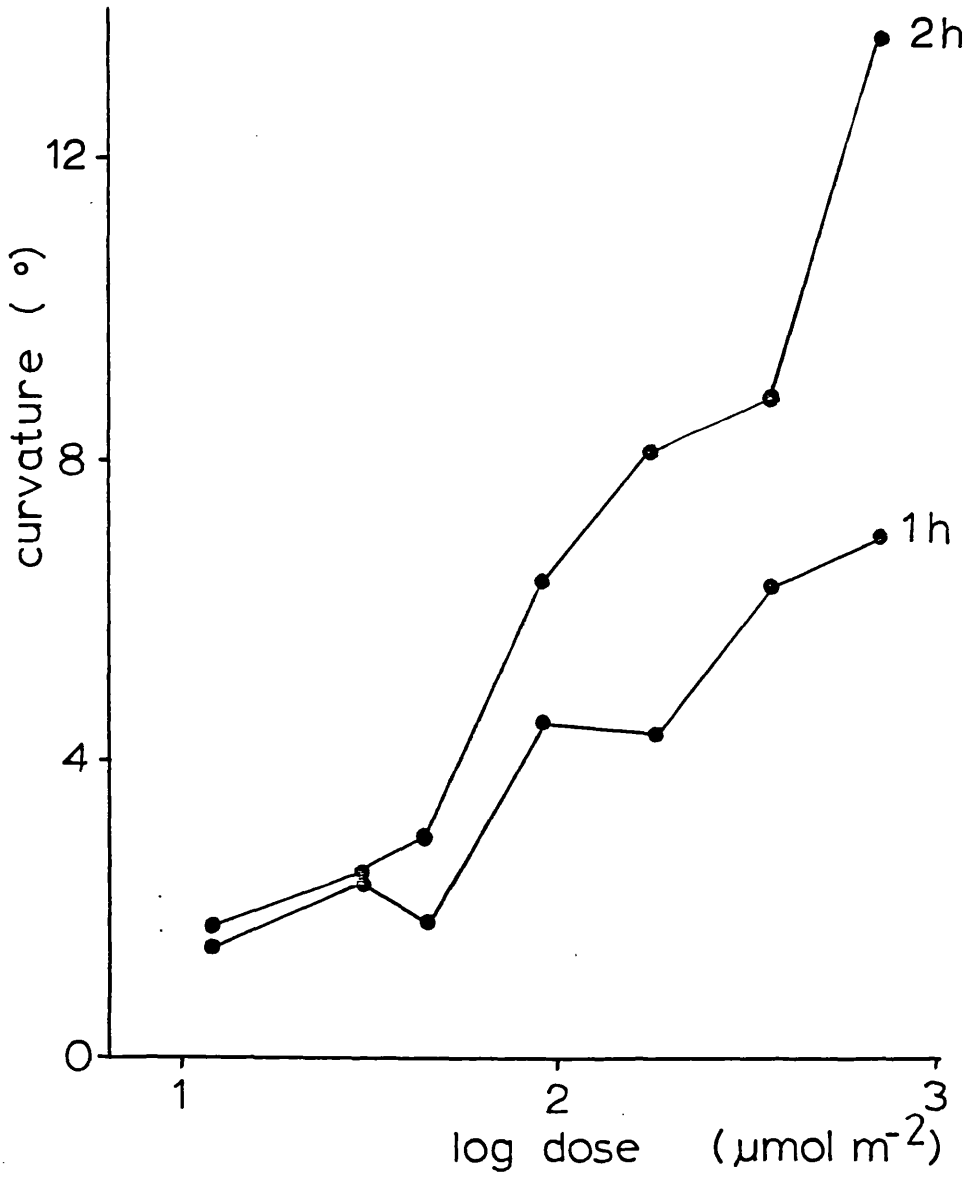

curvature (°)
30
20
10
0
-3
-2
-1
0
1
log stimulus fluence rate (μmol m⁻²s⁻¹)

FIGURE 35

Curvatures at 1 and 2 hours replotted from Figure 33 to show the relatio between light dose and curvature. The different doses were obtained by varying duration of exposure to 0.1 μmol m^{-2} s^{-1}. Points are means of 3 experiments, 10 plants per experiment.


2h
1h
12
8
4
0
curvature (°)
1
2
3
log dose (μmol m-2)

which is at least partly due to the onset of gravitropic compensation and autotropic straightening (Figures 31 and 32). The influence of gravitropism will depend on the sensitivity of the plants and the timing of gravitropism in relation to phototropic curvature. The data of Figure 29 suggest the influence of gravitropism may also be related to the fluence rate of the phototropic stimulus; inhibition of curvature rate is more pronounced at lower fluences. Such an interactive mechanism may give the impression of a continuation of the dose-response curve if responses are measured during this phase. Autotropic straightening appears to be a phenomenon intrinsic to tropic curvatures (Firn & Digby, 1979). Both these phenomena would be expected to contribute to the complexity of dose-response relationships, and must therefore be excluded.

To test for reciprocity would require considerably more detailed measurements of the rate of curvature than have been obtained here. If reciprocity held, the 3 different forms of dose-response curves (varying fluence rate, varying time of exposure, or varying both) would be identical. The fact that Figure 35 differs from Figure 34 is hardly surprising as the curvatures are measured in different phases of the response. Similarly, the apparent complete failure of reciprocity (Table 7) for curvatures measured at 1 hour can hardly be considered significant. Hence, it is not known whether reciprocity does or does not hold in this system, but the results do point towards photobiological conditions under which reciprocity should be tested. For instance, reciprocity would only be expected to hold for stimuli of low fluence rates, or for brief, high fluence rates where the total light dose is below a critical saturating level. Interestingly, these are two of the three conditions under which reciprocity holds in buckwheat (Ellis, 1984). This is, of course,also assuming that other additional complicating factors such as separate light-growth responses,

TABLE 7. A comparison of curvatures at different light doses obtained at 1 hour after phototropic stimulation. The doses were given in a variety of combinations of fluence rate and time. Curvatures are the mean of 3 experiments, each experiment consisting of 10 plants.

Dose (μmol m^{-2})	Fluence rate (μmol m^{-2} s^{-1})	Exposure time	Curvature (degrees)
7200	3.0	40 mins	7.983
2160	3.0	12 mins	4.05
	1.0	36 mins	11.72
720	3.0	4 mins	2.2
	1.0	12 mins	4.93
	0.3	40 mins	8.78
216	3.0	72 secs	1.57
	1.0	3.6 mins	4.36
	0.3	12 mins	5.72
	0.1	36 mins	7.2
72	3.0	24 secs	0.75
	1.0	72 secs	1.9
	0.3	4 mins	0.483
	0.1	23 mins	6.417
	0.03	40 mins	3.48
21.6	1.0	21.6 secs	1.383
	0.3	72 secs	1.4
	0.1	3.6 mins	3.5
	0.03	12 mins	3.35
7.2	0.3	24 secs	0.433

failure to measure comparable stages of response development, changes in the light gradient, autotropism and gravitropism are eliminated. The latter may also explain the third condition under which reciprocity holds in buckwheat; the small final angles of curvature obtained may either not have been sufficient to induce a gravitropic response or may have been attained before the onset of gravitropic compensation.

Some features of the dose-response relationship can therefore be attributed to the way the system is experimentally probed (c.f. Curry, 1969). Clearly, once again, more care must be taken with many experiments! The fact that reciprocity does hold in some systems e.g. Avena (Zimmerman and Briggs, 1963; Steyer, 1967), Zea (Briggs, 1960); Lepidium (Fröschel, 1980; cited in Ellis, 1984), Lens (Steyer, 1967), Pisum, Helianthus and Raphanus (Fuller and Thuente, 1941), Phycomyces (Blaauw, 1909), Fagopyrum (Ellis, 1984) and Arabidopsis (B. Steinitz, pers. comm. 1985), assuming the reciprocity is not coincidence, must be considered highly significant evidence that only one photoreceptor is operating in phototropism. Two additional pieces of evidence from second-positive curvature in Avena where reciprocity does not hold also indicate that only one photoreceptor is operating. Thimann and Curry (1961:see Curry, 1969) found the shape of the dose-response curve was the same for two (436 & 365nm) widely separated wavelengths , and Everett and Thimann (1968) found the action spectrum of second positive curvature was not different from that for first-positive curvature. Both these observations argue against the involvement of multiple photoreceptors and suggest reciprocity failure could again be due to experimental conditions.

CHAPTER 6

THE RELATIONSHIP BETWEEN LIGHT GRADIENT AND PHOTOTROPIC RESPONSE

INTRODUCTION

Phototropic responses can be induced when a sufficient light gradient is established within a plant axis by a heterogeneous light environment. Light gradients are set up by light scattering and absorption in the tissues (Vogelmann & Haupt, 1985), and they are of key importance to the response in two ways. First, the direction of the response is determined by the direction of the greatest relative light gradient across the tissue (Buder, 1920; Darwin & Darwin, 1880), not by the absolute direction of the light beam. Second, the magnitude of the response is also probably determined by the magnitude of the gradient, as curvature can be modified by changing the gradient characteristics (e.g. du Buy, 1934; Bunning *et al.*, 1953; Brauner, 1955, 1957; von Guttenberg, 1959; Pickard *et al.*, 1969; Vierstra & Poff, 1981; Poff, 1983). Zimmerman & Briggs (1963) and Briggs (1963b) however cite evidence against such a relationship and suggest that the magnitude of the response is not affected by the gradient. As Kunzelmann & Schäfer (1985) demonstrated the importance of the light gradient in phytochrome-mediated phototropism (see also Appendix I), and as Figure 29 indicates that the rate of curvature is dependent on both the absolute amount of light and the relative difference in fluence rate on the opposite sides of the hypocotyl, the relationship has been reinvestigated for blue-light-mediated phototropism in light-grown mustard seedlings.

MATERIALS AND METHODS

Plants were grown and treated as described in Chapter 2; 8 plants were used in each experiment, and each experiment was repeated 3 times. Curvature was measured at 0.5, 1.0, 1.5 and 2h after the onset of stimulation. Light sources were as described in Chapter 2 with the exception that the background SOX light was 45 μmol m^{-2} s^{-1}. For unilateral blue light treatments a constant fluence rate (0.11 μmol m^{-2} s^{-1}) reference light was applied to one side. This fluence rate was selected to induce as large a response as possible in unilateral light without saturating the rate of curvature (Chapter 5). For bilateral light treatments, the reference light source was applied to one side simultaneously with addition of a variable fluence rate light source to the opposite side. The fluence rate of the variable light source was varied by altering the distance to the plants.

The % transmission of light through the hypocotyl was measured using a 400W Thorn Graph-X discharge lamp. Hypocotyls (c. 1mm wide) were carefully sandwiched between 2 pieces of black card along narrow slits c. 0.5mm wide, and laid directly over the spectroradiometer head taking care to eliminate stray light. The % transmission (mean of 10 hypocotyls) thus represents light passing through the central core of the hypocotyl.

RESULTS

The experimental relationship between curvature and internal light ratio

Light gradients can be manipulated by either changing the spectral properties of the plant (e.g. du Buy, 1934; Poff, 1983) or the applied light regime (Pickard et al., 1969). The latter technique was selected here as it is non-intrusive. The key assumption is that if the internal

light environment is directly related to the external light environment (an assumption made by anyone analysing plant growth in relation to light), a change in the externally-applied ratio should result in a similar change in the internal light ratio. The internal light ratio is here defined as the ratio of the amount of light in the illuminated half of the hypocotyl to that in the shaded half ("illuminated" and "shaded" being relative differences in light quantity).

The light gradient (or internal light ratio) was manipulated by applying different unequal bilateral light treatments. A constant reference fluence rate (ref.) was added to one side of the plant, and simultaneously a variable fluence rate (var.) light added to the opposite side (for $0 \leqslant$ var. $\leqslant$ ref. μmol m^{-2} s^{-1};note the extremes represent unilateral and equal bilateral treatments respectively). When curvatures are plotted against the applied light ratio calculated as the variable fluence rate divided by the reference fluence rate (i.e. analogous to Pickard et al., 1969), they show an exponential relationship (Figure 36). Curvatures obtained in unilateral light are similar to those reported in Chapters 2 and 5 with the exception of the somewhat lower than expected t=½h point.

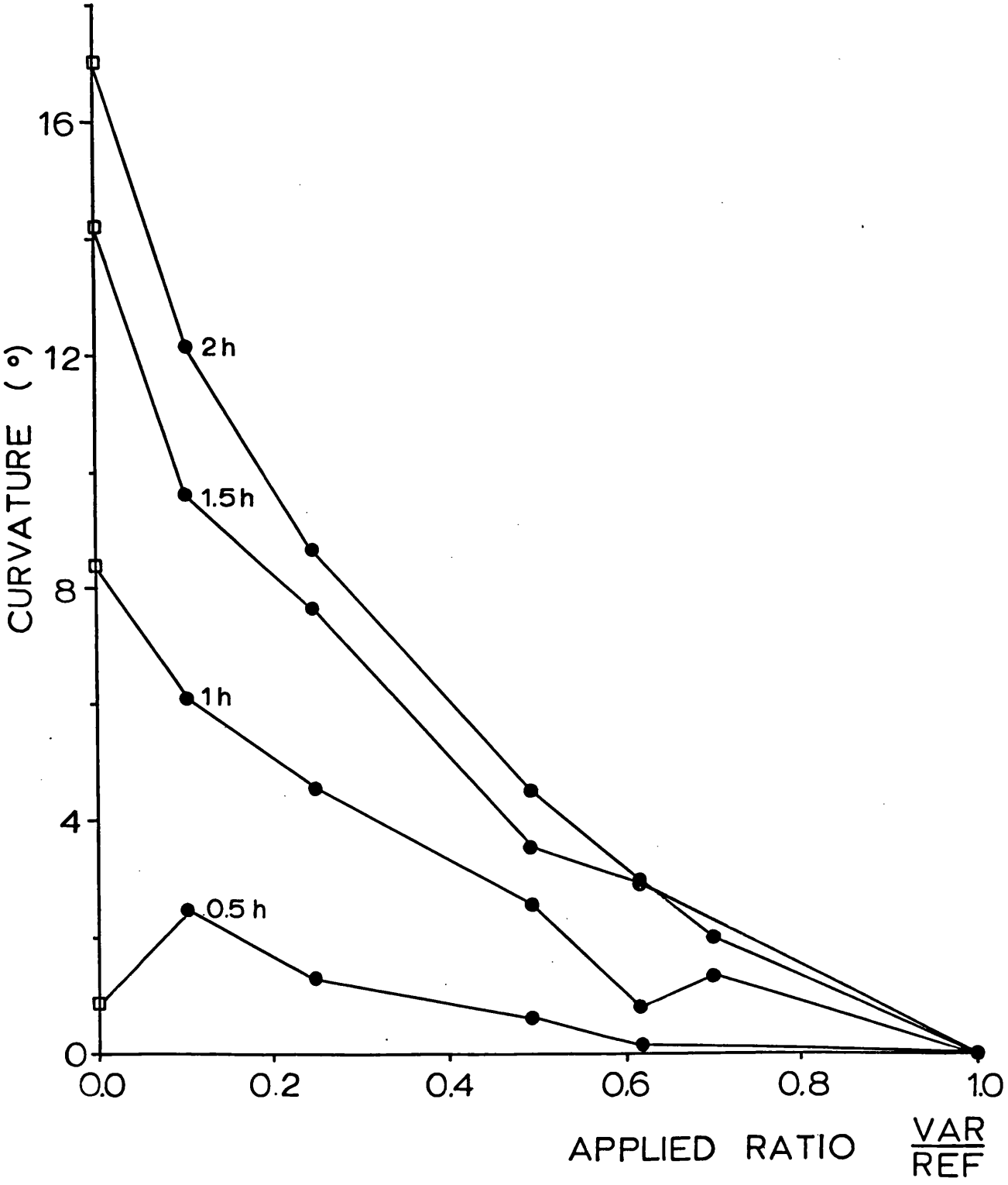
Derivation of the physiologically-significant internal light ratio

Derivation of the physiologically-significant internal light ratio depends on extrapolation of the relationship between curvature and applied light ratio in unequal bilateral light treatments to curvatures obtained in unilateral light, and assumes that the same mechanism controls phototropism under both conditions. The applied ratio model used here is a slightly modified form of the theoretical ratio of Pickard et al (1969), taking into account the fact that the photoreceptor counts photons in a logarithmic fashion (Chapter 5):-

FIGURE 36

The relationship between phototropic curvature and applied light ratio at 30 minute intervals after stimulation. The applied light ratio was calculated as the fluence rate of the variable light source divided by that of the reference light. Note in unilateral light the applied ratio = 0, and in equal bilateral light = 1.

(□) unilateral light treatments, (●) bilateral light treatments.


CURVATURE (°)
16
12
8
4
0
2h
1.5h
1h
0.5 h
0.0
0.2
0.4
0.6
0.8
1.0
APPLIED RATIO VAR/REF

$$\text{Applied ratio} = \frac{\log (\text{var} + K.\text{ref})}{\log (\text{ref} + K.\ \text{var})} \qquad \text{(Equation 7)}$$

where var = fluence rate of variable light source, ref = fluence rate of reference light source and K = transmission coefficient ($0 \leqslant K \leqslant 1$). This equation simply describes the ratio of light at the surface of the shaded side to that on the illuminated side including both incident and transmitted components. Using the assumption that the internal light environment is dependent on the external light environment, the externally applied ratio can be taken as equivalent to the internal light ratio. Hence, if there is a predictive relationship between curvature and internal light ratio, Equation 7 can be rewritten:

$$\text{Curvature} = C. \frac{\log (\text{var} + K\ \text{ref})}{\log (\text{ref} + K\ \text{var})} \qquad \text{(Equation 8)}$$

where C is a constant relating the proportionality of curvature to the internal light ratio. The transmission coefficient (K) can then be taken as a measure of the most important internal light ratio (or proportion transmitted) for phototropism. This physiologically-significant ratio can be found by selecting values for the transmission coefficient (K selected) and then deriving a predicted value for the transmission coefficient (K predicted) from the resulting relationship. The experimental data will fit the model (i.e. Equation 8) best when Kpredicted = Kselected. The values of Kpredicted were derived from the experimental data as follows:-

The curvature kinetics in uneven bilateral light treatments were regressed against the applied light ratio for selected values of K calculated using Equation 7. For all values of Kselected investigated ($0 \leqslant$ Kselected $\leqslant 0.5$) there was a highly significant linear relationship between curvature and applied light ratio (only 1 value of $p > 0.001$). Equal bilateral

light treatments were not included as they are below the threshold gradient required for a response. Figures 37 and 38 show the relationships for $K_{selected}$ 0 and 0.15 respectively. The linear relationship is then assumed to hold for unilateral light treatments. The applied light ratio which would be expected to produce unilateral curvatures can then be found by extrapolating the regression lines to the curvatures actually obtained in unilateral light (c.f. Figures 37 and 38). Predicted values of the transmission coefficient can then be easily calculated from Equation 7 which simplifies as var. = 0 in unilateral light. The mean value of K predicted at the different times was then calculated (ignoring the somewhat erroneous t= ½h value).

K predicted was then plotted against K selected (Figure 39). The data indicate K predicted = K selected at about 0.14. This value indicates the physiologically significant internal light ratio is about 7.1:1.

Experimental testing of the light gradient - curvature model

To test the model relating curvature to the light gradient, curvatures obtained by experiment were compared with those predicted from Equation 8. Two fluence rates were selected and curvatures determined (Figure 40). Curvatures were calculated from Equation 8 using a transmission coefficient (K) of 0.14 (Figure 39) and a curvature proportionality constant derived from the fluence rate-response data in Figure 34. The proportionality constant was taken at 1 and 2 hours as the reciprocal of the expected curvature in unilateral light of the reference fluence rate. The curvatures calculated using Equation 8 are consistently larger than those measured experimentally.

FIGURE 37

The relationship between curvature and applied light ratio as in Figure but with the applied light ratio recalculated using Equation 7 with K=0 (simplifying the Equation to log variable fluence rate divided by log reference fluence rate). Curvatures obtained in unilateral light (■) cannot be plotted directly as the variable fluence rate = 0, hence they are plotted as extrapolations of the relationship (-----) of unequa bilateral light treatments (●).

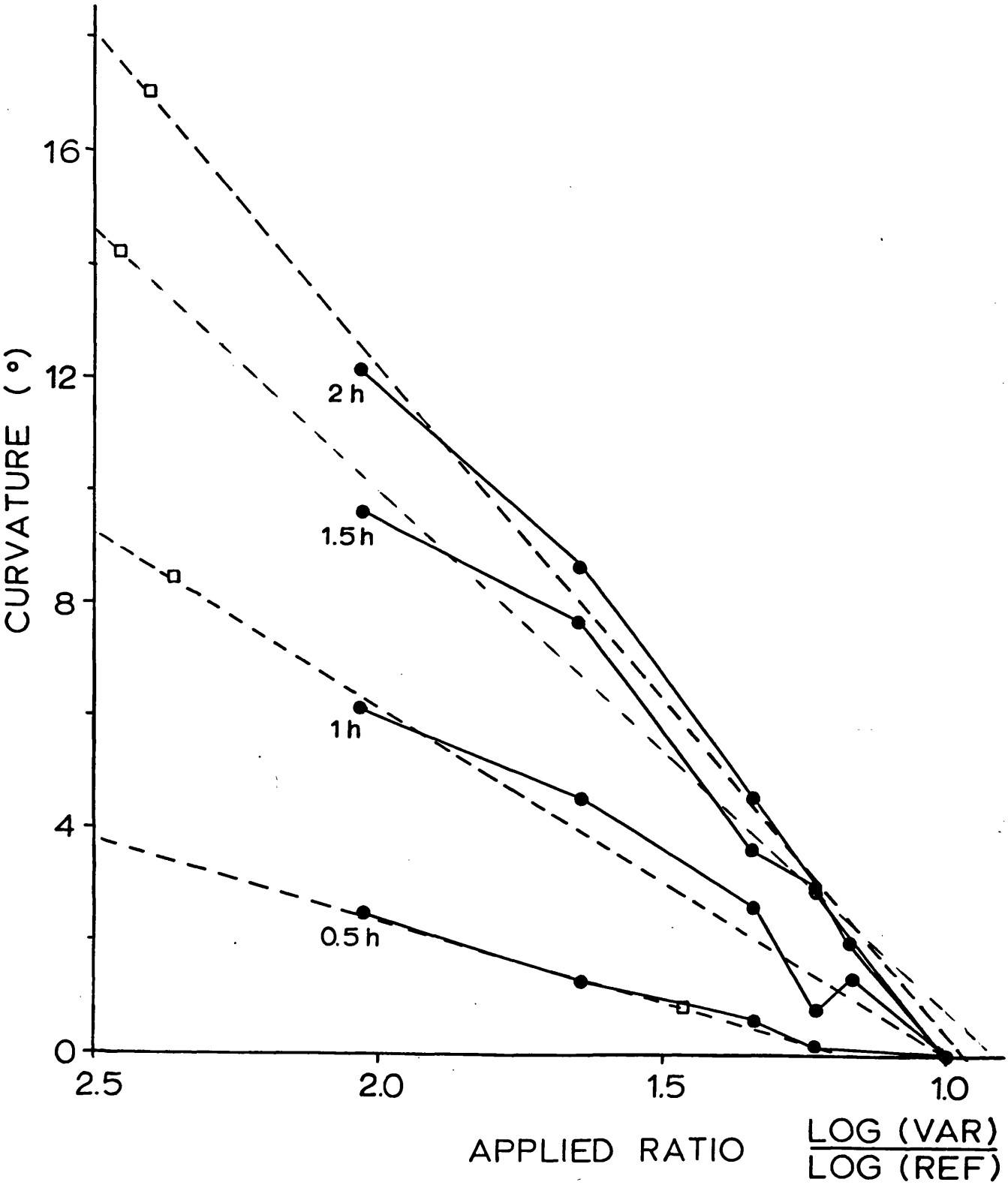

CURVATURE (°)
16
12
8
4
0
2 h
1.5 h
1 h
0.5 h
2.5
2.0
1.5
1.0
APPLIED RATIO
LOG (VAR)
LOG (REF)

FIGURE 38

The relationship between curvature and applied light ratio as in Figures 36 and 37, but with the applied ratio calculated using Equation 7 with K=0.15. Curvatures in unilateral light (□) are plotted as in Figure 3

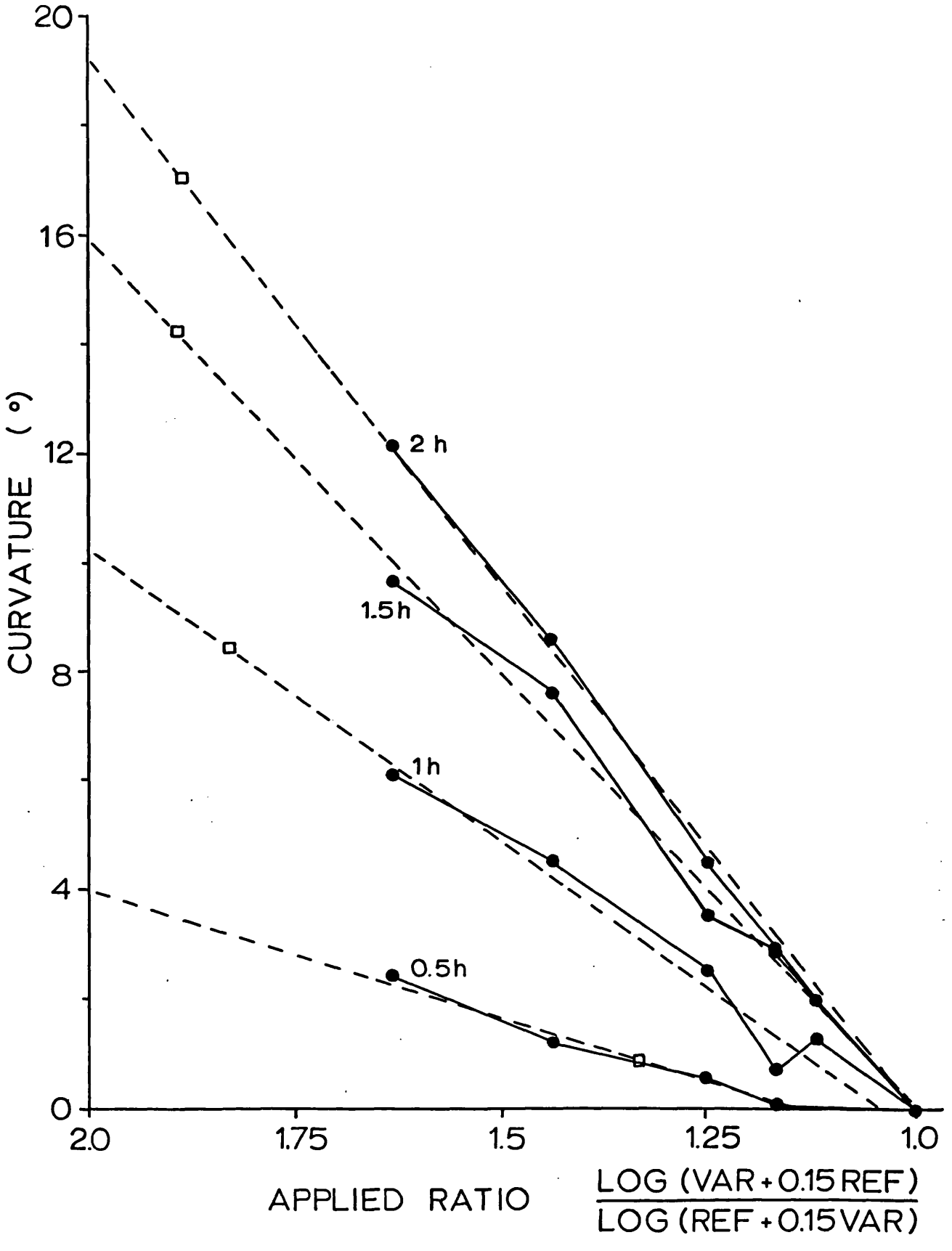

20
16
12
8
4
0
CURVATURE (°)
2 h
1.5h
1h
0.5h
2.0
1.75
1.5
1.25
1.0
APPLIED RATIO
LOG (VAR + 0.15 REF)
LOG (REF + 0.15 VAR)

FIGURE 39

Relationship between the transmission coefficient predicted by the exper data (K predicted), and the transmission coefficient selected to calcula the applied light ratio (K selected). See text for further details.

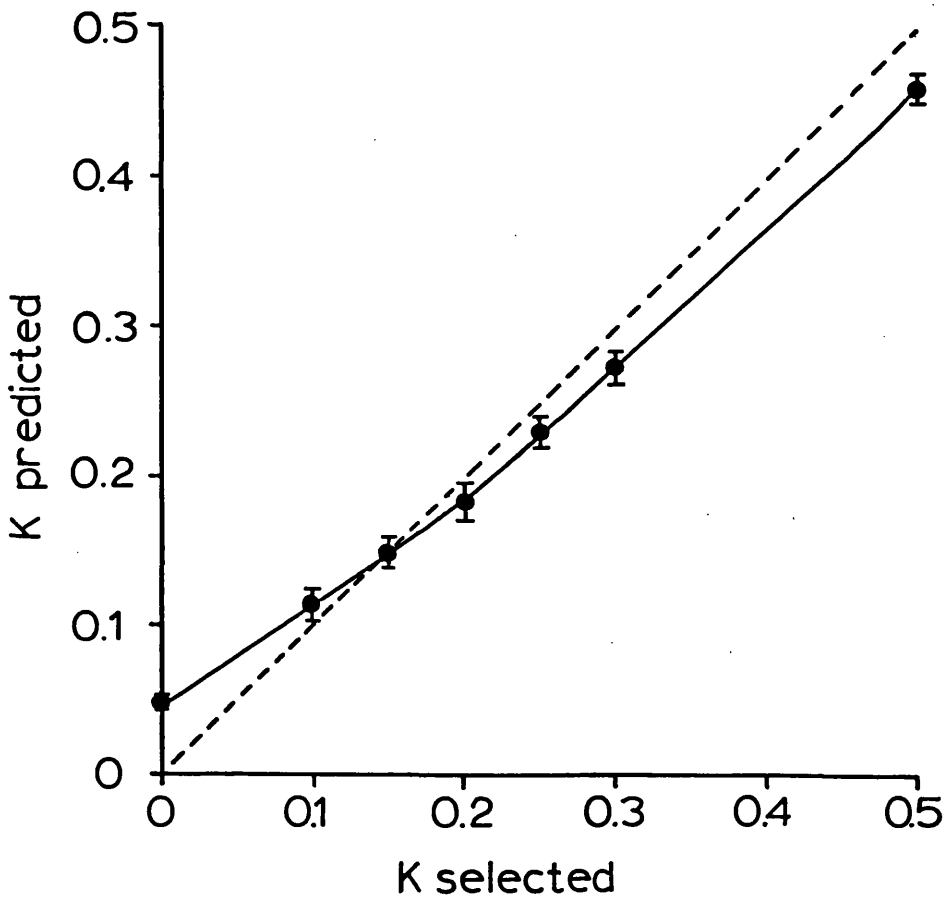
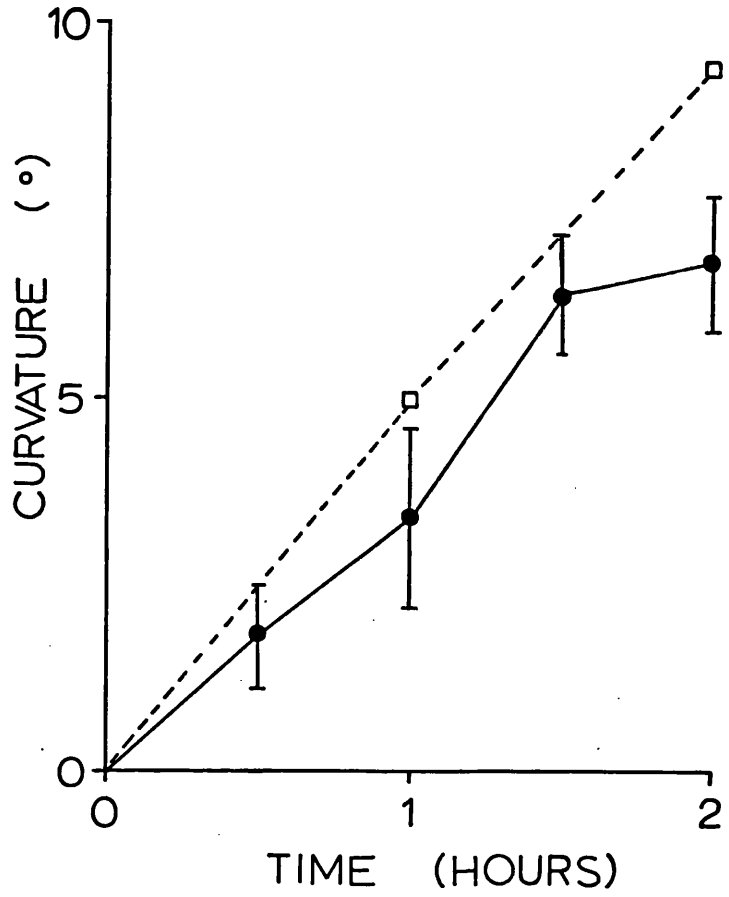

0.5
0.4
0.3
0.2
0.1
0
K predicted
0
0.1
0.2
0.3
0.4
0.5
K selected

FIGURE 40

Comparison of curvatures obtained by experiment (●) with those predicted using Equation 8 (□) using a reference fluence rate of 0.0784 μmol m^{-2} s^{-1} and a variable fluence rate of 0.00225 μmol m^{-2} s^{-1}.

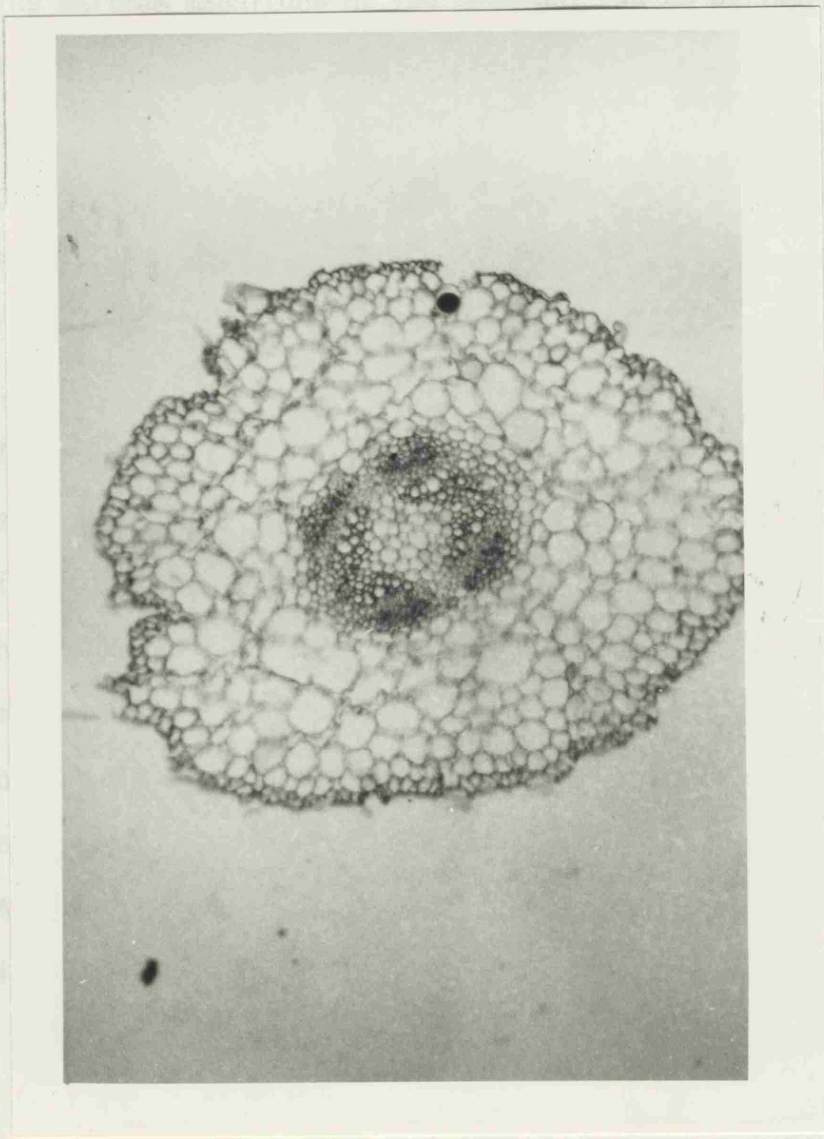

10
5
0
CURVATURE (°)
0
1
2
TIME (HOURS)

Comparison of the physiological internal light ratio with predicted internal light ratios in unilateral light

The characteristics of gradients in unilateral light have been described in detail for a variety of tissues (Parsons et al., 1984; Vogelmann & Haupt, 1985; Cosgrove, 1985b; Kunzelmann & Schäfer, 1985). By fitting appropriate mathematical equations to measured light gradients, it should be possible to calculate estimated internal light ratios. Both linear (Vogelmann & Haupt, 1985) and exponential or hyperbolic (Parsons et al., 1984) gradients have been reported, and both of these are considered here as the shape of the gradient has not been measured for mustard. To derive the necessary mathematical equations, it was necessary to determine the maximum magnitude of the light gradient and the number of cells in a transverse section of the central core of the hypocotyl, where the maximum light gradient will be established. It is assumed no lens effects occur (Vogelmann & Haupt, 1985).

A typical transverse section of the hypocotyl is shown in Plate 2. The section is radially symmetrical and composed of an epidermis, a cortex and a vascular bundle. The epidermis consists of 2 layers of cells which are small and thick-walled in comparison to the cortical cells. The sub-epidermal layer is distinguished from the outer epidermis by the presence of anthocyanin (best viewed under a light microscope). There are 6-8 layers of cortical cells, which make up the bulk of the hypocotyl tissue; they are thin-walled and large, often increasing in size towards the centre of the hypocotyl. The vascular bundle is located in the centre and consists of both xylem and phloem elements. Assuming all the cells behave identically in generation of the light gradient, and ignoring the vascular bundle, light in the central core of the hypocotyl will on average pass through

PLATE 2



Transverse section of a mustard hypocotyl embedded in wax (section prepared by M. Wilkinson).

about 20 cells. If the vascular bundle is included, the predicted internal light ratios are increased slightly.

The maximum magnitude of the gradient can be estimated if the relative amounts of light in the outer epidermal cells on opposing illuminated and shaded sides of the hypocotyl are assumed to equal the percent transmission through the hypocotyl. The absolute amounts of light immediately external to the hypocotyl surface are not directly equivalent to the light in the epidermis however, due to enhancement by backscattering (Vogelmann & Bjorn, 1984; Vogelmann & Haupt, 1985).

The average percent transmission of blue light (350-530nm) through the central core of the hypocotyl was measured as 3.85 $\pm$ 0.24 (s.e.)%. This is of similar magnitude to the 2% reported by Parsons *et al.* (1984) and the 6% calculated by Kunzelmann & Schäfer (1985). The proportion transmitted is wavelength-dependent (Figure 41), as expected since the hypocotyls are green. Parsons *et al.* (1984) reported wavelength-independent gradients for a variety of tissues but only compared 2 wavelengths.

A linear light gradient can be described by a simple straight line equation:-

$$I_n = I_o - m.n. \qquad \text{(Equation 9)}$$

where I_n = amount of light at cell n, I_o = amount of light in outer epidermal cell on illuminated side (i.e. n=0), n = number of cells from outer epidermal cell of illuminated side (for $0 \leqslant n \leqslant 19$), and m = constant.

An exponential gradient can be described using a modified version of Bouguer-Lambert:

$$I_n = I_o . C^{-kn} \qquad \text{(Equation 10)}$$

where I_n, I_o and n are as above, and k = extinction coefficient. The gradients predicted by these equations (assuming I_o = 100% and I_{19} = 3.85%, as above) are shown in Figure 41. The internal light ratios

FIGURE 41

Wavelength-dependent transmission of light through a mustard hypocotyl

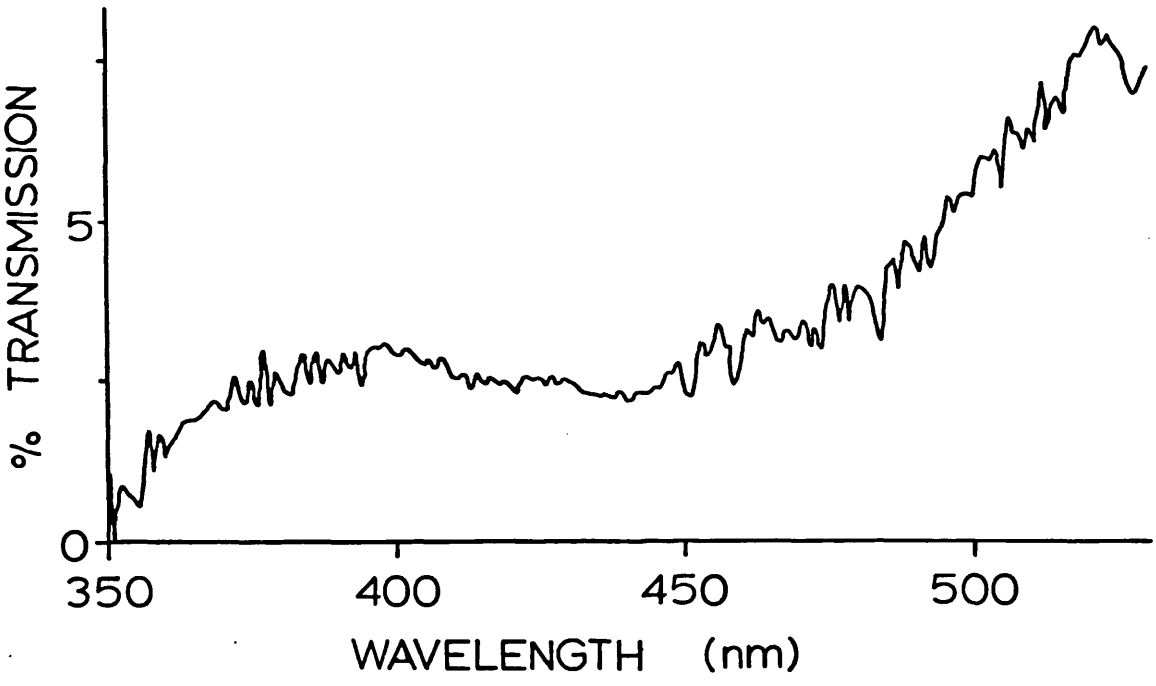
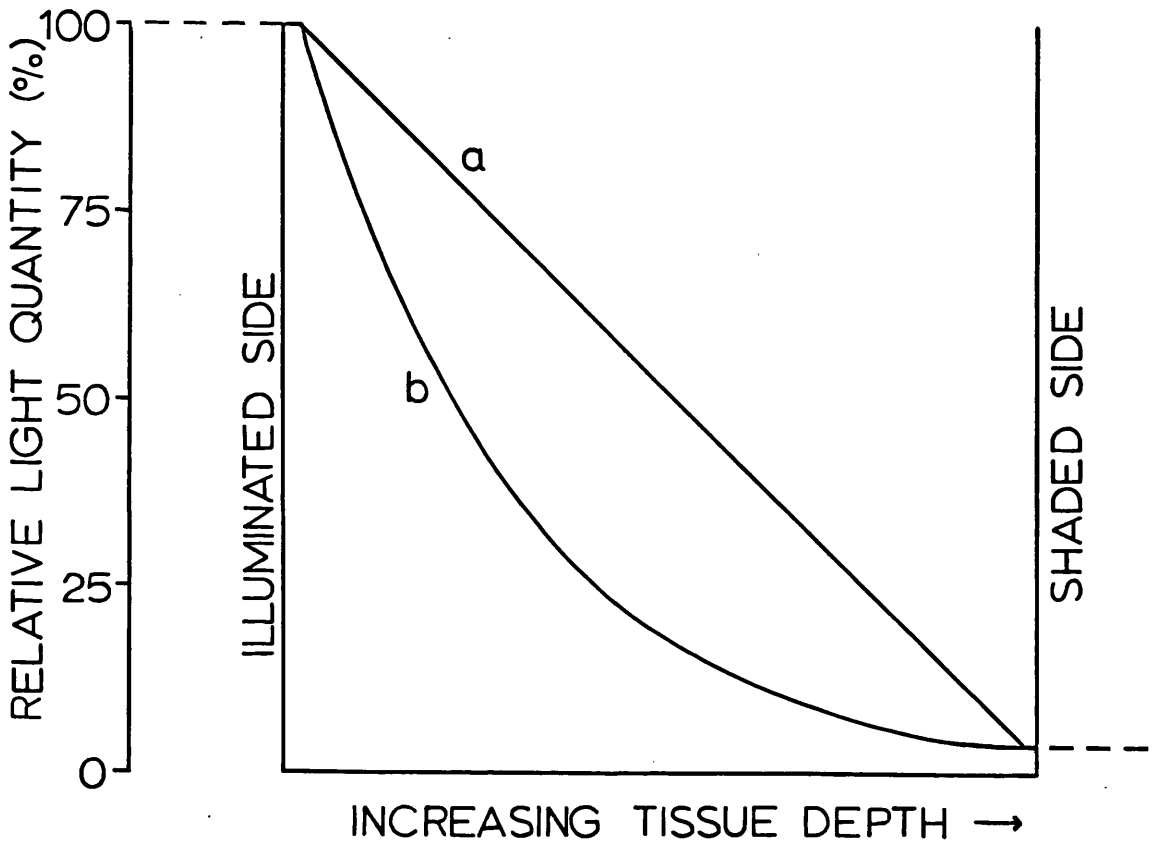

% TRANSMISSION
5
0
350
400
450
500
WAVELENGTH (nm)

FIGURE 42

Linear (a) and exponential (b) light gradients calculated from Equations 9 and 10 respectively, assuming 3.85% transmission.


RELATIVE LIGHT QUANTITY (%)
100
75
50
25
0
ILLUMINATED SIDE
SHADED SIDE
a
b
INCREASING TISSUE DEPTH →

calculated using the equations for a variety of cell and tissue combinations are given in Table 8. Ratios are given for both normal and logarithmic light quantities; the former are more easily compared with ratios in other tissues (e.g. Parsons et al., 1984; Vogelmann & Haupt, 1985), but the latter are more relevant to the model of phototropism (Equation 8) if the photoreceptor detects light in a logarithmic fashion (Chapter 5).

Threshold gradient required for curvature

The minimum applied ratio required to induce curvature was found by extrapolating the K=0.15 regression line at t=1, 1.5 and 2h to zero curvature. A variable fluence rate of 94.864 $\pm$ 2.346 (s.d.) % of the reference fluence rate was then calculated from the applied ratio. This suggests only a $\pm$ 5% imbalance of fluence rate across the hypocotyl is the threshold required for a phototropic response.

DISCUSSION

In contrast to the results of Figure 36, Pickard et al (1969) found a linear relationship between applied intensity ratio and curvature. The difference may be attributable to the different species involved, the different physiological states of the tissues or the experimental conditions, but it should be noted that the unilateral controls reported in the text by Pickard et al (1969) are markedly higher than expected from the linear relationship shown in their Figure.

The data in Figure 36 suggest there is a simple relationship between the applied light ratio, and hence internal light ratio, and curvature. The relationship will obviously depend on the tissue structure, pigment content, etc., of the species concerned. A model describing this

TABLE 8. Internal light ratios calculated for different combinations of cells and different gradients.

Gradient	Cell Combination	Internal Light Ratio	
		Illuminated:shaded	Log illuminated: log shaded
Linear Eqtn. 9	Cortex (8 cells)	2.28:1 1:0.44	1.263:1 1:0.79
	Epidermis (2 cells)	15.3:1 1:0.065	2.07:1 1:0.483
	Outer epidermis only	26:1 1:0.038	3.42:1 1:0.29
	Epidermis and cortex	2.9:1 1:0.345	1.42:1 1:0.7
	Cortex (8 cells)	3.94:1 1:0.254	1.6:1 1:0.625
Exponential Eqtn. 10	Epidermis (2 cells)	21.9:1 1:0.046	3.16:1 1:0.316
	Outer epidermis only	26:1 1:0.038	3.42:1 1:0.29
	Epidermis and cortex	5.55:1 1:0.18	1.81:1 1:0.55

relationship, Equation 8, can be constructed using the transmission coefficient of 0.14 (Figure 39). This model suggests that although the light stimulus must be detected independently on the illuminated and shaded sides of the hypocotyl, perception on both sides is needed to integrate the light signal to induce the response. This again (Chapter 3) implies some form of communication or dependence is involved (Macleod *et al.*, 1985). Two predictions of the model, that an increase in the magnitude of the light gradient should result in greater curvature, and *vice versa*, have already been experimentally demonstrated (e.g. Poff, 1983), but attempts to test the model quantitatively in mustard were less successful (Figure 40)! This may be due to the use of the dose-response data from Figure 34 which would be better replaced with a separate unilateral control at the reference fluence rate.

Castle (1965) derived a theoretical equation relating the relative bending speed in *Phycomyces* to the difference-to-sum ratio of the incident light intensities, a model which incorporates a measure of the absolute difference in fluence rates across the sporangiophore. When the model was applied to mustard, no direct relationship was found even for a variety of transmission coefficients, probably because as previously shown (Figure 30), the curvature is not related to the absolute light gradient.

Explaining in physiological terms how the relative differential excitation of the photoreceptor can influence the magnitude of curvature is another matter. One purely speculative mechanism by which it could be explained could involve lateral transport of a growth limiting substance proportional to the light fluence rate, such that the more light, the faster the transport rate. Under conditions of unequal illumination, a faster rate of transport on the illuminated side would result in an

accumulation of the growth limiting substance on the shaded side, the relative amounts on the two sides being proportional to the relative fluence rates of the light. The extent of redistribution would also be proportional to the total fluence rate, a requirement of the dose-response relationship (Chapter 5).

Zimmerman & Briggs (1963b) rejected any relationship between the magnitude of the light gradient and the magnitude of curvature because the gradient across coleoptile tips was too small to account for the width of the dose-response relationships. Their argument was based on a model which incorporated a consideration of the absolute difference in fluence rate, to which curvature is not related in mustard. Assuming the results are extrapolatable to other systems, it is not surprising that the light gradient cannot explain the dose-response curve. The results of Figures 36 and 38 are evidence of a direct relationship between magnitude of light gradient and magnitude of curvature, thus Zimmerman & Briggs (1963b) kinetic model for phototropic responses (based on an assumption of no relationship) is invalid.

Comparison of the transmission coefficient with the internal light ratios calculated from actual measurements of the light gradient may provide a method of determining how plants integrate the phototropic light signals. That there is some form of integration or communication is indicated experimentally by the opposite directions of the changes in growth rate on the illuminated and shaded sides of the hypocotyl (Chapter 3); the growth rate changes would be in the same direction if each cell responded individually to the light environment it received. What form the integration mechanism takes, and whether it is at a cellular or tissue level, is unknown. Although the differences in light intensity across an individual cell may be small (Parsons *et al.*, 1984), integration

is quite clearly possible as phototropism occurs in unicellular structures such as Phycomyces sporangiophores. Firn & Digby (1980) have suggested the best mechanism would involve integration across the epidermis as this has the greatest relative light gradient. Comparison of the physiological light gradient of 7.1:1 with the calculated internal light ratios (Table 8) does not help indicate the integration mechanism here. The mathematical descriptions of the light gradient from which the ratios were derived are clearly over-simplifications, and it may be possible to attribute the lack of comparison to these equations. Alternatively, and probably more likely, the integration mechanism may not be directly related to the internal light ratio, which would negate comparisons.

The threshold difference of a 5% imbalance of fluence rates of the light sources required to induce curvature is an indication of how sensitive plants are to small differences in the light environment. Observation of plants growing in the constant environment room confirms that the plants can respond to differences in light quantity imperceptible to the human eye. This threshold difference is much smaller than the 20% reported by Pickard et al (1969), but is similar to the 2% imbalance in Phycomyces reported by von Guttenburg (1959).

The fact that curvature rate is dependent on the light gradient has important implications. For instance, the wavelength-dependent transmission (Figure 41) suggests that the different gradients established in monochromatic light may distort action spectra. Changes in the gradient as plants curve (Pickard et al 1969; Chapter 5) would be expected to modify the response, and the variation of gradients within a tissue (e.g. Vogelmann & Haupt, 1985) may also lead to differential expression of curvature within an organ. To what extent these and other gradient-dependent changes influence phototropism remains to be seen.

CHAPTER 7

GENERAL DISCUSSION: THE PHOTOTROPIC TRANSDUCTION CHAIN

To understand any photomorphogenetic system, it is necessary to characterise the entire sequence of events from the first activation of the photoreceptor through to the final expression of the response. For most systems, exasperatingly little is known about most steps in the transduction chain, and what little is known is largely based on circumstantial evidence derived from physiological experiments; it is this evidence which delimits the constraints within which a mechanism must operate.

The main constraints relating to classical blue-light-mediated phototropism, based on the work presented here and that in the literature, are discussed below. It is assumed there is only one basic blue-light-mediated phototropic mechanism in higher plants, and that even if the exact details differ between species, the main features are generally applicable. The discussion centres on the primary transduction chain; many other factors can modify the final response and must ultimately be accounted for (e.g. Phytochrome; Chapter 3), but they are considered here to be of secondary importance. The aim of the discussion is to establish the firm foundations (Firn & Digby, 1980) on which to build models and test theories.

The characteristics of the light stimulus required to induce curvature are determined by the tissue, the photoreceptor and the transduction chain. Action spectra show the stimulus must be "blue" light of wavelengths between 350nm (and probably also shorter wavelengths) and

520 (-550)nm; this character is determined by the chromophore electron energy distributions. The threshold dose is determined by a combination of tissue optical properties, quantum yield and concentration of the photoreceptor, sensitivity of signal amplification, slippage reactions, etc. For mustard, a threshold dose of c. 20 μmol m^{-2} is required (Table 7), though this is considerably smaller in Avena or Phycomyces (7.3×10^{-4} μmol m^{-2} and 500 quanta respectively; Shropshire, 1979). The way in which this dose is given is also critical (Table 7, Blaauw & Blaauw-Jansen, 1970a); the fluence rate must not only be above a critical minimum (10^{-3} μmol m^{-2} s^{-1} in mustard, Figure 34; 0.2 μmol m^{-2} s^{-1} in cucumber, Cosgrove, 1985b) but must also be given for a minimum time, the length of which is partly dependent on the fluence rate (Table 7). These results indicate that perception of the light stimulus is dependent on both light and dark reactions.

A third characteristic of the stimulus is that it must be sufficiently spatially heterogeneous to establish a gradient of light intensity within the tissue, i.e. establish "illuminated" and "shaded" sides. In most experiments this is achieved with unilateral light, but responses may be induced whenever the imbalance of fluence rate across the axis exceeds a threshold (5% in mustard, Chapter 6; 20% in Avena, Pickard et al., 1969). The direction of the greatest relative light gradient in the tissue determines the direction of the response, and the relative magnitude of the gradient determines the extent of the response (Figure 36; Chapter 6), hence the gradient is of critical importance in expression of the response. In essence, the critical feature of the stimulus is that it must establish differential spatial excitation of the photoreceptor within the organ.

A fourth criterion is that the stimulus must be applied to the axis as it is the site of photoperception (Shuttleworth & Black, 1977; Franssen & Bruinsma, 1981). In *Avena*, photoperception and response can occur throughout the coleoptile (Macleod *et al.*, 1984), although the tip is the most sensitive region (Curry, 1969).

The precise identity of the photoreceptor is unknown. Comparison of the action spectrum of first-positive phototropism in *Avena* with the *in vitro* absorption spectra of putative photoreceptors indicates the pigment is either a flavin or a carotenoid, but small differences between the spectra do not allow further clarification. Assuming only one photoreceptor (Chapter 5, but see Löser & Schäfer, 1986), the differences could be attributed to the techniques employed during determination of the action spectra (*c.f.* Chapters 3, 5 and 6), but the general consistency between the *Avena* action spectrum and those of other blue-light-mediated responses (Presti, 1983) suggests they reflect differences between the *in vivo* and *in vitro* absorption spectra. A consensus based on other evidence currently favours a flavin as the photoreceptor (Dennison, 1979; Briggs & Iino, 1983; Presti, 1983), probably closer in structure to riboflavin than FMN or FAD (Mohr & Shropshire, 1983). Additional knowledge about blue-light-absorbing pigments and their primary reactions is largely derived from other photomorphogenetic systems, but is probably also applicable to the phototropism photoreceptor.

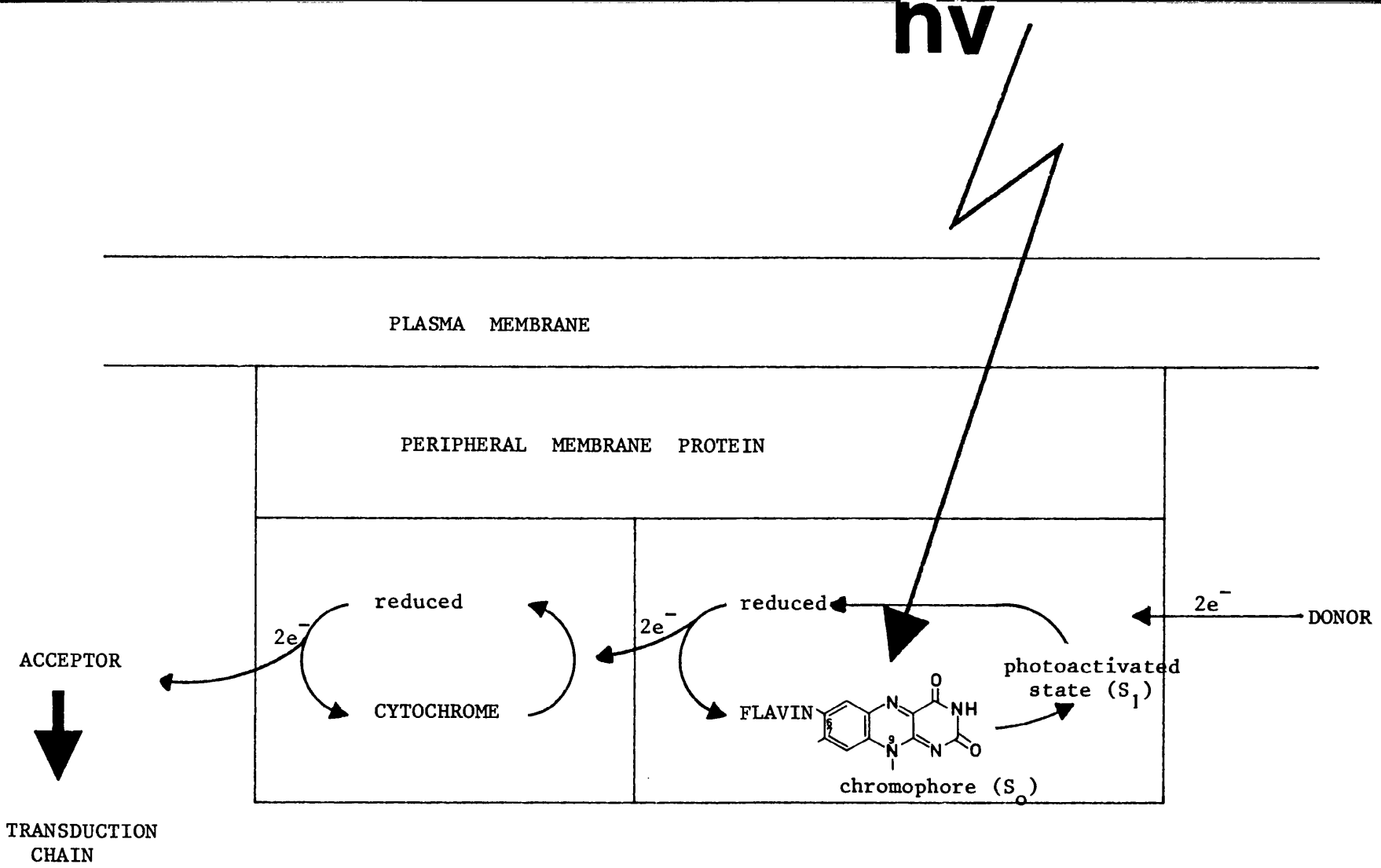
Briggs & Iino (1983) discuss evidence which suggests the photoreceptor may be a flavoprotein-cytochrome complex associated with the plasma membrane. The action spectrum for light-induced reduction of a *b*-type cytochrome accompanied by simultaneous reduction of a flavoprotein in *Neurospora* closely resembled the action spectra of other blue-light-mediated responses

(Muñoz & Butler, 1975). In Zea, the photoreceptor for a similar reduction reaction was firmly bound to membranes (Goldsmith et al., 1980), and Leong & Briggs (1981) showed that the photoactivity was associated with the plasma-membrane fraction. They also showed that when the membrane preparation was washed with mild detergent, the photoactive component could be separated without markedly affecting its activity, suggesting that the flavin and cytochrome are located on the same protein and that it is a peripheral rather than an intrinsic membrane protein. Additional evidence for location of the photoreceptor in or near the plasma membrane comes from experiments with polarized light or microbeam irradiation (Schmidt, 1983).

Flavins are noted for their ability to undergo reversible redox reactions to both half-reduced ($1e^-$ equivalent) and fully-reduced ($2e^-$ equivalent) forms (Presti, 1983), and photoactivation of the flavin probably results in reduction of the molecule by electron transfer from a suitable donor. Unusually, this probably occurs from an excited singlet state of the flavin as phototropism is unaffected by triplet state quenchers (Vierstra et al., 1981). The transduction chain is probably then initiated by transfer of one or two electrons to a specific acceptor molecule (Delbruck et al., 1976), regenerating the flavin. Although there is no evidence that reduction of the cytochrome moiety is involved in the transduction chain, its close coupling to flavins in other electron transfer chains and proximity to the flavin in the complex make it a likely candidate for consideration in the primary reaction. A simple tentative model showing these initial steps is given in Figure 43. Flavin photobiology and physiology are discussed in more detail by Presti (1983) and Schmidt (1983).

FIGURE 43

Tentative model for primary reactions of the blue-light-absorbing photoreceptor. Note the cyclical regeneration of the flavin and cytochrome. A possible chromophore (Mohr & Shropshire, 1983) is also shown. See text for further details and references.


hν
PLASMA MEMBRANE
PERIPHERAL MEMBRANE PROTEIN
reduced
2e⁻
CYTOCHROME
ACCEPTOR
TRANSDUCTION CHAIN
reduced
2e⁻
FLAVIN
chromophore (S_o)
photoactivated state (S_1)
2e⁻
DONOR

Little evidence is available to indicate the events between flavin reduction and the changes in wall rheological properties that lead to differential growth (c.f. Chapter 4), but a number of processes must be involved. First, the rapidity of responses (e.g. 3-4 minutes in Fagopyrum; Ellis, 1984) suggests that phototropism may result from changes in membrane transport properties rather than gene expression. The localisation of the photoreceptor on the plasma membrane and the changes in wall yielding properties suggest membrane transport must occur at least once during the transduction chain.

Second, in common with other transduction chains, amplification of the initial signal is required. Delbruck et al (1976) suggest that a light-induced change in membrane voltage could provide a suitable amplification system if membrane conductance was exponentially related to the voltage. A simple enzyme cascade system could also be used to amplify the signal.

Third, the pattern of differential growth indicates that some integration mechanism must operate because the changes in growth rate are in opposite directions on the illuminated and shaded sides of the hypocotyl (Chapter 3); cells do not directly regulate their growth according to the amount of light they receive (as envisaged by the Blaauw theory). Integration probably occurs at some stage after amplification but before the induction of the mechanism that changes wall extensibility. Integration also must take place at the tissue or organ level as the cells respond in a coordinated fashion, though the stimulus perception is at the cellular level. This is probably a key step in understanding the mechanism of phototropism.

In some phototropic responses there is evidence of spatial separation of sites of photoperception and response, and some mechanism must be incorporated in the transduction chain to explain the transmission of the stimulus. For instance, in first-positive phototropism in Avena (Went and Thimann, 1937) and Zea (Iino and Briggs, 1984) irradiation of the tip causes a progressive onset of curvature down the coleoptile. In other cases the major phototropic response is restricted to the illuminated region (Macleod et al., 1984), and in mustard, cucumber (Cosgrove, 1985b) and second-positive phototropism in Avena (Curry, 1969) where curvature starts ± simultaneously along the axis, the sites of perception and response are essentially congruent and no transmission mechanism is required.

Various other processes have been implicated in the transduction chain, but there is little evidence yet to establish whether they are directly involved, or whether they are by-products. Transverse bio-electrical polarization of coleoptiles (Schrank, 1946) and membrane hyperpolarization (Racusen & Galston, 1980) have been reported following unilateral light stimuli, but at present there is simply insufficient knowledge to determine even whether such bioelectrical changes are causally involved in photomorphogenesis as a whole (Racusen & Galson, 1983). Redistribution of cations has also been demonstrated following unilateral light, but Goswani & Audus (1976) concluded movement was neither a result of, nor cause of, curvature. Redistribution of calcium is of particular interest in relation to its general role in plant development (Hepler & Wayne, 1985) and may therefore merit more detailed investigation. The role of other secondary messengers (e.g. cyclic AMP) also remain to be investigated. Whilst it is clear that many metabolic events may occur during the signal processing and that their analysis is crucial to understanding the mechanism of phototropism, there seem to be few ways available at present critically to establish their role in the transduction chain.

The biophysical/biochemical parameters that change to alter wall rheological properties are not known for phototropism, or indeed for any growth response. The biochemical structure and bonding of the wall dictate its mechanical properties. A typical dicot primary cell wall is thought to consist of cellulose microfibrils embedded in a matrix of polysaccharide and glycoprotein, held together by hydrogen and covalent structural bonds (for full details see Preston, 1979; Darvill et al., 1980; and also McNeil et al., 1984; Taiz, 1984). Wall extensibility is probably governed at least in the short term by stress-bearing bonds in the matrix, but which bonds are broken, or how, is not known.

There are two schools of thought regarding the nature of the stress bearing bonds (Taiz, 1984). One school views wall extensibility as being limited by covalent bonds which require enzymic cleavage, and the other school attributes the matrix mechanical properties to the combined strength of many weak interactions amongst polymers and ions, with loosening effected by enzymic and/or physical means. It is evident from the literature however that there is little agreement about the processes involved, and the complexity of bonding in the wall at present defies definitive analysis. Preston (1979) interpreted the yield threshold in terms of a physical force needed to break strong bonds before the wall polymers can slip past one another through breakage and synthesis of weaker bonds (i.e. creep). An alternative explanation is that stress is needed simply to cause sufficient relative movement of molecules to prevent resynthesis of the original bonds (Cleland, conference lecture 1985). Current evidence does not support a role for weakening of hydrogen bonds in wall extension (Taiz, 1984), and although some consider loosening probably involves covalent bond cleavage (Lamport, 1970), others (Sellen, 1980), do not. In any

case, growth is probably under multifactorial control and it is likely there is more than one mechanism for controlling wall extension. It is also likely, however, that specific bonds in the wall are broken and remade in order to maintain some structural regularity, and the fact that wall extensibility is at least partly metabollically determined and maintained ("biochemical creep"; Preston, 1979) indicates that both biological and physico-chemical processes are involved. Wall extensibility itself is also a function of more than one process (Cosgrove, 1985a) suggesting more than one bond population may be involved; one model of the cell wall (Monro et al., 1979) even incorporates at least 2 different matrix components. The influence of both water and ions (both important components of the wall) on wall bonding does not seem to have been analysed in detail.

Wall extensibility and strength is then probably a function of the total number and rate of breakage and/or synthesis of bonds in the matrix. A number of mechanisms have been proposed to mediate bond breakage, but again definitive evidence is lacking in many cases. The pattern of differential growth and presence of a lag are evidence against a direct effect of light on the wall as envisaged by Towers and Abeyasekera (1984). The short lag time suggests that direct control of growth by regulation of synthesis and/or incorporation of wall material is unlikely, and similarly, there are no known wall enzymes with suitable kinetics (Cleland, 1971) to cause the observed changes in growth rate. Whilst there is little doubt that wall acidification can change growth rates, the pattern of differential proton efflux measured during phototropism (Mulkey, Kuzmanoff & Evans, 1981), is inconsistent with the pattern of differential phototropic growth (c.f. Chapter 3), though this was not measured for the same tissue. The roles of cations (especially Ca^{2+}), lectins, wall proteins and phenolics

require further elucidation before they can be assessed. Further details of the biochemistry of wall expansion are discussed by Taiz (1984). Elucidation of the mechanism responsible for changes in wall rheological properties is a second key step in understanding phototropism.

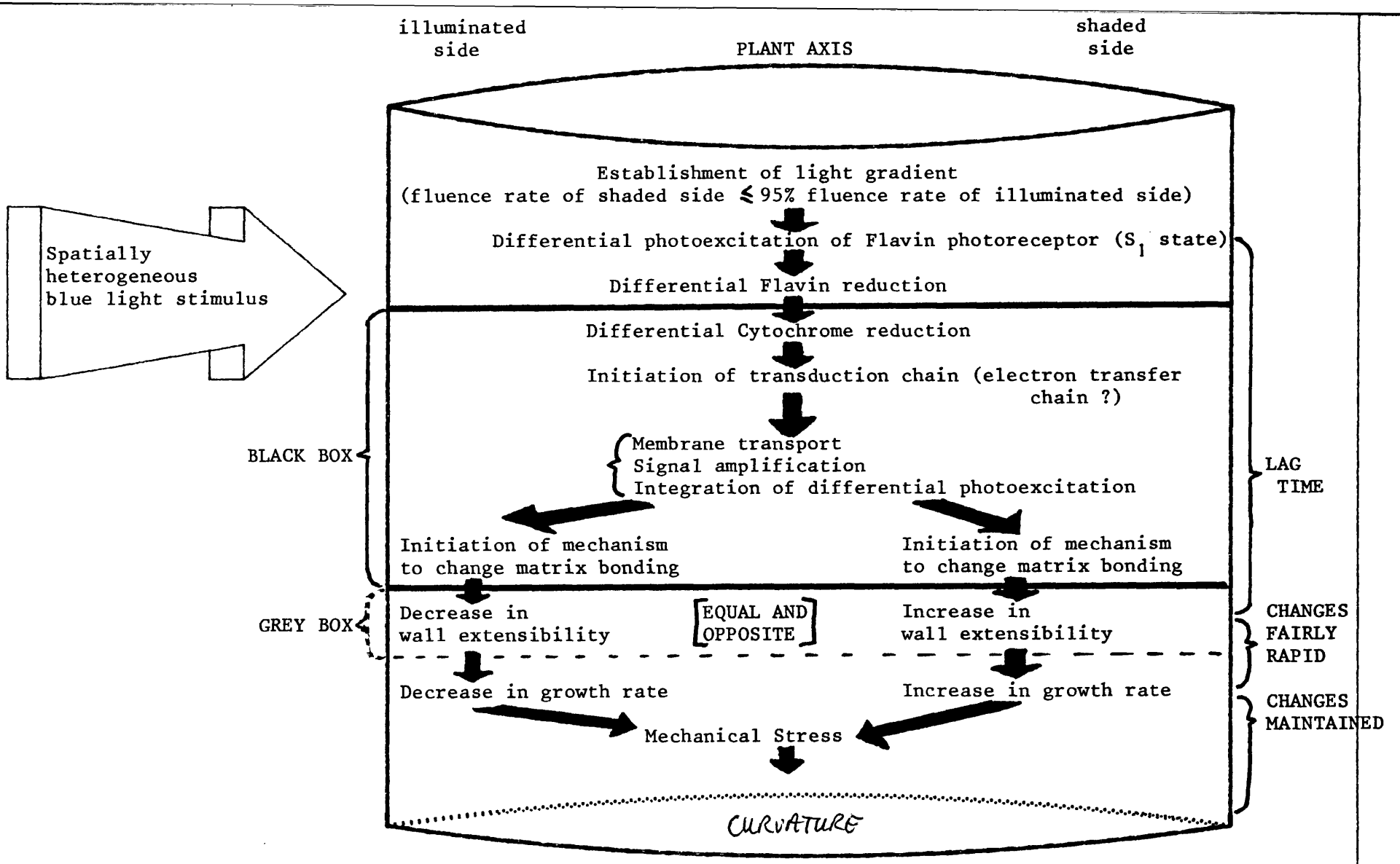
The pattern of differential growth indicates that the changes in wall extensibility (and hence growth rate) are in opposite directions on the illuminated and shaded sides of the axis. Once established, the differential growth rates generate mechanical stress within the axis which leads to the most obvious expression of the phototropic response, curvature.

There are also a number of quantitative constraints which can be used to indicate potential mechanisms. First, any proposed mechanism must be able to induce curvature within the lag period, and must do so with suitable kinetics to explain both the true mean lag time (e.g. 20 minutes in mustard; Chapter 4 and Appendix II) and the first detectable response (5 minutes). Second, the mechanism must cause the changes in growth rate to be complete within an average of about 10 minutes, and then to be maintained for at least a further 30-60 minutes without further change (Chapter 5). Third, the mechanism must induce changes in growth rate on the two sides of the hypocotyl which are opposite in direction but equal in magnitude (Chapter 3). Net growth remains unaltered.

The main features of the transduction chain are summarised in Figure 44. It is evident that whilst some features of the transduction chain are reasonably well characterised and understood (e.g. stimulus, photoreceptor, pattern of differential growth), others, particularly those at the biochemical level, are not (e.g. integration mechanism, wall extensibility changes).

FIGURE 44

Summary of the blue-light-mediated phototropism transduction chain (see text for full details and references). Tentative and unknown steps are placed in grey and black boxes respectively.


illuminated side
PLANT AXIS
shaded side
Establishment of light gradient
(fluence rate of shaded side ≤ 95% fluence rate of illuminated side)
Spatially heterogeneous blue light stimulus
Differential photoexcitation of Flavin photoreceptor (S_1 state)
Differential Flavin reduction
Differential Cytochrome reduction
Initiation of transduction chain (electron transfer chain ?)
BLACK BOX
Membrane transport
Signal amplification
Integration of differential photoexcitation
LAG TIME
Initiation of mechanism to change matrix bonding
Initiation of mechanism to change matrix bonding
GREY BOX
Decrease in wall extensibility
EQUAL AND OPPOSITE
Increase in wall extensibility
CHANGES FAIRLY RAPID
Decrease in growth rate
Increase in growth rate
CHANGES MAINTAINED
Mechanical Stress
CURVATURE

The Cholodny-Went theory offers the only extant explanation of phototropism consistent within the constraints of the transduction chain outlined above. Although considerable doubts have been expressed about many aspects of the theory (Firn & Digby, 1980; Trewavas, 1981), it has not yet been possible either to disprove the model, or to answer the criticisms in full. The work presented here provides a suitable framework within which to investigate the theory further, but as the relationship between auxin and growth rate has not been examined in mustard, it is not possible to evaluate critically the Cholodny-Went model in more detail. All that is required is one incontrovertable piece of evidence inconsistent with the Cholodny-Went theory to set the study of phototropism back 60 years. A suitable approach to find such a piece of evidence would be to examine the amount of auxin and the kinetics of redistribution using immunochemical techniques (e.g. Mertens et al., 1985) in parallel with measurements of growth rates in light-grown mustard seedlings under conditions independent of phytochrome.

APPENDIX I

PHYTOCHROME-MEDIATED PHOTOTROPISM

INTRODUCTION

For many years there has been an apparent anomaly in the literature as to why phytochrome-regulated extension growth but did not also mediate phototropism in higher plants. This anomaly was based on the observation that wavelengths longer than about 520nm did not induce curvature (e.g. Galston, 1959; Dennison, 1979) but were effective at regulating extension growth (e.g. Elliot & Shen-Miller, 1976). Although phototropic responses to red light had occasionally been noted (e.g. Atkins, 1936), they were generally regarded as either a tailing off of the blue-light response, or due to blue light contamination of the light sources (e.g. Shuttleworth & Black, 1977), and were out-numbered by reports documenting a lack of response to red light (e.g. Mohr & Peters, 1960; Shropshire & Mohr, 1970; Elliot & Shen-Miller, 1976). This state of affairs was maintained until Iino, Briggs & Schäfer (1984) documented the first detailed, authoritative account of phytochrome-mediated phototropism in higher plants. Curvature was originally noted in maize mesocotyls grown in a low-fluence-rate red-light growth room which could not be explained by the blue-light-absorbing photoreceptor, and further investigation suggested that mesocotyl curvature was probably mediated by phytochrome.

The curvature in maize mesocotyls (Iino, Briggs & Schäfer, 1984) was induced by subsaturating doses of both unilateral red and blue light, though the fluence-response curve for blue light required 100-fold higher fluences than that for red light. This shift probably reflects the quantum efficiency of phytochrome phototransformation at the different wavelengths

(Pratt & Briggs, 1966; Kunzelmann & Schäfer, 1985). However, as both the lag time and the rate of curvature were fluence-dependent, the exact fluence response relationships depends on when the curvature is measured (see also Chapter 5). Red light pretreatment eliminated the response whilst far-red light pretreatment did not, although far-red given after a red light stimulus decreased the magnitude of the response. Unilateral far-red light alone caused curvature towards the light, but when applied after vertical red light caused negative curvatures. The absolute curvatures obtained in blue light were greater than those in red light due to the wavelength-dependent light gradients (Kunzelmann & Schäfer, 1985). Iino, Schäfer & Briggs (1984) showed the major site of photoperception of the response was the bending zone of the mesocotyl itself.

The mesocotyl curvatures were explained in terms of localised phytochrome-mediated inhibition of axis extension growth (Iino, Briggs & Schäfer, 1984; Kunzelmann & Schäfer, 1985). Unilateral irradiation with subsaturating doses of light produces a gradient of Pfr across the axis. As axis extension rate is related to the amount of Pfr (though not directly), different localised concentrations of Pfr result in differential growth and hence curvature.

The data presented in this Appendix can probably be explained by a similar mechanism. The data are incomplete and preliminary, but, as they were obtained independently from the results above, are nonetheless considered of sufficient interest to merit inclusion in the thesis, despite their inadequate state. The appropriate photobiological tests to corroborate phytochrome in the responses have not been carried out, and as the plants are probably subject to gravitropism and autotropism in a similar fashion to blue-light-mediated responses (Chapter 5), the data are best regarded as qualitative only.

MATERIALS AND METHODS

Mustard seedlings were grown as described in Chapter 2. Pea and Gourd cv 'Mammoth' were also investigated. Pea seedlings were grown for 7 days in a dark growth room at 25°C and de-etiolated for 24 hours under 118 μmol m^{-2} s^{-1} continuous white light in the constant environment room. Gourds were grown for 9 days in the dark and de-etiolated for 24 hours. Plants were equilibrated overnight in background SOX.

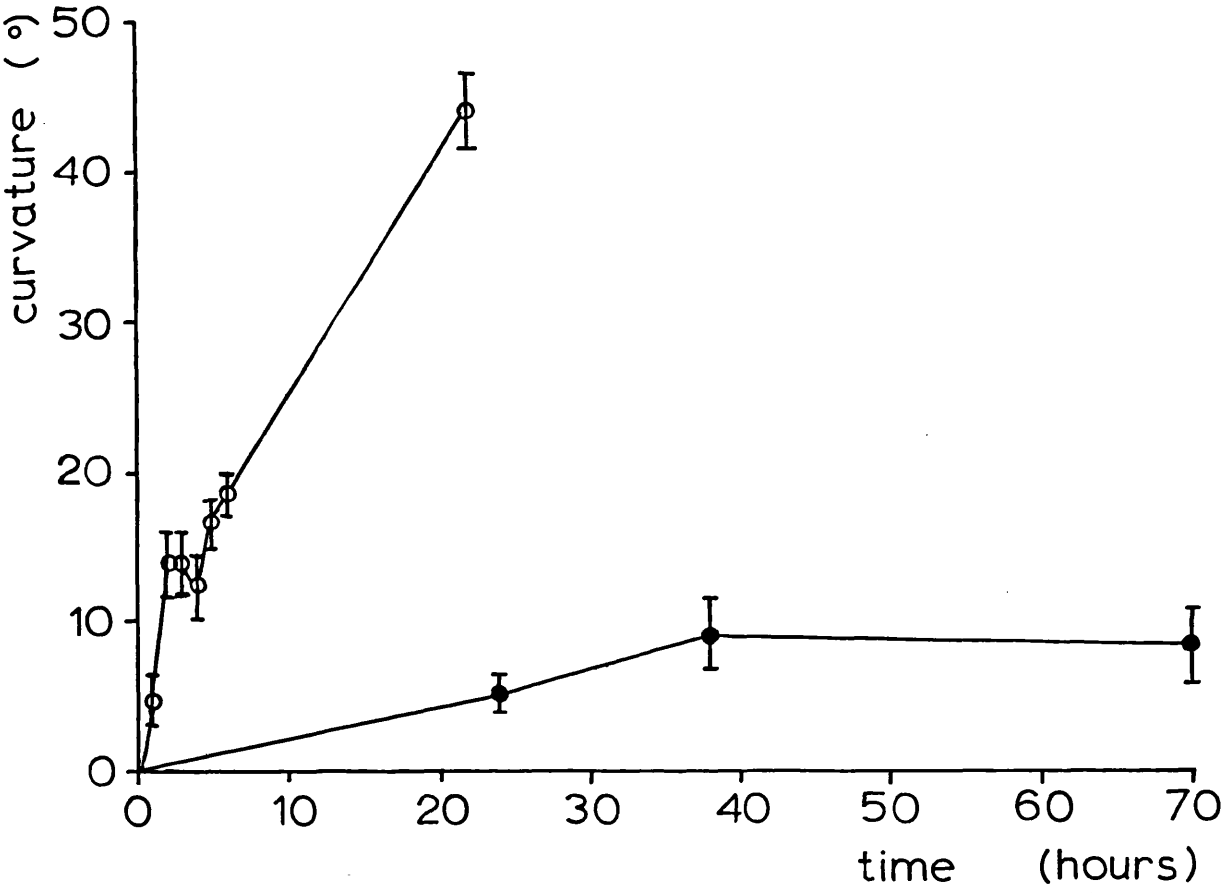
For fluence rates used, see Figure legends. The SOX and far-red light sources were as described in Chapters 2 and 3 respectively. In addition, for unilateral SOX treatments, mustard plants were placed in a box with a window of 2 layers of orange Cinemoid; only light above 558nm was transmitted into the box (data not presented) hence blue light contamination is eliminated. The pattern of differential phytochrome-mediated phototropic growth was investigated as in Chapter 2.

RESULTS

The development of curvature towards unilateral SOX light is shown in Figure 45. Curvature in response to unfiltered SOX light is due to 0.098 μmol m^{-2} s^{-1} "blue" (300-530nm) light contamination and is similar in kinetics to data in previous chapters. In contrast, curvature towards filtered SOX is slower and smaller in magnitude, though direct comparison is not strictly possible as the fluence rates differ. Shuttleworth & Black (1977), Franssen & Bruinsma (1981) and Iino et al., (1984) also found red light induced curvatures were small in comparison to classical blue-light phototropism. Plants treated with unilateral far-red light alone elongated markedly and showed variable, distorted curvatures with no consistent direction (data not presented).

FIGURE 45

Time courses for development of curvature towards unilateral SOX light. o unfiltered SOX light (70 μmol m^{-2} s^{-1}), • SOX light filtered through 3 layers of orange Cinemoid (45 μmol m^{-2} s^{-1}). Points are means ± s.e. (n=15).

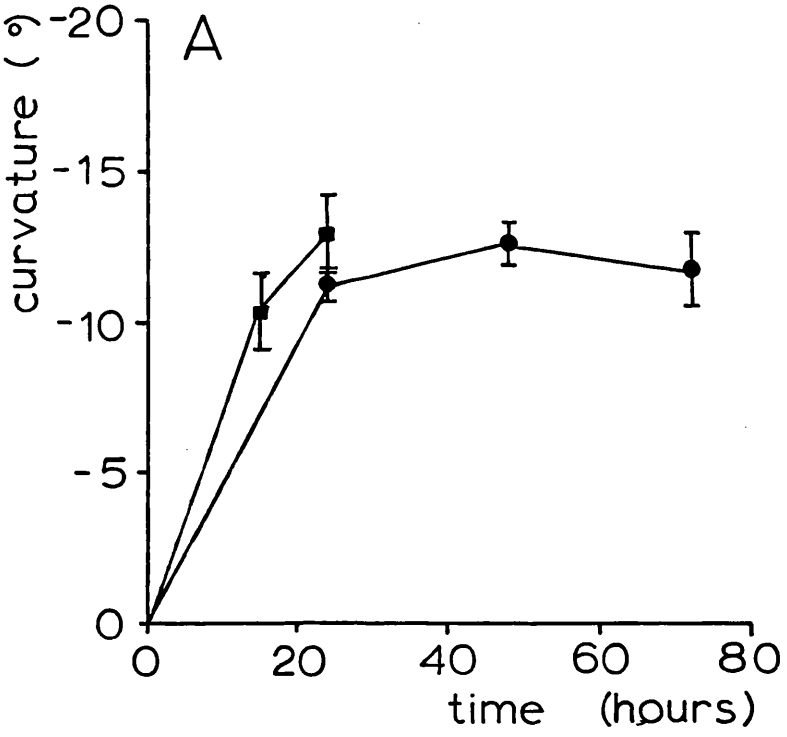

curvature (°)
50
40
30
20
10
0
0
10
20
30
40
50
60
70
time (hours)

When plants in a background of SOX light were treated with unilateral far-red light, curvatures away from the far-red source were observed. Figure 46a shows the development of curvature of mustard and gourd seedlings. Curvatures of about 10° were measured after 24 hours in both cases, and also in 1st stem internode of Pea (data not presented). A more detailed time course for mustard (Figure 46b) under different (though unfortunately not precisely defined!) conditions indicates significant curvatures away from far-red light can be measured at 6 hours. All these plants showed an even distribution of curvature down the length of the hypocotyl, similar to curvatures in blue-light phototropism. Iino, Schäfer & Briggs (1985) found curvature in maize restricted mainly to the apical region of the mesocotyl.

Measurement of the pattern of differential growth did not prove entirely successful as it was not possible to distinguish between the growth rates of the illuminated and shaded sides of the hypocotyl (Figure 47). The data do show a marked increase in net growth rate following addition of unilateral far-red light, showing the plants are responding to the far-red light, some plants doubling their length after 72 hours. These data indicate that, as plants curve away from the light source, curvature must be established by a differential acceleration of growth and that the growth rate of the illuminated side must be greater than that of the shaded side. Measurement of the changes in growth rate should provide a better measure of the response than curvature in this case (e.g. Firn et al., 1983), but the small differences in growth rate are difficult to quantify accurately.

FIGURE 46

Time courses for development of phytochrome-mediated phototropism. Plants were placed in a background of SOX light applied from above, and unilateral far-red light added from one side. Plants curved away from the far-red light. A. Development of curvature in mustard (●) (n=15), and gourd (■) (n=10) in a background of 100 μmol m^{-2} s^{-1} SOX and 91 μmol m^{-2} s^{-1} unilateral far-red light. B. More detailed time course for development of curvature in mustard (●) (n=15) in a background of 45 μmol m^{-2} s^{-1} SOX light in response to unilateral FR light (probably 90 μmol m^{-2} s^{-1}). Controls (o) (n=15) received no unilateral FR. Points are means $\pm$ s.e.


A
curvature (°)
-20
-15
-10
-5
0
0
20
40
60
80
time (hours)

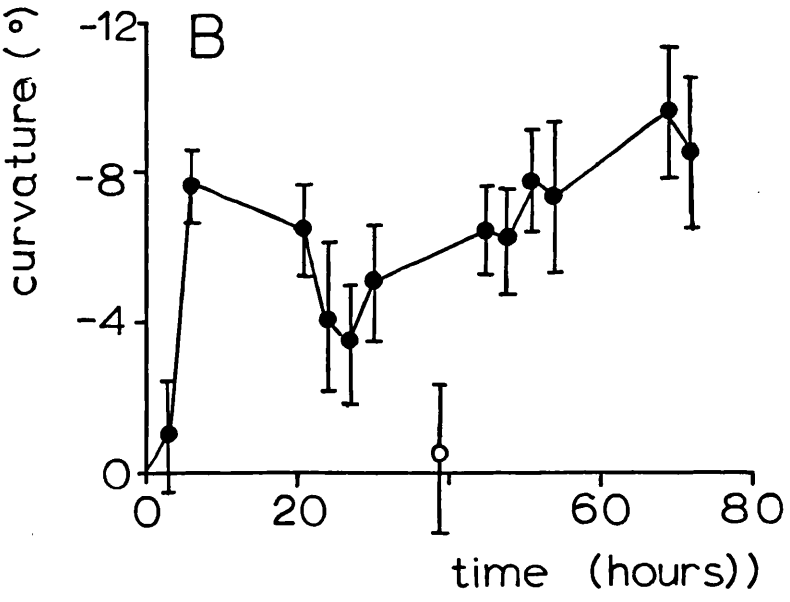
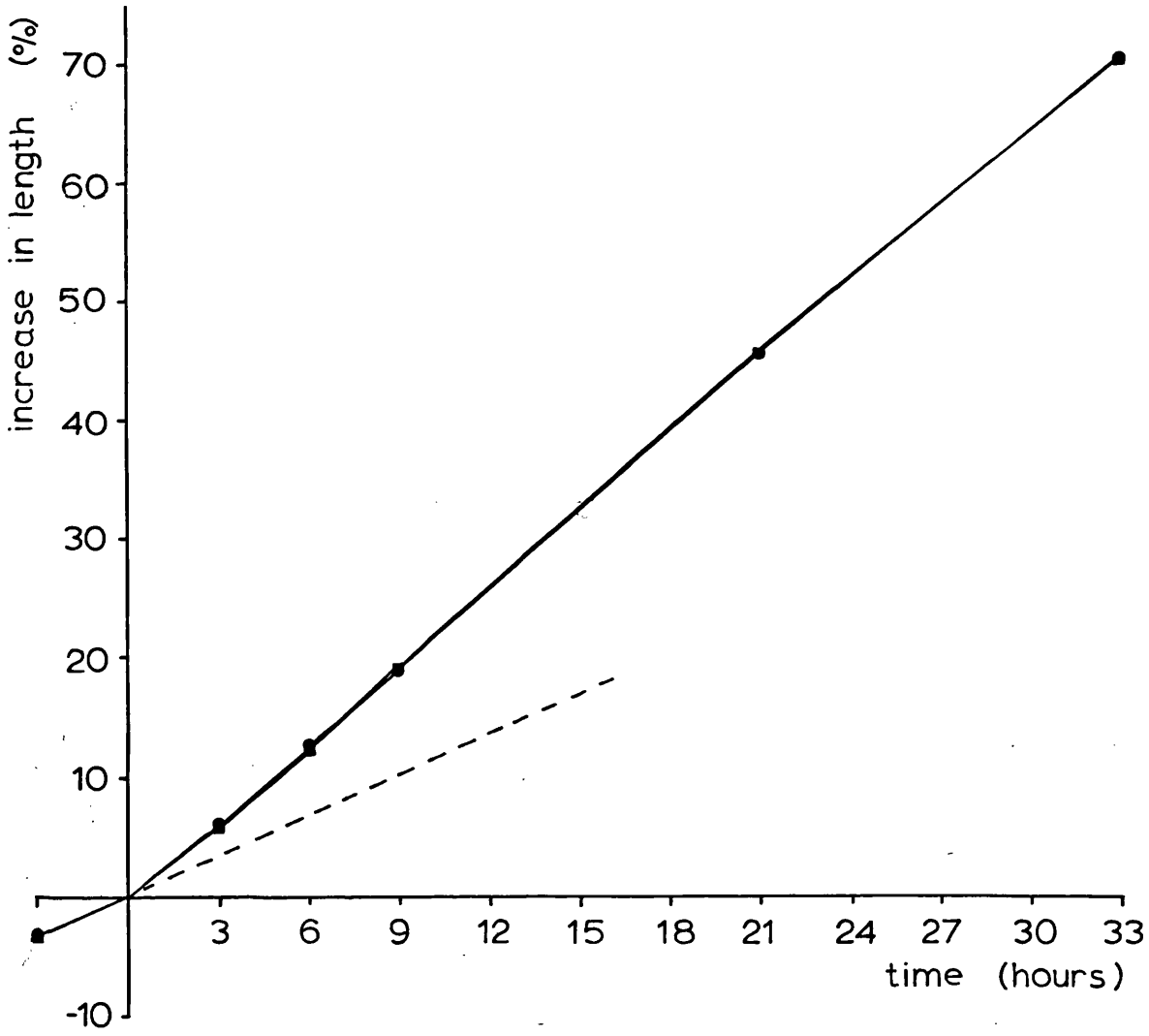

B
curvature (°)
-12
-8
-4
0
0
20
60
80
time (hours))

FIGURE 47

Attempted measurement of differential phytochrome-mediated phototropic growth in mustard seedlings, probably treated as in Figure 43a! ■ light side, ● shaded side, --- prestimulation growth rate. Points are means of 3 experiments, each experiment with 10 plants.


increase in length (%)
70
60
50
40
30
20
10
-10
3
6
9
12
15
18
21
24
27
30
33
time (hours)

DISCUSSION

The curvature towards filtered unilateral SOX light (Figure 45) is surprising in view of the lack of response usually reported for wavelengths above 520nm (Galston, 1959). The mechanism by which it is mediated is unclear. Steinitz, Ren & Poff (1985) found a "tailing-off" of the blue photoreceptor response in Arabidopsis and Lactuca for wavelengths 510-550nm, but were unable to detect curvatures at longer wavelengths. The Cinemoid filters used here let through no detectable light below 558nm and therefore presumably the response is not attributable to the blue photoreceptor. One possible explanation is that some phytochrome-mediated growth mechanism is involved, but there are several problems. Unilateral SOX light at the fluence rate used would probably establish the same photoequilibrium (ϕ = 0.86: Table 1) on both sides of the hypocotyl, hence any growth mechanism involving different photoequilibra within the axis is ruled out. The absolute fluence rates will, however, differ on the opposing sides of the hypocotyl, and hence some fluence-rate-dependent mechanism (e.g. Heim & Schäfer, 1982; Beggs et al., 1980; Holmes et al., 1982) could occur to establish different growth rates, though Figure 11 suggests that at least in the short term, hypocotyl growth is not controlled by a phytochrome irradiance-dependent response, hence no curvature would be expected. It is possible that differential illumination of the cotyledons could lead to phytochrome-mediated changes in hypocotyl growth (e.g. Shuttleworth & Black, 1977). Further characterisation is required.

The simplest hypothesis to explain the curvatures obtained by addition of unilateral far-red light to the background SOX light (Figure 46) is that the light treatments establish different photoequilibria across the hypocotyl which cause different rates of localised extension growth, and hence curvature. The actual photoequilibria established within the hypocotyl will depend on the relative absolute fluence rates of SOX and far-red light. Assuming the fluence rates of SOX light are the same for both sides of the hypocotyl, the difference in photoequilibria will be determined by the magnitude of the far-red light gradient. In maize, the relative light gradient is approximately 1:0.33 (Kunzelmann & Schäfer, 1985), hence extrapolating to mustard, a lower photoequilibrium would be expected on the illuminated side of the hypocotyl. The relationship between photoequilibrium and growth rate is well-known; very crudely, the lower the photoequilibrium, the higher the growth rate (c.f. Figure 6); hence the absolute growth rates, and therefore the difference between them, will depend strongly on the amount of far-red light added. In principle, therefore, the unilateral far-red light added in a background of SOX would be expected to establish a lower photoequilibrium (and hence higher growth rate) on the illuminated side of the hypocotyl than on the shaded side, leading to curvature away from the light source; Figure 46 shows this indeed to be the case. Although Iino, Schäfer & Briggs (1984) demonstrated the site of photoperception was essentially congruent with the site of curvature in maize, it is not possible to rule out some additional influence of the cotyledons (e.g. Shuttleworth & Black, 1977) in mustard.

This is essentially the same light-growth hypothesis advanced by Kunzelmann & Schäfer (1985) for phytochrome-mediated phototropism in maize mesocotyls, with the exception that here the growth rates increase (Figure 44) rather than decrease. The fact that curvature can be induced in mustard, gourd and pea suggests that the phenomenon can occur in a wide range of species, and it is therefore all the more surprising that phytochrome-mediated phototropism has not been more widely observed. It is possible that as the response is smaller and slower to develop than blue-light mediated phototropism, it has been overlooked, but it is more likely attributable to the experimental light treatments used to investigated phytochrome-mediated phototropism. In order to see curvature different growth rates must be established and maintained within the axis, and it is possible that some light treatments do not in fact achieve this. Two previous reports investigating phytochrome-induced curvature (Mohr & Peters, 1960; Shropshire & Mohr, 1970) attributed the lack of response to unilateral red light in mustard to either an insufficient light gradient or a diffusible material (e.g. gibberellins) preventing the establishment of differential growth. The fact that curvature does occur in other situations rules out the second possibility, but it is possible that a small light gradient would not establish a sufficient difference in irradiance-dependent responses to establish curvature, or even that irradiance-dependent responses do not occur at all (e.g. Figure 11). Explanation of these apparent anomalies may be resolved when the phytochrome-control of hypocotyl growth, especially in relation to irradiance-dependency, is more fully understood.

Phytochrome-mediated phototropism therefore differs from classical blue-light phototropism in time course, magnitude and pattern of differential growth. Apart from the fact that it is mediated by phytochrome and not

the blue-light-absorbing photoreceptor, it is essentially identical to Blaauw's (1914, 1915) light-growth mechanism. This makes it all the more surprising that such a system does not also operate for blue-light-mediated inhibition of axis extension (c.f. Chapter 2). It is also interesting to note that, despite only having been established as a phenomenon for 2 years, probably more is known about the mechanisms and primary transduction chain of the phytochrome-mediated phototropism than that of classical blue-light-mediated phototropism!

APPENDIX II

COMPARISON OF LAG TIMES IN PLANT PHYSIOLOGY

One of the classical approaches of plant physiology is to infer mechanisms from a comparison of time courses. In this approach, it is often important to determine the 'lag time' or 'latent period' between the initial induction and subsequent development of a response, particularly when there is reason to suspect that individual responses may be part of a causative sequence. Equally, when attempts are made to pursue an investigation to the biochemical level, it is clear that in order to qualify as a putative causal event, any postulated metabolic change must precede, or at most coincide with, the expression of the response. Such an agreement between the lag times may suggest, but can never prove, cause and effect. On the other hand, a significant discrepancy between lag times may be considered as definitive evidence against a causal relationship. For example, the acid growth hypothesis of auxin action predicts that auxin should induce proton flux from auxin-sensitive tissue, and that the latent period for the efflux should be no longer than the latent period for enhancement of growth. The examples where this has been shown to be the case may be cited as circumstantial evidence in favour of a causal relationship between auxin, acid and growth (Evans, 1985), but the lack of agreement in time between cell elongation and medium acidification kinetics reported by Kutschera & Schopfer (1985) is incompatible with such a theory.

Comparison of lag times is a well-established and widely-used experimental principle. In order to draw conclusions from such comparisons, the variance of any lag times should be known. Thus the most useful estimate of a lag time is a population mean with a measure of the variance to account for the inherent variability of biological material and experimental error. Surprisingly, there are relatively few data with this statistical information in the literature.

Lag times are usually derived in one of two ways. The most widely used method is to estimate the lag time by extrapolation from a plot of mean response against time. The lag time is usually taken as the time subsequent to induction at which the mean response curve departs from zero or exceeds the limit of experimental variation. Less frequently a second method is used. The time between induction and first expression of a response is determined for each individual replicate, and then averaged to give a mean lag time for the population.

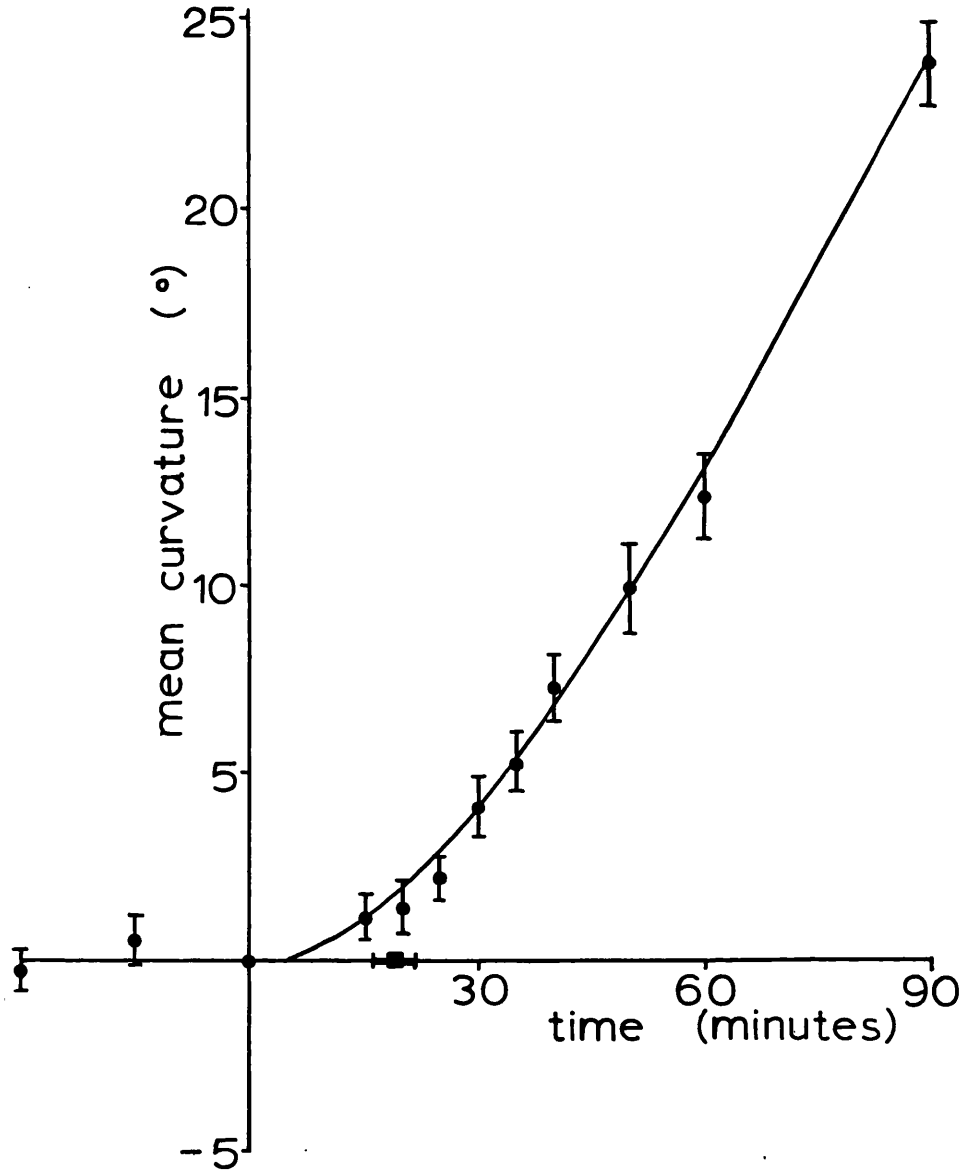
The difference between these two techniques may be illustrated by the following hypothetical example: Consider two plants with different lag times for a response, the first with a lag of 10 min, the second 30 min. The true mean lag time is clearly 20 min. An estimate of the lag time from a plot of mean response against time, however, will be only 10 min because the response of the first plant obscures the lack of response in the second plant. Lag times derived by this latter method measure expression of the response rather than lack of expression. They reflect the lag time of the fastest responding plants in the population only, and will result in an under-estimate of the true mean lag time.

This simple argument can also be applied for any further number of plants or to real experimental data. Figure 48 shows the development of phototropic curvature with time for light-grown mustard (Sinapis alba L.) seedlings, grown and essentially treated as previously described by Rich, Whitelam & Smith (1985).* An estimate of the lag time measured for individual plants and then averaged is 18.8 $\pm$ 10.3 (s.d.) min. These individual lag times show a broad,

* see Chapters 2 & 3.

FIGURE 48

The development of mean phototropic curvature for mustard hypocotyls as a function of time in response to 0.64 μmol m^{-2} s^{-1} continuous, unilateral blue light in a background of 45 μmol m^{-2} s^{-1} SOX light (●). The curve is a third-degree polynomial fitted by computer. Data before t=0 give a measure of circumnutation and experimental error in the population. The true mean lag time calculated on an individual plant basis is also shown (■). All data are mean ± s.e. (n=15).


25
20
15
10
5
-5
mean curvature (°)
30
60
90
time (minutes)

positively skewed distribution with a median of 16-20 min. In contrast, an estimate of the lag time from a solution of the polynomial describing the mean response curve for zero curvature is 5.0 min.

The conclusions drawn from a comparison of lag times may then depend on which estimate is taken. For instance, according to the Cholodny-Went theory of phototropism (Went & Thimann, 1937), growth curvatures induced by light are brought about by lateral transport of auxin. The theory predicts that the lag time for action of auxin on cell elongation growth should be no longer than the lag time for phototropism. If the two lag times for phototropism in mustard above are compared with a typical lag for auxin action of about 10 min (Evans, 1974; but note many of the data on which this is based are derived from averaged response data themselves), then two contradictory, though not necessarily mutually exclusive, conclusions can be reached. If the 5 min average response lag time is taken, then auxin cannot be directly involved in phototropism, and the Cholodny-Went theory is not an adequate explanation. If the 18.8 min true mean lag time is used, then auxin is still a potential mediator and the Cholodny-Went theory is still valid. A survey of ten papers in the literature shows only three that have reported true mean lag times for phototropism based on individual replicates. Darwin & Darwin (1880) give a lag time of 4-9 min for Phalaris coleoptiles, and Fox & Puffer (1977) report a lag time of less than 6 min for a single plant of Stapelia. Baskin et al. (1985) analysed growth rates during phototropism at the cellular level, and found true mean lags of 22 min for the change in rate on the illuminated side of maize coleoptiles, and 30 min for the change on the shaded side. It is unclear how the lag time reported

by Curry (1969) was derived, but Pickard et al. (1969), Hart & MacDonald (1981), Britz & Galston (1983), Iino & Briggs (1984), Ellis (1984) and Cosgrove (1985) have all derived lag times from graphs of average curvature. The reason that these latter workers have derived values in this manner is probably because circumnutation causes difficulty in defining when the curvature actually begins, and averaging the response attempts to minimise this source of error. Although this averaging approach solves one problem, it creates another in terms of the decreased usefulness of the resultant estimate of the lag time.

As the lag times derived from graphs of averaged response are biased by the faster responding plants, they may not be representative of the population. They are still of use, however, because again, the fastest lag time for a presumed causative biochemical change still has to be no longer than the fastest lag for the response itself, irrespective of agreement between the means. For example, the fastest lag time for auxin action on elongation growth of mustard hypocotyls would have to be shorter than, or equal to, the 5 min averaged response lag time for phototropism if the Cholodny-Went theory is correct. This argument also applies to individual replicates, where a potential physiological mechanism still has to be capable of causing the fastest response observed. A comparison of such lag times may therefore prove valuable, but their poor statistical basis suggests caution in interpreting their significance.

It may also be possible crudely to compare true lag times with the lag times derived from averaged responses if the variation of individual lag times in the population is known. The difference between the two will depend on the type of data distribution and on the deviation from the mean. A Poisson, or positively-skewed, distribution will give much less difference than a normal, or

negatively-skewed, distribution. Similarly, the difference will be less when the standard deviation is small rather than large. If two conditions are met a plot of average response against time can be used to derive a mean lag time. First, the response must start abruptly, and second, that once begun, the response must continue at a constant rate for the length of time investigated. The lag time may then be simply derived by extrapolating the linear curve back to zero. If the first condition does not hold, then only an upper limit for the mean lag time can be derived through extrapolation. This approach has been used recently by Bleiss & Smith (1985). More precise resolution can only be obtained with knowledge of the average time taken to achieve a constant rate of response. Generally, however, it is not possible to obtain a reliable estimate of the lag time from graphs of average response because the variation on an individual plant basis is not known, and hence comparison is not possible.

The fact that these two methods give different measures of the lag time that are neither directly comparable nor statistically compatible appears to have been widely overlooked, and in many cases lag times obtained by the two methods have been indiscriminately compiled and compared (eg. Evans, 1974; Firn & Digby, 1980). It is clear that more care should be taken in the correct use of the different lag time estimates and to this end we suggest that the terms lag and latent should be used strictly to define the period of time between induction and expression of a response for each individual replicate. True mean lag times can then be easily calculated. Lag times derived from averaged response should then be redefined as "first detectable responses in the population" or some other suitable descriptive term. The

danger of a broad definition covering both cases is that it is not possible to distinguish between them without reference to the original data, and the first detectable responses in a population may be taken to represent a true mean lag time even if not meant in that sense originally.

The potential scale of this problem is apparent from a cursory survey of lag times reported in Plant, Cell and Environment, Planta and Plant Physiology during the last 5-8 years. Over half the papers we have seen from many areas of plant physiology clearly reporting or using lag times should be ascribed to the "first detectable response" category. It will be interesting to see if conclusions based on comparison of such data stand the test of time themselves.

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PHOTOTROPISM IN LIGHT-GROWN MUSTARD (*SINAPIS ALBA* L.) SEEDLINGS

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This thesis is an investigation of the mechanisms of blue-light-mediated phototropism in higher plants. Phototropism was analysed in light-grown mustard (*Sinapis alba* L.) seedlings under low pressure sodium (SOX) lamps to minimise the involvement of phytochrome. Light-grown mustard seedlings do not show a blue-light-mediated inhibition of axis extension growth and the Blaauw theory must therefore be rejected as an explanation of phototropism. Phototropic curvature was established by an inhibition of growth on the illuminated side of the hypocotyl accompanied by an equal but opposite acceleration on the shaded side, with little or no change in net growth. This pattern of differential growth can be modified by light-growth responses separate from those involved in phototropism. Preliminary results from a biophysical analysis of cell growth using micro-pressure probes indicates phototropic growth responses are caused by changes in wall rheological properties, possibly by wall extensibility alone. There is a complex relationship between the kinetics of phototropism and stimulus fluence rate. The lag time is independent of fluence rate. The initial rate of curvature was directly proportional to log fluence rate between threshold and saturation fluence rates. Following this initial phase, gravitropic compensation and autotropic straightening modify curvature, which can no longer be used as a quantitative measure of phototropism. Implications for measurement of the response and selection of appropriate photobiological conditions are outlined. The magnitude of the internal light gradient influences the rate of curvature. The experimental results are discussed in relation to other knowledge of the phototropic transduction chain. The results are not inconsistent with the Cholodny-Went theory of phototropism. Preliminary data indicating phytochrome-mediated phototropism are presented, and the comparison of lag times in plant physiology is also discussed.