

PHOTOCONTROL OF INTERNODE EXTENSION GROWTH IN  
Sinapis alba L.

by  
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## ABBREVIATIONS AND DEFINITIONS

B:	blue light
°C:	degree Celsius
cm:	centimetre
D:	darkness
d:	day
FR:	far-red light
g:	gram
h:	hour
kDalton:	kilo Dalton
LSD:	least significant difference
µm:	micrometre
µmol m <sup>-2</sup> s <sup>-1</sup> :	micromole per metre squared per second
mm:	millimetre
mg:	milligram
min:	minute
nm:	nanometre
No:	number
P:	total phytochrome
PAGE:	polyacrylamide gel electrophoresis
PAR:	photosynthetically active radiation
Pr:	red-absorbing form of phytochrome
Pfr:	far-red-absorbing form of phytochrome
Pfr/P:	phytochrome photoequilibrium (Hayward, 1984)
R:	red light
SDS:	sodium dodecyl sulphate
sem:	standard error of the means
SOX:	light from low pressure sodium lamps
WL:	white light

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PHOTOCONTROL OF INTERNODE EXTENSION GROWTH IN  
Sinapis alba L.

Jorge José Casal

This study is concerned with the responses of internode extension rate in white-light-grown Sinapis alba seedlings to light: the kinetics, nature and organ localization of the photoreceptors and possible transduction chains. Phytochrome status was modified either by means of red, or far-red, light pulses given at the end of the photoperiod, or by supplementing white fluorescent light with different fluence rates of far-red light during the photoperiod. The status of specific blue light-absorbing photoreceptor(s) was modified by supplementing a background of blue-deficient light with different fluence rates of blue light.

Low Pfr/P established in the leaves induced a promotion of internode extension rate that persisted for ca 24 h after the plant returned to high Pfr/P. The effect of Pfr/P during dark periods is due mainly to this persistent response. Several fluence-rate-dependent responses were revealed: (a), blue light reaching the leaves modulates the responsiveness of the internode to a reduction of Pfr/P restricted to the internode itself; (b), blue light reaching the leaves inhibits internode extension in subsequent darkness; (c), for a given period in darkness, internode growth rate is higher if Pfr/P is reduced before, rather than at the beginning of darkness, or at the beginning of darkness, rather than later; (d), the transition from light to darkness causes a rapid reduction (lag < 10 min) of internode extension rate.

A correlation (kinetics and localization) between the extracellular activity of one moderately acidic peroxidase isoform extracted from the internodes, and the effect of Pfr/P on internode extension rate is presented. The activity of another extracellular peroxidase isoform was unaffected by Pfr/P, but showed a rapid increase after wounding. This treatment caused no significant effects on the isoform controlled by Pfr/P.

Present findings are discussed in relation to the ecological significance and the physiological basis of the control of internode extension growth by light.

## CHAPTER 1

### GENERAL INTRODUCTION

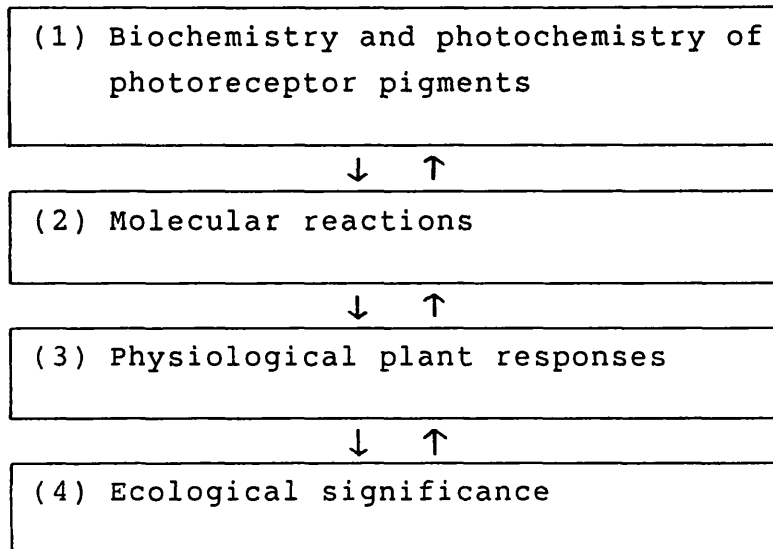
The light environment exerts a profound influence on plant growth and development. On the one hand, visible light (400-700 nm) provides the energy that makes possible growth and development of green plants, and indeed, makes possible their existence. The processes of light energy capture by the plant and the subsequent transformation in chemical energy are called photosynthesis. In addition, light is also a source of information for the plant. Unlike photosynthesis, the latter is also true for many animals, including man. We can, for instance, distinguish the red from the green traffic lights and react accordingly. Plants, through processes that share many common features with human vision, can sense the increase in the proportion of FR (i.e. 700-800 nm) resulting from the presence of neighbouring plants, and react to this information. The way in which we, or the plants, react to the light signals received has consequences that, in some cases, could cost the life of the individuals involved.

Plants receive different sorts of light signals, characterised by their intensity, duration, spectral composition, and direction (Smith, 1984). These signals can be received and "interpreted" by the plant only if the relevant photoreceptors are present and active. The photoreceptors perceive and transform the light signals into biochemical information that, through further biochemical and biophysical changes, leads to the final responses. Photomorphogenesis is a term used to define some of these processes, mainly the perception of, and the responses to, the information provided by light quantity and spectral composition (Smith, 1984) and also the consequences of such responses. In many cases it is not easy to make a clear cut distinction, but those processes related to light duration and light direction signals are called photoperiodism and phototropism, respectively, and are excluded from photomorphogenesis defined in a strict sense (Smith, 1984).

As with many issues in plant science,



photomorphogenesis can be investigated at different levels, ranging from the study of photoreceptor molecules to the ecological consequences of photomorphogenic responses in nature. The scheme below shows some of these levels, but it must be borne in mind that, as with any model, this scheme is a simplification of the real world.



↓↑: flux of knowledge.

The early work in photomorphogenesis involved the study of plant responses (3) such as stem growth and flowering under different light treatments. A turning point was reached as a result of the physiological studies conducted by Hendricks and Borthwick in the United States Department of Agriculture at Beltsville. They grew Pinto bean plants under normal photoperiods from fluorescent lamps, terminated with R or FR pulses of low fluence rates. These plants did not show any response to a R pulse immediately before D, but were responsive (increase in internode length) to a FR pulse. They became responsive to R, however, immediately after they received FR. The response depended on whether a R or a FR pulse was the last in a series of pulses. From these data, and the results of other experiments, the

authors deduced the existence of a R/FR photoreversible pigment that today we call phytochrome (e.g. Borthwick et al., 1952; Downs et al., 1957). Several years later, the Beltsville group, at that time including W. Butler and W. Siegelman, isolated the predicted pigment (Butler et al., 1959). Immediately after, studies began to be conducted in order to understand how phytochrome works (2). Today we know that some responses involve the regulation of gene expression (Schäfer et al., 1986), and others, changes at membrane level (Haupt, 1986). Nevertheless, essential aspects of the nature of the molecular events involved in phytochrome mediated responses (2) are still an enigma.

During the last half of the 70's, several groups, but especially that led by H. Smith, first in Nottingham and later in Leicester University, drew attention to the ecological implications of the phytochrome responses in natural canopies (4) (Smith, 1982). This opened a new perspective: it began to be attractive to understand not only how phytochrome works, but also why it is present in nature.

The existence of plant responses to the fluence rate of B (3) was documented in very early reports (reviewed by Stolwijk, 1954). With the discovery of phytochrome, investigations were initiated to determine whether that pigment was also mediating B responses such as stem growth. Perhaps surprisingly, it took almost 20 years to obtain solid evidence for the existence of a specific blue light photoreceptor involved in the photomorphogenesis of higher plants (Thomas and Dickinson, 1979; Gaba and Black, 1979). Nevertheless, our understanding of B responses is still very poor.

The present work is concerned with one group of photomorphogenic responses: the photocontrol of internode growth in light-grown mustard seedlings. Part of this thesis is concentrated in the acquisition of background information, such as the nature of photoreceptors involved in the responses to light

signals, the loci of perception of these signals, the kinetics of the responses and the interactions between them (i.e. 3). In addition, attempts to move towards a more "molecular" level of understanding (i.e. 2) are also described. The aim of the present studies is to aid a rational approach to two main issues: a), the mechanisms involved, at the whole plant, organ and biochemical levels, in the different stem extension responses to light, and b), the ecological significance of such responses. For those purposes several questions were addressed:

a) Is internode extension growth affected by the status of phytochrome during preceeding periods or, alternatively, is it only affected by the current Pfr/P? (Chapter 2).

b) Is there any interaction between the responses to Pfr/P and possible responses to fluence rate (e.g. to the fluence rate of B)? If so, what are the characteristics of the response to different Pfr/P established before darkness compared to the response to different Pfr/P established under continuous background WL described previously (Child and Smith, 1987)? (Chapters 3, 4 and 5).

c) Are the changes in extension growth rate caused by different Pfr/P correlated with differences in extracellular proteins that could account for the effects on growth? (Chapter 6).

In the last two chapters (7 and 8), the results presented in previous chapters are integrated and discussed in relation to their relevance for ecological and physiological studies respectively.

## CHAPTER 2

PERSISTENT EFFECTS OF CHANGES IN PHYTOCHROME STATUS  
ON INTERNODE GROWTH IN LIGHT-GROWN MUSTARD:  
OCCURRENCE, KINETICS AND LOCUS OF PERCEPTION<sup>\*</sup>.

<sup>\*</sup>Casal and Smith (1988) Planta 175: 214-220

## INTRODUCTION

In photosynthetically-competent mustard plants grown under WL, irradiation of the first internode with supplementary FR from fibre-optic light guides causes a rapid increase in extension growth (Morgan et al., 1980; Child and Smith, 1987). This effect is largely mediated by a reduction in Pfr/P in the growing internode, as indicated by both the reduction in growth stimulation by simultaneously applied R (Morgan et al., 1980) and by its linear relationship with the Pfr/P measured in etiolated tissue placed in the same position as the stem (Child and Smith, 1987). If FR is switched-off, the mean time for extension growth to return to the level before FR switch-on is 16 min, and this is not affected by the duration of the treatment between 35 and 180 min (Child and Smith, 1987). Furthermore, mustard plants can respond to successive brief FR pulses directed to the internode with no consistent carry-over effects from previous FR treatments (Child and Smith, 1987). From these data a model could be proposed in which internode growth during a period of several hours is a linear function of the average Pfr/P in the growing internode during that period, even if the Pfr/P is modified by light treatments during the considered period (as long as growth capacity remains constant). A consequence of this model is that, in the natural environment, the information provided by previous shade-events would be only stored in the accumulated growth but not in the phytochrome-transduction chain system. However, it is well known that phytochrome effects on the growth of etiolated mustard are strongly affected by light pretreatments perceived by phytochrome itself (Beggs et al., 1980; 1981). Furthermore, recent results obtained with young WL-grown seedlings, show that hypocotyl growth between 7 and 24 h after WL-D transition is not only affected by light pulses given at 7 h, but also by pulses given immediately before the beginning of D.

Several explanations can be proposed to resolve this apparent conflict: (i), long term effects are characteristic of very young, etiolated or briefly de-etiolated seedlings but are not found in photosynthetically-competent plants; (ii), long term effects are characteristic of phytochrome action during dark incubations but are not found when the R:FR of continuous WL is modified; (iii), long term effects are caused by changes in phytochrome status in the leaves but not in the growing internode and (iv), long term effects require changes in phytochrome status of longer duration than those produced in the transducer experiments (less than 180 min, Child and Smith, 1987). The experiments shown in this paper are directed to answer the above questions.

#### MATERIALS AND METHODS

Plant material. Mustard (*Sinapis alba* L., Asmer seeds, Leicester, U. K.) were sown in Petersfield (Leicester, U. K.) No 2 commercial range potting compost in 120 cm<sup>3</sup> pots, watered with tap water and grown under fluorescent WL. Temperature (22 or 25 °C), PAR (86-325  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and age (11-12.5 d) prior to the beginning of treatments varied among different kinds of experiments and are indicated in the legends of figures and tables.

Light sources. WL was provided by Osram or Thorn EMI (both Birmingham, U. K.) warm white 65/80 W fluorescent tubes (Fig 2.1A). When the plants were grown under different R:FR ratios of the WL, background WL was provided by Thorn EMI Pluslux Blanc 3500/36 W (Fig. 2.1A) and FR (Fig. 2.1B) was provided by Philips (Belgium) 7785 R/500W incandescent quartz lamps in combination with 4 cm circulating water and a black acrylic filter (Plexiglass FR F 700 West Lake Plastics Co, Lem, PA, USA). Different R:FR of the WL were provided in two separate compartments (light- but not air-tight) of the same growth chamber. R (4  $\mu\text{mol m}^{-2}$

s<sup>-1</sup>) for whole plant pulses was provided by a bank of 6 Thorn EMI Deluxe Natural /40W fluorescent tubes filtered through 1 cm deep copper sulphate solution (1.5 % w/v) and one red Cinemoid (Rank Strand, Isleworth, Middlesex, U. K.) (No. 14) sheet (Fig. 2.1B). FR (102  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) was provided by a bank of 10 water-cooled 100 W incandescent bulbs with a black acrylic filter (Fig. 2.1B). A mixture of R+FR (260  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) was provided by one Phillips 7785 R/500W incandescent quartz lamp with a 2 cm running water, and a red Cinemoid (No 14), filter (Fig. 2.1B). R or FR (13  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) for localised irradiation was provided by Schott Mainz KL 150 B projectors (supplied by Carl Zeiss, Welwyn Garden City, Hertfordshire, U. K.) with a heat absorbing glass filter and either a 659.5 nm Ealing (Watford, U. K.) interference filter or a sandwich of two red (No 14), one orange (No 5) and one green (No 24 A) Cinemoid filters (Fig. 2.1C) respectively.

Light measurements and Pfr/P calculations. The fluence rates and spectral photon distributions of light sources were recorded with a calibrated LI-COR LI-1800 spectroradiometer (Lincoln, Nebraska) by placing the cosine-corrected remote probe head horizontally at plant height or facing the fibre optic tip. Pfr/P was calculated from the spectral photon distributions (Hayward, 1984).

Locus experiments. Localised irradiations were given by means of fibre optic probes (Schott Mainz, 80 cm length, 0.5 cm tip diameter) directed to the internodes. Since light emerged from the fibre optics with a wide angle, black rigid sheets (8 x 10 cm<sup>2</sup>) supported by the fibre optic bundles were placed parallel to soil surface both above and beneath the edges of the probes (i.e. separated by 0.5 cm). A slot (3 mm width) was made in the sheets to place the plant 1 cm distant from the tip of the fibre optics. In another set of experiments the primary leaves were covered by means of two black plastic envelopes (5 x 7 cm<sup>2</sup>) supported by a planar

surface placed at leaf height.

Plant measurements and statistics. The length of the first internode from the cotyledonary node to the first leaf pair node was measured to the nearest 0.5 mm with a ruler, all measurements being taken by the same person. A systematic sequence of measurements for the different treatments was avoided. When possible, throughout measurement each plant was coded so as to hide the treatment it received. Each experiment (except that shown in Table 2.1B) was repeated 3 to 7 times with each plant assigned at random to the different light regimes; thus, all the individuals of the different experiments were used as replicates in the statistical analysis (the actual number is indicated in the legends of Tables and Figures). When the plants were grown in the growth chamber providing different R:FR ratios of the WL (see light sources section), the compartment to which FR was added was randomised in each experiment. Since the results were consistent among experiments, no obvious "compartment effect" was found and the assumption of independence amongst plant replicates was regarded as reasonable.

## RESULTS

Effect of phytochrome status during a period of incubation in D on the subsequent growth in D. Mustard plants were grown under WL and received a R or FR pulse at the end of the 11th d. After 3.5 h D, R or FR pulses were given in factorial combination with the end-of-day pulse. Growth in D, between 3.5 and 15.5 h after the end of the day was affected not only by the current, but also by the previous phytochrome status in D (Table 2.1A). The lack of effect of the R pulse 3.5 h after the end-of-day FR pulse was not due to the permanence of low Pfr/P, as a R pulse given immediately after the end-of-day FR pulse reversed the promotion of growth caused by the latter (Table 2.1B).



Effect of phytochrome status during a period of incubation in D on extension growth during a subsequent period under WL. Mustard seedlings were grown under WL for 11 d and received either a R or a FR pulse before being transferred to D. The duration of the dark period varied from 0 to 9 h. Growth in D was promoted by a FR, compared to a R, pulse (Fig. 2.2A). Furthermore, growth during the subsequent 12 h under continuous WL was also faster in those plants receiving a FR pulse at the beginning of the previous dark period (Fig. 2.2B). This effect was mediated by phytochrome since a R pulse given immediately after the FR pulse fully reversed the long term promotion caused by the latter (i.e.  $\Delta$  length (mm) during 6 h under WL, following 3 h of dark incubation = R:  $1.46 \pm 0.24$ ; FR:  $2.28 \pm 0.24$ ; FR  $\rightarrow$  R:  $1.00 \pm 0.14$ ; n = 14). Therefore, growth under WL was affected by the previous phytochrome status in D.

A FR pulse followed by dark incubations of less than 60 min caused no long-term promotion compared with either plants under continuous WL, or plants incubated for 60 min in D after a R pulse (Fig. 2.3). Therefore, a minimum duration (i.e. 60 min) of low Pfr/P was required to effect a long term promotion. A dark incubation with low Pfr/P of 2.5 h duration saturated the long term effect (Fig 2.2B and 2.3). More rapid growth of plants pretreated with low Pfr/P during 3 h persisted up to ca. 24 h after the pretreatment, slowly decreasing during that period (Fig. 2.4). Beyond 24 h the promotion disappeared and no further significant differences were observed (Fig. 2.4). A R pulse followed by a dark incubation period caused a significant reduction of the average growth during the first hours of subsequent WL, compared with those plants under continuous WL (Fig. 2.2 and 2.3).

Effect of phytochrome status during a period of WL on the extension during the subsequent period in D. Eleven-day-old mustard plants were exposed for 12 h to WL or WL+FR. Lowering the R:FR ratio promoted the

growth of the first internode (i.e.  $\Delta$  length (mm) WL :  $1.06 \pm 0.06$ ; n= 155; WL+FR:  $1.99 \pm 0.08$ ; n= 158). At the end of the 12 h WL the plants received saturating light pulses providing different Pfr/P and were incubated in D for another 12 h. Growth in D was not only promoted by pulses providing low Pfr/P at the end of the day, but also by low, compared to high, R:FR ratios during the previous WL period (Fig. 2.5). The effect of the R:FR ratio during the WL pretreatment was larger in the plants receiving FR as end-of-day pulse.

Effect of phytochrome status under WL on the subsequent growth under WL. Mustard plants were exposed to WL or WL+FR for 12 h. During this period internode growth was promoted by low R:FR ratios (Fig. 2.6). After the 12 h period, half of the seedlings of each group were moved to the opposite light regime. Under WL+FR, extension rate was high regardless of the R:FR received during the pretreatment (Fig. 2.6). However, the plants moved from WL+FR to WL continued growing significantly faster than the controls under continuous WL, during the following 24 h (Fig. 2.6).

Locus of perception of the light signal triggering the long term promotion. One experimental approach to this question consisted of irradiating the whole plant with R or FR and, for half of the plants of each group, reverting the Pfr/P in the internode by means of fibre optic probe irradiations. After these treatments, the seedlings were incubated in D during 3 h and growth was measured during the subsequent 12 h under continuous WL. Extension rate under WL was higher in FR- than in R-treated plants (Fig. 2.7A). A FR pulse given only to the internode did not cause any promotion of extension growth (Fig. 2.7A), indicating that the growing internode would not be the main locus of signal perception. However, a R pulse given to the internode partially reversed the long term effect (Fig. 2.7A). Therefore, an interaction between the leaves and the stem can not be ruled out. In other experiments, it was

found that covering the leaves before giving the FR pulse caused a significant reduction of growth during the 12 h under WL following the dark incubation (Fig. 2.7B). The promotion caused by low Pfr/P in parts of the shoot other than the first leaf pair was three-fold smaller than that found when the whole shoot (i.e. including the first leaf pair) received the FR pulse. This confirms that the first pair of leaves are important for the perception of the stimulus causing the long term effect.

### DISCUSSION

These results indicate that extension growth of fully de-etiolated mustard seedlings, both under WL (Figs 2.2 and 2.6), and in D (Fig. 2.5, Table 2.1A), may be affected not only by the current Pfr/P but also by the Pfr/P established during previous periods either in D (Fig. 2.2, Table 2.1A) or under WL (Figs 2.5 and 2.6).

The growth of de-etiolated mustard seedlings seems to be under the control of phytochrome through at least two different pathways which may be differentiated on the basis of (i), the duration of the effects after the return to high Pfr/P; (ii), the duration of low Pfr/P required to effect the promotion and (iii), the site of perception.

(i) Duration of the effect. If FR is given for short periods (i.e. up to 180 min) directly to the internode of plants growing under background WL, extension rate measured by linear displacement transducer is promoted with a lag of ca. 10 min, returning to the original value by approximately 16 min after FR switch-off (Child and Smith, 1987). The results presented here show that, in addition to the short duration effect, a long term promotion may be triggered. The latter is manifested as different growth rates in plants having the same current Pfr/P, but having experienced different Pfr/P during previous periods. The differences established by either

3 h of dark incubation after a FR, compared to a R, pulse or 12 h under WL+FR compared to WL, persisted during ca. 24 h after the return to the high Pfr/P provided by WL (Figs 2.4 and 2.6). Beyond 24 h no further significant effects were noticed. These effects suggest that the plant has a "memory" of previous irradiation conditions.

(ii) Duration of low Pfr/P required to effect the promotion. FR given to the growing internode of plants under background fluorescent WL promotes growth with a lag of ca. 10 min., an effect which may be triggered by pulses as short as 1 min (Child and Smith, 1987). Approximately 60 min after the onset of continuous FR, a more-or-less steady elevated rate is reached which persists, without further increase while FR is on (Child and Smith, 1987). The present results show that low Pfr/P must be maintained for more than 45 min (at least in D) in order to trigger a long-term promotion of internode growth and that this promotion increases with the duration of the low Pfr/P pretreatment until 2.5 h (Fig. 2.3).

(iii) Site of perception. The rapid (i.e. 10 min lag) promotion of growth is triggered if the internode itself receives a low R:FR, whilst irradiating the first pair of leaves alone does not promote growth until 3 h of treatment (Morgan et al., 1980). Conversely, stem irradiation with FR does not cause a long-term growth promotion, which is only fully-triggered when the first pair of leaves receives a low R:FR treatment (Fig. 2.7 A and B). The involvement of the hypocotyl and the cotyledons, and of the growing internode and the leaves situated above and below it, in the control of hypocotyl growth in Cucumis sativus (Black and Shuttleworth, 1974) and in 4th internode growth in Chenopodium polyspermum (Lecharny, 1979) respectively, has already been demonstrated. Black and Shuttleworth (1974) also provided evidence for different photoreceptors acting in the cotyledons and the hypocotyl. Present and previous

(Morgan et al., 1980; Child and Smith, 1987) results with mustard show that in this species the photoreceptive organs can be differentiated by the demonstration of different kinetics of internode extension.

Present data can be accounted for by a model in which a growth-promotive substance is accumulated as a result of low Pfr/P reaching the leaves. In fact, in all the experimental systems used the promotion caused by low Pfr/P pretreatments persisted beyond the duration of the pretreatments. The involvement of changes in the responsiveness towards Pfr/P, as found in etiolated seedlings (Beggs et al., 1981), can not be fully excluded. However, the fact that the same kind of pretreatments led in some cases to a smaller difference between contrasting treatments (Fig. 2.6) and in others to a larger difference (Fig. 2.5) is certainly not in favour of this idea. The lack of effect of a R pulse given 3.5 h after the end-of-day FR pulse (Table 2.1A) can not be explained by the endogenous rhythmic change in responsiveness towards pulses providing contrasting Pfr/P that was reported for hypocotyl growing seedlings (Wilderman et al., 1978; Schäfer et al., 1984). In fact, in young seedlings a R pulse is inhibitory even when given 3 to 9 h after an end-of-day FR pulse (Schäfer et al., 1984). At present, a theoretical increase in phytochrome levels due to low Pfr/P pretreatments (Kilsby and Johnson, 1981; Smith, 1981) can not explain the long term growth promotion. It should be noted that the latter is also unlikely to be the mere consequence of the differences in growth during the pretreatment -i.e. the comparison of plants of different heights and therefore different capacities to capture light at the beginning of the period under consideration. In fact, (i), low Pfr/P during the pretreatment promoted extension growth not only under WL but also in D and (ii), the long term promotion is lost after ca. 24 h.

The nature of the messengers in the transduction chain

between phytochrome photoconversion and growth response has not yet been elucidated. The long-term promotion reported here implies a sequence of events beginning with the perception of the environmental signal in the leaves, followed by the accumulation (Fig. 2.3), and transmission of a biological signal to the growing internode, and culminating in its reception therein and the initiation of the ultimate growth response. One or more substances could be involved as messengers in this chain. A priori, hormones (i.e. gibberellins), or photoassimilates, could be considered as likely candidates. A R pulse followed by a dark incubation reduced extension growth after return to continuous WL, compared to plants which received no light interruption (Fig. 2.2 and 2.3). A simple interpretation of this effect could be based upon a reduction in stored assimilates during D.

The R:FR ratio within vegetation canopies is not constant throughout the photoperiod (Holmes and Smith, 1977; Deregibus et al., 1985; Casal et al., 1986; Ballaré et al., 1987). Even in relatively dense canopies, low R:FR ratios can be interrupted during relatively long periods due either to unfiltered direct sunlight penetrating through gaps at high solar elevations or to larger proportions of unfiltered diffuse light at the extremes of the photoperiod. Present results suggest that the shadelight signal could be stored in some messengers in the chain between phytochrome photoconversion and the growth response. A persistent promotion triggered when the R:FR is low, would allow the high rates of stem extension to continue when the R:FR is high. The capacity to avoid future neighbour shade would be therefore increased.

Table 2.1. Effect of R and FR pulses (10 min) given at the end of the photoperiod (0 h) and 3.5 h after the beginning of D on the extension growth of the first internode during 12 h in D after the second (A) or the first (B) light pulse. Before treatments the plants were grown during 11 d under 12 h photoperiods of WL (PAR=  $325 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Temperature= 25 °C. Each data is a mean of 59-60 (A) or 8 (B) plants  $\pm$  sem. (A) and (B) correspond to different experiments.

A)		
Pulse at 0 h	Pulse at 3.5 h	$\Delta$ length (mm) between 3.5 and 15.5 h
R	R	1.63 $\pm$ 0.12
FR	R	2.89 $\pm$ 0.14
R	FR	2.82 $\pm$ 0.14
FR	FR	3.21 $\pm$ 0.17
B)		
Pulse at 0 h		$\Delta$ length (mm) between 0 and 12 h
R		1.75 $\pm$ 0.4
FR		2.93 $\pm$ 0.2
FR $\rightarrow$ R		1.81 $\pm$ 0.3

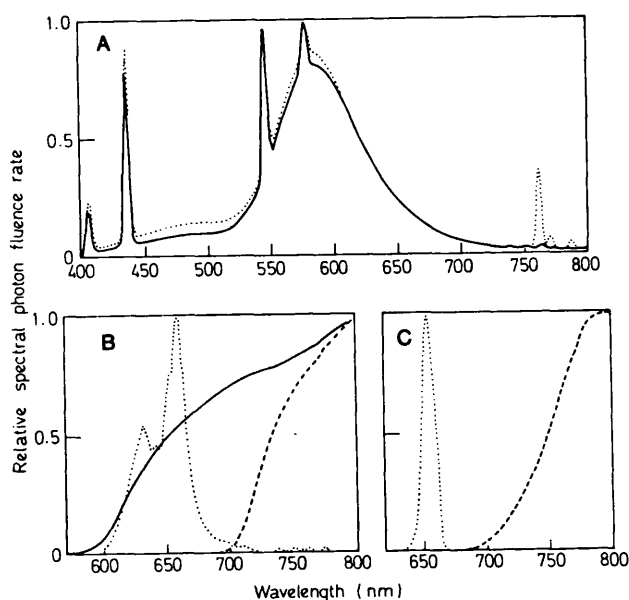


Fig. 2.1. The relative spectral photon fluence rates of the light sources. (A), WL provided by warm white (solid line) and Pluslux (dotted line) fluorescent tubes. (B), R (dotted line), FR (broken line) and R+FR (solid line) sources used for whole plant light pulses and also (in the case of FR) to reduce the R:FR ratio of WL. (C), R (dotted line) and FR (broken line) sources used for internode irradiations.

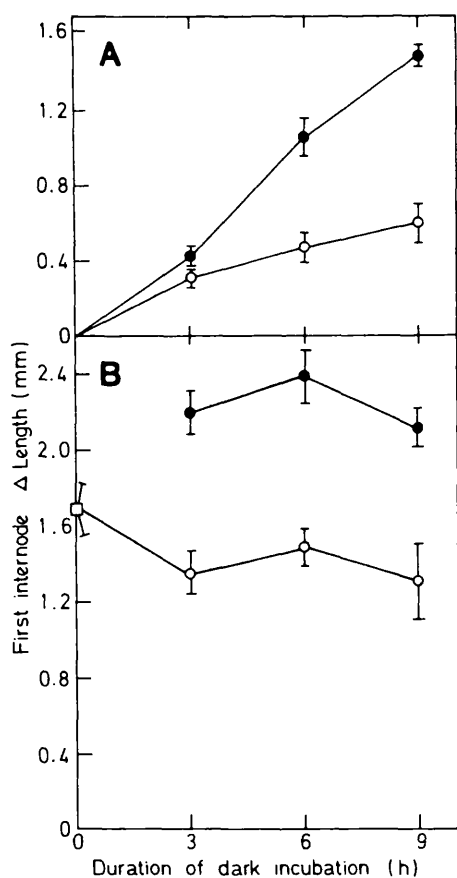


Fig. 2.2. Effect of R (open circles) or FR (closed circles) pulses (5 min duration) given after WL on the extension growth of the first internode (A), during the dark period immediately following the pulses and (B), during the subsequent 12 h under WL ( $\text{PAR} = 86 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) beginning at the end of the dark incubation. Square symbol = control plants under continuous WL. Before treatments the plants were grown for 11 d under continuous WL ( $\text{PAR} = 86 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Temperature  $22^\circ\text{C}$ . Each point is a mean of 43-62 plants  $\pm$  sem.



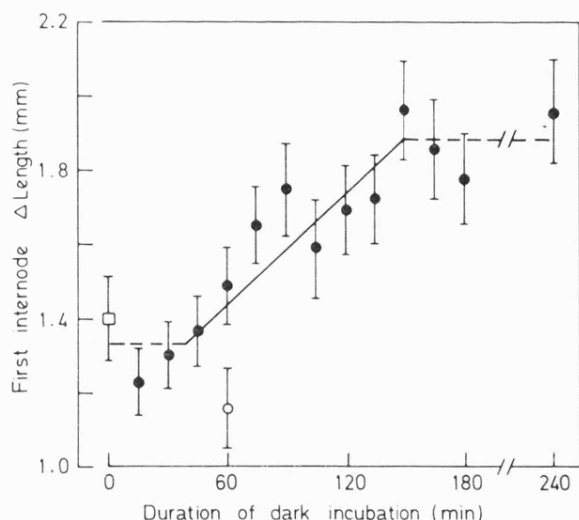


Fig. 2.3. Effect of R (open circle) or FR (closed circles) pulses (5 min duration) given at the beginning of a dark period on the extension growth of the first internode during the subsequent 12 h under WL (PAR=  $86 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) beginning at the end of the dark incubation, plotted as a function of the duration of the dark incubation. Square symbol= control plants under continuous WL. Before treatments the plants were grown for 11 d under continuous WL (PAR=  $86 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Temperature  $22^\circ\text{C}$ . Each point is a mean of 50-70 plants  $\pm$  sem.

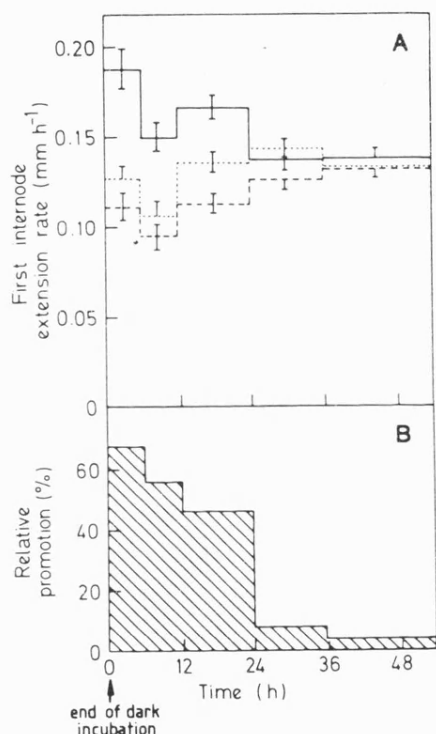


Fig. 2.4. Effect of a R (broken line) or a FR (solid line) pulse (5 min duration) given after continuous WL and followed by 3 h of dark incubation on the extension growth of the first internode after return to continuous WL (A). Dotted line= control plants without dark incubation. (B), apparent growth promotion caused by the FR pulse-dark incubation pretreatment ( $[\text{growth rate FR} - \text{growth rate R}] / \text{growth rate R}$ ). Before treatment the plants were grown for 11 d under continuous WL (PAR=  $86 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Temperature  $22^\circ\text{C}$ . Each rate is a mean of 130-145 plants  $\pm$  sem.

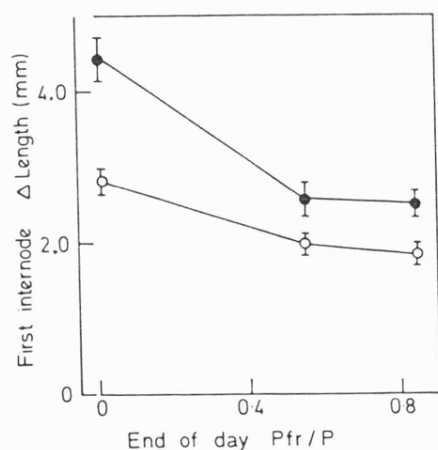


Fig. 2.5. Growth in darkness (12 h) as affected by the calculated Pfr/P provided during the previous 12 h under WL (PAR=  $130 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and the Pfr/P established by the light pulses (15 min duration) given before transferring the plants into darkness. Open symbols= WL (calculated Pfr/P= 0.7); closed symbols= WL+FR (calculated Pfr/P= 0.5). Before the pretreatments the plants were grown during 12 d under 17 h photoperiods of WL (PAR=  $90 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and for 12 h under WL (PAR=  $130 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Temperature  $25^\circ\text{C}$ . Each data is a mean of 50-71 plants  $\pm$  sem.

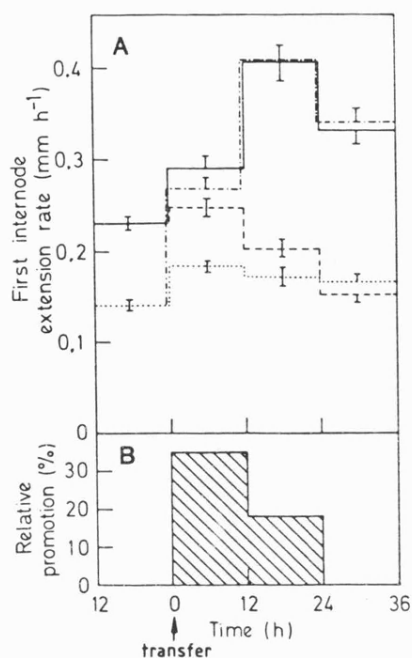


Fig. 2.6. Extension growth of the first internode under continuous WL. Dotted line= WL (calculated  $Pfr/P = 0.7$ ); solid line= WL+FR (calculated  $Pfr/P = 0.5$ ); broken line= plants transferred from WL+FR to WL at 0 h; broken-dotted line= plants transferred from WL to WL+FR at 0 h. (B), Apparent promotion caused by a WL+FR pretreatment after the transfer to WL ( $[\text{growth rate WL+FR pretreatment} - \text{growth rate WL pretreatment}] / \text{growth rate WL pretreatment}$ ; under WL). Before the pretreatments (-12 h) the plants were grown for 10 d under 17 h photoperiod of WL ( $PAR = 90 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and 1.5 d under continuous WL ( $PAR = 130 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Temperature = 25 °C. Each data is a mean of 155 (before 0 h) or 76-79 plants  $\pm$  sem.

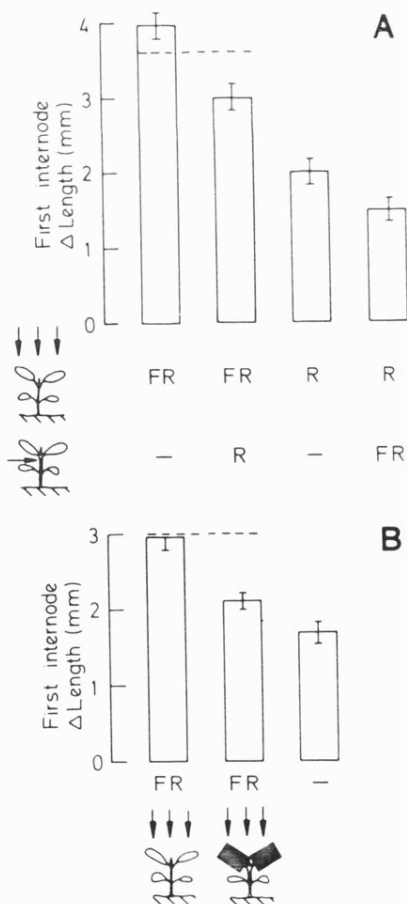


Fig. 2.7. The effect of localized R or FR irradiations followed by 3 h of incubation in darkness on the extension growth of the first internode during the subsequent 12 h period under WL ( $PAR = 110 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). (A), the plants received 5 min R or FR pulses from above, followed in half of them by a 3 min pulse of the contrasting wavelength given to the internode. The dotted line represents the level of control plants which after the FR pulse from above were placed in the fiber optic device without receiving any pulse directed to the internode. (B), the plants received a 5 min R pulse from above followed by the indicated treatment. The dotted line represents the level of control plants in which the first leaf pair was covered after instead during the FR pulse. Each data is a mean of 32-79 plants  $\pm$  sem.

### CHAPTER 3

THE LOCI OF PERCEPTION FOR PHYTOCHROME CONTROL OF  
INTERNODE GROWTH IN LIGHT-GROWN MUSTARD. PROMOTION BY  
LOW PHYTOCHROME PHOTOEQUILIBRIA IN THE INTERNODE IS  
ENHANCED BY BLUE LIGHT PERCEIVED BY THE LEAVES<sup>\*</sup>.

<sup>\*</sup>Casal and Smith (1988) Planta 176: 277-282

## INTRODUCTION

Extension growth in green plants of many species, including mustard, is affected by the Pfr/P provided both at the end of the photoperiod by R-FR pulses (Wildermann et al., 1978a; Chapter 2), and under continuous WL (Morgan, O'Brien and Smith, 1980; Chapter 2) or green light (Wall and Johnson, 1981) as a result of different fluence rates of supplementary FR. However, whether the physiological steps involved in one case and the other are exactly the same is not known. Among other differences, PAR, B and phytochrome absorbable radiation are not present after the various Pfr/P are established by light pulses at the end of the day, in contrast to daytime treatments. All these factors have been proposed to interact with Pfr/P in the control of extension growth (Johnson and Tasker, 1979; Lecharny and Jacques, 1979; Attridge, Black and Gaba, 1984; Drumm-Herrel and Mohr, 1984; Gaba, Black and Attridge, 1984; Smith and Hayward, 1985).

One of the approaches to investigate the physiology of phytochrome action at the whole plant level is a study of the locus of perception. In photosynthetically-competent mustard plants, both the leaves (Morgan, O'Brien and Smith, 1980; Chapter 2) and the internode itself (Morgan, O'Brien and Smith, 1980; Child and Smith, 1987) are involved in the perception of the light stimuli in phytochrome mediated extension growth responses. In the present study, evidence is presented that the relative contribution of these organs is different for the responses to changes in Pfr/P at the end of the day or under continuous WL. The causes of these differences were also investigated.

## MATERIALS AND METHODS

Mustard (Sinapis alba L., Asmer seeds, Leicester, U. K.) were grown under a PAR of ca  $90 \mu\text{mol m}^{-2} \text{s}^{-1}$  provided by

Thorn EMI (Birmingham, U.K.) warm white fluorescent tubes as described previously (Chapter 2). Temperature was 22 °C. Treatments began after 13-14 d.

End-of-day treatments. Plants received 5 min R or FR irradiations. The light sources, spectra, fluence-rates, fibre optics and envelopes used to cover the leaves or the cotyledons were as previously described (Chapter 2). The device located at the fibre optic tip in order to prevent the light given to the internode reaching other organs was modified as follows. The internodes were placed close to the fibre tip between two vertical black cardboard sheets supported by the fibre bundle (Figs 3.1a and b). A U-shaped piece of black cardboard was placed downwards over the upper edges of the vertical sheets thus preventing light from reaching the primary pair of leaves. The two vertical sheets were joined with a clip which set the position of the U-shaped cardboard piece, while the latter kept the internode close to the fibre tip (Figs 3.1a and b). Temperature was set at 25 °C.

Light treatments under continuous white light. Plants were irradiated from above with fluorescent WL ( $\text{PAR} = 130 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) with or without supplementary FR ( $170 \mu\text{mol m}^{-2} \text{s}^{-1}$  between 700 and 800 nm) in a growth chamber previously described (Chapter 2). Calculated  $\text{Pfr/P}$  were 0.50 and 0.69 respectively. The internodes were irradiated with WL ( $\text{PAR} = 10 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) with or without supplementary FR ( $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) transmitted from the top of the growing area of the corresponding chamber compartment by means of fibre optics and internode cover cardboards similar to those used for end-of-day treatments. Calculated  $\text{Pfr/P}$  for the light given to the internode was 0.40 and 0.69 respectively. In other words, the experiments consisted of a factorial combination of the light given from above and to the internode. In addition, plants under WL, or WL+FR, without the fibre optic and covers were included in order to evaluate any influence of the latter.

Temperature was set at 25 °C.

Influence of the irradiation provided from above on the effect of Pfr/P provided to the internode. In some experiments, the plants received either continuous WL (PAR 12  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; calculated Pfr/P= 0.86) or continuous WL+FR (PAR= 11  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; FR= 17  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; calculated Pfr/P= 0.54) directed to the internode by means of the fibre optics and internode covers, or remained without these treatments thus receiving only the light provided from above. Continuous WL (0 to 95  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; calculated Pfr/P= 0.86) or orange light (94  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; calculated Pfr/P= 0.84) supplemented with different fluence rates of B (0 to 8.4  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) were given from above. WL+FR was provided by 15 W incandescent bulbs (Wotan, U.K.), WL by Deluxe Natural fluorescent tubes (Thorn EMI, Birmingham, U.K); orange light by 135 W SOX (Thorn EMI) and B by Deluxe Natural fluorescent tubes (Thorn EMI) filtered through one layer of No 32 medium blue "Masterline Cinemoid" (Rank Strand, Isleworth, Middlesex, U.K). Spectral scans are shown in Fig. 3.2a and, for SOX, in Rich, Whitlam and Smith (1985). The fluence rates of WL and B were varied by modifying the number of tubes or by using muslin covers. Temperature was 17 °C.

In other experiments, the plants were defoliated before applying the treatments and only one leaf of the first pair remained attached to the stem. Half of the plants received a 5 min FR pulse and all the individuals were placed in boxes made with two black plastic plant pots (No 7 square growers pot, B.E.F. Products, Essex, U.K.), while the attached leaf remained out of the box (Fig. 3.1c). The petioles were surrounded by Blu-Tack (Bostick Limited, Leicester, U.K.) in order to prevent them from being damaged by the edges of the boxes. The space between these edges was finally sealed by means of black adhesive plastic tape ensuring D inside the boxes. While the stem remained in D, the leaves were exposed to a saturating R pulse followed by D, to WL (Thorn EMI

65/80 W warm white fluorescent tubes) or to WL (Thorn EMI Pluslux Blanc 3500/36 W fluorescent tubes) filtered through one layer of yellow Chromoid (N<sup>o</sup> 101, Strand Lighting, Isleworth, Middlesex, U.K.), which removed radiation below 480 nm (Fig. 3.2b). The calculated Pfr/P was 0.87 for both WL and filtered WL and PAR was 73 and 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$  respectively. Even though the remaining leaf was previously exposed to FR, the duration of the low Pfr/P in this organ was always less than 10 min in order to ensure that the differences between control and FR treated plants were due to different Pfr/P in the darkened internode and not to a long term promotion triggered in the leaf (Chapter 2). The nature of the results allows any obvious possibility that this assumption was mistaken to be discarded. Temperature (outside the boxes) was 22 °C.

Light and plant measurements and statistics. Light measurements; Pfr/P calculations,  $\Delta$ length measurements (24 h periods) and statistical analysis were performed as described previously (Hayward, 1984; Chapter 2). Leaf biomass was obtained by drying (3 d at 80 °C) and weighing the material.

## RESULTS

Locus of perception for end-of-day treatments. Extension growth during a 24 h period of D was promoted by a FR- compared to a R-pulse given at the end of the preceding period of continuous WL (Fig. 3.3a). This effect was reversible by a subsequent R-pulse, indicating the involvement of phytochrome (see Chapter 2). A FR pulse given only to the growing internode did not cause any promotion, nor did a R pulse to the internode significantly revert the promotion caused by immediate prior FR from above (Fig. 3.3b). Covering the first leaf pair before giving the FR pulse significantly reduced the extent of the growth stimulation (Fig. 3.3b). Covering the leaves after, instead of during, the FR

pulse does not cause any obvious effect (see Fig 2.7B).

Surgical removal of the first and second leaf pair and the cotyledons, before the light pulses, reduced both growth in R-treated plants and the extent of response to the FR treatment in absolute and relative terms. Both growth and the absolute response to low Pfr/P were directly and linearly related to the dry weight of the remaining foliage tissue (Fig. 3.4). Plants fully defoliated continued growing but they did not respond to the Pfr/P established in the remaining shoot (stem) (Fig. 3.4). From these results (Fig. 3.3 and 3.4) we conclude that the "end-of-day" response to Pfr/P in mustard is triggered in the leaves and cotyledons while no significant effect can be attributed to the growing internode.

Loci of perception for treatments under continuous white light. Extension growth under continuous WL was promoted by the reduction of the R:FR caused by supplementary FR given from above (Fig. 3.5a). Low R:FR provided only to the internode in the same geometry as in the "end-of-day" treatments promoted growth (Fig. 3.5b). This effect was not an experimental artifact caused by the internode covers as the latter did not affect growth when WL, instead WL+FR, was transmitted through the fibre optics (Fig. 3.5b). The largest promotion corresponded to the plants receiving WL+FR both to the internode and from above (Fig. 3.5b).

Effect of the light environment surrounding the leaves on the extent of response of the internode. The different effect of lowering the Pfr/P in the internode by light pulses followed by D compared to continuous light treatments could be caused by: (i), the continuous WL+FR reaching the internode; (ii), the continuous WL reaching the leaves, while the internode receives the low Pfr/P treatment or; (iii), the continuous WL reaching both kind of organs, while the internode receives the low Pfr/P treatment. In order to distinguish between the first interpretation and the



other two, plants were transferred into D but received continuous WL+FR to their internodes. This treatment did not promote growth compared to plants in full D (Fig. 3.6; fluence rate of WL= 0). However, increasing the fluence rate of WL given from above both promoted growth and established the difference between control plants and those receiving light with a low R:FR in their internodes (Fig. 3.6). These results allow the first interpretation above to be discarded. When WL was replaced by SOX light (with a PAR equivalent to that found effective for WL) growth was promoted to a similar extent, but the difference between control plants (those receiving either WL to the internode, or no internode treatment) and those plants receiving WL+FR to the internode, was not displayed (Fig. 3.7, B fluence rate= 0). The addition of relatively small amounts of B to the SOX light (both given from above) restored the promotion caused by low R:FR given the internode (Fig. 3.7).

In order to investigate whether, in addition to WL reaching the leaves, continuous light in the internode was also necessary to trigger the growth promotion by low Pfr/P in the internode, the stems were darkened, with or without a previous FR pulse, while one primary leaf was exposed to different treatments (all other leaves and cotyledons were defoliated). The effect of low Pfr/P in the internode (a FR pulse followed by D), compared to high Pfr/P (dark controls) depended on the light reaching the leaves. Low Pfr/P was promotive when the remaining leaf was exposed to continuous WL, but no significant differences were found when the leaves received either a R pulse followed by D or continuous light deficient in B (Table 3.1).

## DISCUSSION

Different loci for the perception of changes in Pfr/P under continuous white light or as a result of "end-of-day" treatments. Under continuous WL, internode

extension in mustard plants is promoted by low Pfr/P established both in the internode and/or the leaves (Morgan et al., 1980 and Fig. 3.5). The promotion by an "end-of-day" FR pulse was triggered only in the leaves and cotyledons, while a pulse of FR given either to the growing internode alone (in the same geometry as in the experiments under continuous WL) (Fig. 3.3), or from above to fully defoliated plants (Fig. 3.4), was totally ineffective. The latter result is different from previous findings in younger mustard plants (hypocotyl growth, Jose and Schafer, 1980), in beans (Downs, Hendrick and Borthwick, 1957; Jose and Schafer, 1978); in sunflower (Garrison and Briggs, 1975), in Chenopodium (Lechamy, 1979) and in Vigna sinensis (García-Martínez et al., 1987).

Effects of B perceived by the leaves on the response to different Pfr/P provided to the internode. Continuous WL+FR given only to the internode was also ineffective if the rest of the shoot remained in D (Fig. 3.6). Both the background stem growth and the growth promotion caused by treatments establishing low Pfr/P in the internode (either continuous WL+FR or a FR pulse followed by D) were increased along with increasing fluence rates of WL given from above (i.e. received by the leaves) (Fig 3.6; Table 3.1). However, SOX light or blue-deficient fluorescent light promoted growth to approximately the same extent as WL, but did not evoke the response to the different Pfr/P in the internode (Fig 3.7; Table 3.1). A specific B effect is indicated since a small addition of B was sufficient to evoke the response to the internode treatment (Fig 3.7). No differences in calculated Pfr/P were found as a result of supplementary B and this agrees with previous measured and calculated Pfr/P data using the SOX+B technique (Thomas and Dickinson, 1979; Rich et al., 1985)

Blue light inhibition of hypocotyl extension growth in mustard is a transient phenomenon which disappears after

de-etiolation (Beggs et al., 1980; Thomas, 1980; Drumm-Herrel and Mohr, 1985). The results of this paper, involving a totally different experimental approach, indicate that B continues to be effective in fully de-etiolated plants. Its effect, however, is not to inhibit growth, but to set the responsivity of the internode to Pfr/P. The interaction between phytochrome and the B-absorbing photoreceptor(s) has already been shown for several plant responses, including extension growth (Attridge et al., 1984; Drumm-Herrel and Mohr, 1984; Gaba et al., 1984). It is worthy of notice that in those cases both B, and high Pfr/P, inhibited extension growth, while in the present case B reaching the leaves enhanced the promotion caused by low Pfr/P in the internode. Black and Shuttleworth (1974) reported that hypocotyl growth in etiolated Cucumis sativus seedlings was inhibited by R perceived in the cotyledons, and by B directly perceived in the hypocotyl. Our results do not match that situation since, in mustard, changes in phytochrome-absorbable radiation can be perceived both in the leaves and the internode, but the latter is only effective if B is reaching the leaves.

Internode growth in mustard is promoted by low Pfr/P through at least two different pathways (see Chapter 2). The "rapid" promotion is characterised by its short duration (<16 min) once FR is switched-off under continuous WL, and by being triggered in the internode (Child and Smith, 1987). The "long term" promotion persists ca. 24 h after the time in which the plants return to a high Pfr/P treatment, and is triggered mainly in the leaves (Chapter 2). It appears that the "rapid" effect has a negligible contribution to the end-of-day control of growth by Pfr/P in mustard, as a FR pulse given to the internode was ineffective to promote growth in fully-darkened plants, as measured with a ruler (Fig. 3.3). Furthermore, since B was only effective above a given threshold, and the absolute B irradiance is lower at the extremes of the photoperiod,

the B-modulation of internode responsivity to Pfr/P could prevent internodes in mustard from exerting a full rapid response to the twilight drop in the R:FR (Holmes and Smith, 1977a). On the other hand, during daytime when B irradiance is higher, a dramatic response to small reductions of R:FR caused by neighbour plants would be possible (Morgan et al., 1980; Ballaré et al., 1987). In fact, the latter response can be detected well before the leaves became shaded by other individuals (Ballaré et al., 1987)

Table 3.1. Effect of a FR pulse followed by D in the internode compared to the control in which the internode was darkened directly after continuous WL, as affected by the treatment received by the remaining leaf: D= 15 min R followed by darkness; WL= white light (PAR= 73  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ); WL-B= WL from which light below 480 nm was removed (PAR= 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Each datum point is a mean of 22-46 plants  $\pm$  sem.

Leaf treatment	First internode $\Delta$ length (mm)		Relative promotion <sup>1</sup> (%)
	control	FR	
D	1.0 $\pm$ 0.1	1.1 $\pm$ 0.2	0 $\pm$ 10 b
WL	4.2 $\pm$ 0.4	6.5 $\pm$ 0.5	38 $\pm$ 10 a
WL-B	4.8 $\pm$ 0.3	5.6 $\pm$ 0.5	9 $\pm$ 9 b

<sup>1</sup> The relative promotion was calculated as the slope + se of the linear regression of  $\ln [(\text{first internode } \Delta\text{length})+1]$  against  $\underline{x}$ , where  $\underline{x}$  was =0 for control plants and =1 for FR treated plants (Kleinbaum and Kupper, 1978). The slopes followed by different letters are significantly different ( $P<0.05$ ).

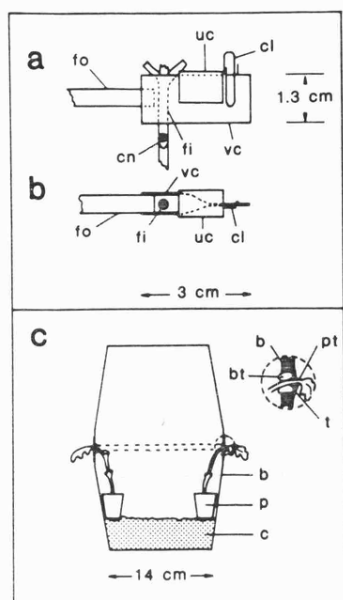


Fig. 3.1. (a), Scheme of the tip of the fibre-optics and covers used in all internode irradiations. (b), Scheme of the tip from above. (c), Scheme of the boxes used to darken the internode while irradiating one of the leaves. b= black box; bt= blu-tack; c= compost; cl= clip; cn= cotyledonary node; fi= first internode; fo= fibre-optic; p= pot; pt= petiole; t= black tape; uc= "u-shaped" cover; vc= vertical cover.

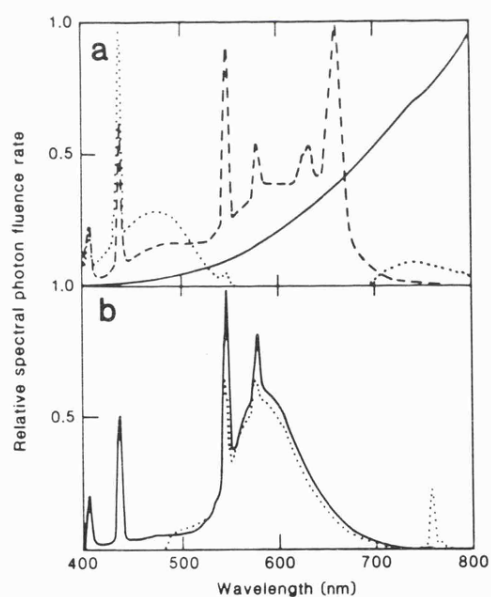


Fig. 3.2. Spectral photon distribution of light sources. (a), WL+FR used for internode irradiation in the experiments shown in Figs 6 and 7 (solid line), fluorescent WL provided from above in the experiments shown in Fig. 6 or directed to the internode in the experiments shown in Fig. 7 (broken line), and B (dotted line). (b), WL (solid line) or WL minus B (dotted line), used in the experiments shown in Table 1.

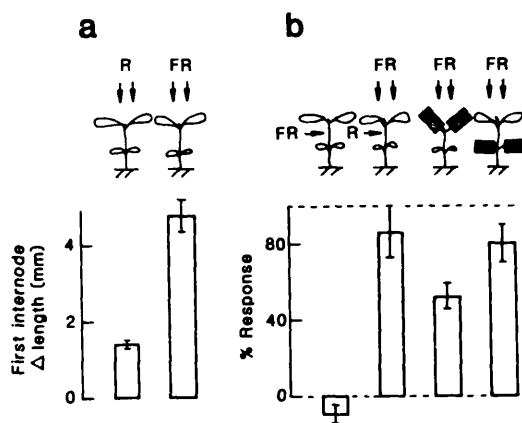


Fig. 3.3. (a), Effect of R or FR pulses provided to the whole shoot at the end of continuous WL on the growth during the subsequent 24 h in D. (b), Relative promotion  $([\Delta \text{length treatment} - \Delta \text{length R}] / [\Delta \text{length FR} - \Delta \text{length R}])$ , caused by localised irradiations. The arrows indicate the main direction of the light pulses and the shaded plant zones show the organs covered during the irradiations. When light was given from above (FR) and to the internode (R), the R pulse followed the FR one. Data are means of 29-30 (a) or 17-24 (b) plants  $\pm$  sem.

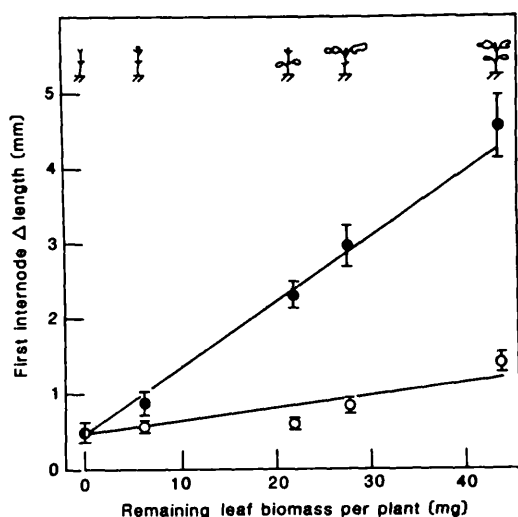


Fig. 3.4. Effect of R (open symbols) or FR (closed symbols) pulses provided at the end of continuous WL on the growth of the first internode during the subsequent 24 h in D. The first or second leaf pair, the cotyledons, or various of these organs were defoliated as indicated in the diagrams and growth was plotted as a function of the leaf biomass remaining after clipping. Each datum point is a mean of 15-38 plants  $\pm$  sem.

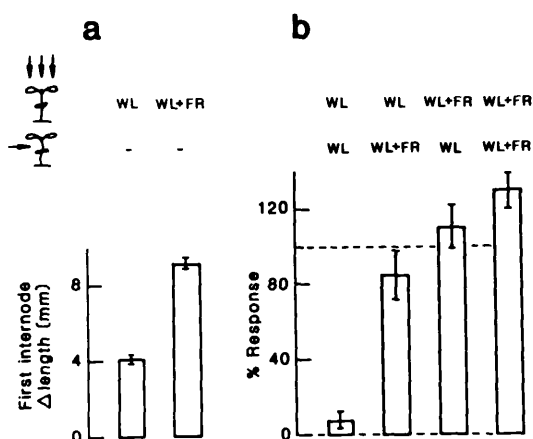


Fig. 3.5. (a), Effect of continuous WL with or without FR on internode growth during the 24 h treatments (plants without fibre-optic and internode cover devices). (b), Relative promotion  $([\Delta \text{length treatment} - \Delta \text{length WL}] / [\Delta \text{length WL+FR} - \Delta \text{length WL}])$  caused by localised irradiations (all the plants, except WL and WL+FR control used for calculations, had the fibre optic and internode cover devices). The arrows indicate the main direction of the light. Data are means of 79-104 (a) or 17-36 (b) plants  $\pm$  sem.

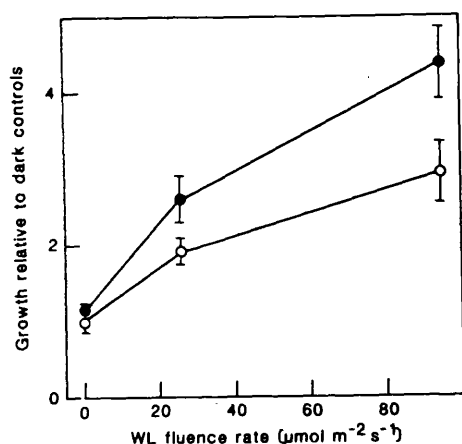


Fig. 3.6. Effect of the irradiance of continuous WL provided from above on the growth of plant with (closed symbols) or without (open symbols) continuous WL+FR given to the internode. Growth is expressed in relation to the growth of plants placed in D during the treatment period. Data are means of 17-18 plants  $\pm$  sem.

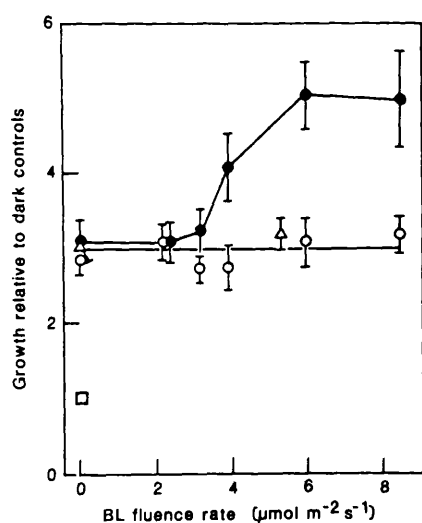


Fig. 3.7. Effect of different fluence rates of B added to background SOX light ( $94 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) on the growth of plants with WL (triangles) or WL+FR (closed circles) given to the internode, or plants without internode treatment (open circles) (*i. e.* receiving at the internode the B + SOX light provided from above). Growth is expressed in relation to the growth of plants placed in D during the treatment period (square). Data are means of 17-48 or 131 (D controls) plants  $\pm$  sem.



## CHAPTER 4

THE "END-OF-DAY" PHYTOCHROME CONTROL OF INTERNODE  
ELONGATION IN MUSTARD. KINETICS, INTERACTION WITH THE  
PREVIOUS FLUENCE RATE, AND ECOLOGICAL IMPLICATIONS\*.

\*Casal and Smith (1989) Plant Cell and Environment 12  
(5) (in press)

## INTRODUCTION

In photosynthetically-competent mustard seedlings, end-of-day light pulses providing different Pfr/P in the leaves modify internode growth rate during the dark period and also during the subsequent photoperiod (Chapters 2 and 3). The latter effect is presumably due to the accumulation of a signal in the transduction chain between phototransformation and growth response, whose expression persists beyond the duration of the differences in Pfr/P (see Chapter 2). These results lead to the conclusion that, in addition to the rapid promotion of internode extension by FR directed to the internode, which disappears quickly after the FR is removed, (Morgan, O'Brien and Smith, 1980; Child and Smith, 1987) there is a persistent promotion of extension growth triggered by photoperception in the leaves. An end-of-day pulse given to the first internode alone does not cause a growth promotion, measurable by ruler, either in D, or in the following photoperiod (Figs 2.7 and 3.3).

Detailed kinetic studies of internode extension growth in photosynthetically-competent mustard seedlings to date have only been conducted under continuous background WL (Morgan et al., Child and Smith, 1987). In order to obtain a more complete understanding of the physiological nature and ecological implications of phytochrome action in light-grown plants, a full characterisation of the extension growth responses after the transition into D is necessary. Previous studies of the phytochrome control of elongation in D have concentrated on seedlings at the hypocotyl stage, using either etiolated seedlings (Shopfer and Oelze-Karov, 1971; Kristie and Jolliffe, 1987), or seedlings briefly de-etiolated with WL (Wildermann et al., 1978 a, b). In the experiments reported here we monitored, by means of linear-voltage-displacement-transducer and ruler measurements, the extension growth of the first internode of light-grown mustard seedlings in D after

pulses which provided different Pfr/P. In addition, the interaction between Pfr/P and the PAR levels at which the plants were grown, was also investigated as the latter had previously been shown to modify the extent of several responses to end-of-day pulses (Sánchez and Cogliatti, 1975; Lechamy and Jacques, 1979). Finally, we compared, over the same period in D, the efficacy of a reduction in Pfr/P at the end of continuous WL with that of a similar reduction during continuous WL (advanced treatment) or several hours after the WL to dark transition (delayed treatment).

## MATERIALS AND METHODS

Plant material and growing conditions. For ruler-measurement experiments, mustard (*Sinapis alba* L, Asmer Seeds, Leicester UK) seedlings were grown for 13 d under continuous WL (spectrum is shown in Fig. 2.1). PAR was  $126 \mu\text{mol m}^{-2} \text{s}^{-1}$  (unless otherwise indicated) and air temperature was 22 °C under WL and 25 °C in D. For transducer experiments, the seedlings were grown for 12 d (or 72 h for seedlings at the hypocotyl stage) under continuous WL (PAR=  $86 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; temperature 22 °C), mounted in the transducer apparatus and equilibrated overnight under WL (PAR=  $70 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; temperature 24 °C) before starting the treatments.

Light treatments. Treatments consisted of 20 min light pulses provided immediately before transferring the plants into D (unless otherwise indicated). The light sources providing Pfr/P= 0.85 (R); 0.01 (FR) and 0.54 were previously described (Chapter 2). A Pfr/P = 0.32 was obtained by filtering the light from the Pfr/P= 0.54 source through a "pea green" (86 A) Supergel (Rosco, London, U.K.) filter. WL in combination with a No 14 red Cinemoid (Rank Strand, Isleworth, Middlesex) filter and WL+FR filtered through a red Cinemoid plus a "pea green" Rosco filter were used to obtain Pfr/P= 0.64 and 0.13 respectively. The chamber providing WL with or without

supplementary FR is described in Chapter 2. A Pfr/P= 0.44 source was provided by two Schott Mainz KL 150 B projectors (Carl Zeiss, Welwyn Garden City, Hertfordshire, U.K.) with red Cinemoid and blue Perspex (No 703) filters.

In the transducer experiments light pulses were given from one side of the plant (outside the acrylic growth chamber) to the whole shoot, or by means of fibre optics selectively directed to the internode (Child and Smith, 1987). For the other experiments, the plants were irradiated from above in single compartments within cardboard boxes. This was to prevent the light reflected from the leaves (i.e. with a spectral composition different from that provided by the source) from reaching the other plants. Fluence rates for the different R+FR mixtures varied from 25 to 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$  but were not correlated to the calculated Pfr/P that they provided, and no obvious effects of these differences were observed. Pfr/P calculations and light measurements were as described previously (Hayward, 1984; Chapter 2). Spectral distributions of the sources used are presented in Fig. 4.1.

Growth measurements. The growth of the first internode was recorded either with a ruler (see Chapter 2) or by means of a linear voltage displacement transducer (Child and Smith, 1987). Plant responses varied among individual plants both in magnitude and time of occurrence. In previous studies using transducer methodology results were presented as average rates at fixed times (Morgan et al, 1980; Gaba and Black, 1985; Child and Smith, 1987). Following that procedure, the differences in timing of the responses appear as differences in rate for a given time. A consequence of this is that the changes in growth appear much smoother than those shown by each plant and that, while the variability of the magnitude of the responses is overestimated, no information is provided about the variability in timing. To overcome these inconveniences

a different procedure was followed in the present study. The time course of growth rate was investigated for individual plants. Thus, the maximum and minimum rates consistently found in the different individuals were characterised in terms of average magnitude and average time of occurrence. The period between two of these events was divided in two or more fractions of equal duration and the average rate at those times was calculated. After the last maximum or minimum observed, the subsequent average rates for 5 min intervals were calculated.

## RESULTS

Growth rate after transition from WL to D: Effect of pulses providing different  $P_{fr}/P$ . After the beginning of D, the extension rate of control plants dropped rather abruptly to about 60 per cent of the previous rate under continuous WL, with a lag of less than 10 min (Fig. 4.2). No further changes were observed during the period of experimentation (Fig. 4.2). The time course of extension rate was markedly different when a 20 min FR ( $P_{fr}/P = 0.01$ ) pulse was given from the time of WL off (Fig. 4.3A). A sharp growth promotion peaked at ca 25 min, followed by a deceleration which led to a growth rate minimum at ca 45 min (Fig. 4.3A). Finally, growth recovered to reach a more-or-less steady elevated rate beyond 60 min (Fig. 4.3A). The timing of these events was not significantly affected by light pulses which reduced  $P_{fr}/P$  to a different extent (Fig. 4.3 A, B, C). When the FR pulse was directed to the internode, the early kinetics of the growth promotion were virtually the same as when FR was directed to the whole shoot (Fig. 4.3A, open circles). However, growth rate gradually declined and became lower in internode-treated than in whole-shoot treated plants (Fig. 4.3A inset), and closer to that of control seedlings that did not receive any pulse after WL. As expected from the

behaviour of control plants, in which growth rate decreased after about 10 min in D, in some plants growth rate decreased before the first maximum peak caused by a reduction in Pfr/P (Fig. 4.3).

Relationship of extension rate to Pfr/P, and to preceding WL fluence rate. During the first 9 h in D, growth rate was virtually constant (Fig. 4.4) and inversely and linearly related to the Pfr/P provided by the end-of-day pulse, at least for Pfr/P values below 0.6 (Fig. 4.5). Both in R and FR treated plants, the rate of internode extension was a function of the WL PAR received during the preceding 13 d (Fig. 4.6) as was the absolute promotion caused by FR. In R-treated plants, growth rate showed no significant changes during 48 h in D (Fig. 4.6A). However, as the dark period advanced, the rate of growth declined in FR-treated plants, this reduction being earlier and of a higher magnitude in plants pretreated with low, rather than high, PAR (Fig. 4.6B). Furthermore, the magnitude of this reduction was more important for the lowest Pfr/P level (Fig. 4.7). The promotion caused by a Pfr/P = 0.54 neither increased nor decreased significantly during the period of experimentation (Fig. 4.7).

Effect of FR and R pulses given after periods of D. A FR pulse was still effective in promoting growth when delayed 8 h into the dark period ( $\Delta$ length between 8 and 32 h in D after a R pulse at 0 h: control =  $0.96 \pm 0.09$ ; R pulse at 8 h =  $0.97 \pm 0.17$ ; FR pulse at 8 h =  $1.45 \pm 0.17$ ). However, if a 24 h period was interposed between the end-of-day R pulse and the FR pulse, the latter was not significantly promotive, even though the growth rate in end-of-day R-treated plants was lower than that of the end-of-day FR-treated plants between 24 and 48 h (Fig. 4.8). A R pulse after 24 h in D did not increase the inhibition caused by the end-of-day R pulse (Fig. 4.8).

Effect of a reduction in Pfr/P during or at the end of continuous WL on the subsequent growth in D.

White-light-grown seedlings were exposed for 7 h to

either WL or WL+FR (Fig. 4.9, bottom). At the end of these pretreatments (time= 0 h), different groups of seedlings were transferred from WL to WL+FR, from WL to D with or without a previous WL+FR pulse, and from WL+FR to D. Other groups remained under continuous WL or WL+FR. Growth rate between 0 and 18 h was higher under WL+FR than under WL and, for the seedlings exposed to WL+FR, there was only a small effect of the pretreatment (Fig. 4.9, left). However, for those seedlings transferred to D growth rate was higher if the Pfr/P was reduced during the pretreatment (i.e. since -7 h) than only immediately before D (i.e. 10 min before 0 h) (Fig. 4.9, right).

Recovery of extension growth in subsequent WL. When the end-of-day R-treated plants were returned to continuous WL, extension growth showed some recovery (Fig. 4.10). The recovery appeared to be quicker in those plants in which the dark period was shorter. Growth continued at a higher rate in those plants in which the Pfr/P had been reduced at the beginning of the previous dark period (Fig. 4.10). In contrast with the R-treated plants, growth under WL was faster when the duration of the previous dark period was longer (Fig. 4.10).

Comparison with seedlings at the hypocotyl stage. In contrast with the observations in older seedlings, seedlings at the hypocotyl stage, when transferred from continuous WL to D, showed a gradual increase in extension rate (Fig. 4.11). A FR pulse given before the onset of D promoted growth, but the growth rate increased with time over several hours rather than reaching a steady value (Fig 4.11).

## DISCUSSION

Growth in D of plants with a high Pfr/P at the end of continuous WL. In briefly de-etiolated mustard seedlings transferred from WL to D with a high Pfr/P, hypocotyl

growth showed a gradual recovery (Fig. 4.11), and this agrees with previous observations showing that in both etiolated, and briefly de-etiolated, seedlings, growth recovery is caused by a gradual loss of inhibition by phytochrome (Schopfer and Oelze-Karow, 1971; Wildermann et al., 1978a, b; Kristie and Jolliffe, 1987). A first distinctive feature of photosynthetically-competent mustard seedlings is that internode growth rate decreased after transition from WL to D with a lag of less than 10 min, and remained relatively stable for at least 48 h (Figs 4.2, 4.4 and 4.6A). Furthermore, growth rate under WL, before the transition to D, increased with increasing fluence rate (Fig. 4.6A, inset). The rapid reduction in growth rate after a WL to dark transition could be the result of a reduction in photoassimilate translocation from the leaves. In fact, a rapid decline in carbohydrate translocation has been found in soyabean plants transferred into D (Moorby, Ebert and Evans, 1963). However, the involvement of other light effects should not be excluded. The lack of detectable growth recovery could be accounted for by a stable pool of phytochrome controlling the response (as indicated by the promotion caused by a FR pulse given after 8 h in D).

The duration of the inhibition caused by high Pfr/P at the beginning of D can be strongly affected by the extent of de-etiolation, or by whether the hypocotyl or the first internode is considered. These differences could be of ecological significance. A young seedling, with an hypocotyl of less than 1 cm above the soil level, is still likely to be covered again as a result of the soil or litter being disturbed by animals, wind or rain. Under these circumstances the resumption of rapid growth could increase the chance of reaching sunlight again. A situation where recovery of growth in D could be of advantage for older seedlings is not so obvious.

The response to lowering the Pfr/P at the end of



continuous WL. A FR pulse given immediately before D promoted internode growth compared to control plants (R pulse or no light pulse after WL). The kinetics of the promotion were essentially similar to those observed when FR is added to background WL (Morgan et al., 1980; Child and Smith, 1987). The growth response was apparently biphasic, with an initial sharp acceleration peaking ca 25 min after WL off, followed by a transient deceleration leading to a growth rate minimum ca 45 min after WL off, followed by a final increase to reach, beyond 60 min, a more-or-less steady elevated rate (Fig. 4.3A). The early response was also produced by FR directed to the internode alone (Fig 4.3A). However, the growth promotion persisted only if the FR pulse reached the whole shoot (Fig. 4.3A, inset). In contrast, the response to continuous FR given only to the internode of plants grown under background WL persists throughout the duration of the low Pfr/P in that organ (Morgan et al., 1980; Child and Smith, 1987). The different magnitude of the promotion caused by low Pfr/P in the internode alone, either under continuous background WL or at the end of the day, is at least partially due to the lack of B reaching the leaves after the transition to D (see Chapter 3). A FR pulse given to the whole shoot of younger plants promoted hypocotyl growth, but growth rate continued to increase for several hours (Fig. 4.11) (Wildermann et al., 1978a) rather than reaching a steady state.

The average extension rate (ruler measurements) was almost constant during the first hours in D (Fig. 4.4), but was inversely and linearly related to the Pfr/P established at the beginning of the dark period, at least for  $Pfr/P < 0.6$  (Fig. 4.5). Similarly, a linear relationship between the Pfr/P provided at the end of the day and stem growth over a period involving several light-dark cycles was found by Vince-Prue (1977) and Morgan and Smith (1978) for Fuchsia hybrida and Chenopodium album respectively. In other words, pulses

reducing Pfr/P to a different extent modified the magnitude but not the timing of the growth promotion (Figs 4.3 and 4.5). This picture is different from that found in seedlings at the hypocotyl stage, where light pulses followed by D had a larger effect on the time before the growth recovery occurred than on the subsequent rate of extension (Fig. 4.11), (Schopfer and Oelze-Karow, 1971; Wildermann et al., 1978a). Obviously, the "threshold" or "all-or-none" model of phytochrome action postulated for hypocotyl growth in etiolated seedlings (Schopfer and Oelze-Karow, 1971) does not hold for the control of internode growth in older, more mature, seedlings.

The response to lowering Pfr/P several hours before or after the transition from WL to D. FR was effective in promoting growth even when delayed 8 h in D. This agrees with previous results in WL pretreated plants (Downs, Hendricks and Borthwick, 1957; Vince-Prue, 1977; Wildermann et al., 1978a; Gaba and Black, 1985). However, light pulses given after 24 h in D provided a particularly revealing result. Neither a FR nor a R pulse caused any effect on internode growth between 24 and 48 h compared to control plants receiving only a R pulse after the end of WL (Fig. 4.8). During the same period, the plants receiving a FR pulse at the end of WL grew faster than the controls (Fig. 4.8). In other words, growth rate between 24 and 48 h was greater in the seedlings receiving a FR pulse at 0 h than at 24 h (Fig. 4.8). Thus, the incubation in D reduced the ability to respond to FR more than that to continue the response to FR given before D. This phenomenon can not be explained at present.

Of particular ecological importance is the fact that, when compared over the same period in D, growth promotion by low Pfr/P was greater if the Pfr/P was reduced by adding FR to the WL for 7 h before D (i.e., during the day), than by a 10 min saturating pulse of W+FR given after WL prior to D (i.e., end-of-day) (Fig.

4.9). This was not the result of the response lag, as the latter is very short (Fig. 4.3) and not detectable by ruler measurements (Fig. 4.4). Furthermore, there was only a small additional promotion as a result of an advanced (i.e. daytime) reduction of Pfr/P when the plants remained under continuous WL between 0 and 18 h (Fig. 4.9). It is therefore possible that, under natural conditions, a seedling could have a high internode extension rate during the night if the R:FR ratio was reduced by neighbour shade during the day. In contrast, a similar reduction of the R:FR ratio restricted to the end of the photoperiod (i.e. caused by atmospheric factors (Holmes and Smith, 1977a) or very distant neighbours) would be much less effective.

Duration of the differences caused by Pfr/P at the end of continuous WL. Plants receiving a FR, compared to a R, pulse before D grew more rapidly for at least 48 h (Fig. 4.6). During the first hours in D, the differences in rate were relatively stable (Fig. 4.4) and related to the previous PAR (Fig. 4.6). However, while growth continued at about the same rate in R-treated plants, a gradual deceleration was found in FR-treated ones (Fig. 4.6). This decrease was earlier and of higher magnitude in the plants grown under low, compared to high, PAR (Fig. 4.6). The fall in growth rate was more important in the plants receiving light establishing low, compared to intermediate, Pfr/P (Fig. 4.7). In consequence, the relationship between Pfr/P and extension growth became flatter in the range of lower Pfr/P with time in D.

The promotive effect of low Pfr/P at the beginning of D continued after the plants were transferred again to continuous WL (Fig. 4.10). In previous experiments, however, a FR pulse followed by a dark incubation longer than 3 h caused no additional promotion of internode growth during a subsequent 12 h period under WL (Fig. 2.3). In the present experiments, PAR was 46 per cent larger, and dark incubation longer than 3 h did cause an additional promotion, especially after a pulse providing

an intermediate Pfr/P or after several hours subsequent WL (i.e. between 14 and 22 h) (Fig. 4.10). These results tend to suggest that the lack of additional promotion by long incubation in D with a low Pfr/P in previous experiments could be caused by the postulated promotive substance being accumulated above a saturating level, rather than by a relatively constant level of the promotive substance beyond 3 h of dark incubation.

### CONCLUSIONS

(1), The transition from WL to D causes a rapid reduction of extension growth. Following this, growth rate remains virtually constant for at least 48 h and is related to the previous PAR level. In other words, "re-etiolation" is not observed for internode extension growth in mustard.

(2), The reduction of Pfr/P at the end of continuous WL causes a growth promotion that resembles the promotion observed as a result of a reduction in Pfr/P under continuous background WL in the following issues: a rapid promotion of growth rate is followed by a transient drop and by a final recovery to a relatively stable rate; different Pfr/P levels affect the magnitude, but not the lag of the promotion; the rapid promotion is triggered by FR perceived by the internode itself; the extent of the promotion depends on the previous PAR.

(3), Darkness imposes a double limitation to the relative promotion caused by low Pfr/P. On the one hand, when compared over the same period in D, growth rate is higher if the Pfr/P is reduced before, rather than, at the end of WL, or at the end of WL rather than after several hours in D. On the other hand, the promotion caused by end-of-day FR given to the internode alone is transient and that caused by end-of-day FR given to the whole shoot decays after several hours (depending on the previous PAR).

(4), Since growth rate is reduced by the transition from WL to D, and the relative efficacy of a reduction in Pfr/P is also lowered by D (see also Morgan and Smith, 1978), we suggest that, for a mustard plant under natural conditions, the effects caused by a drop in the R:FR ratio near to the end of the photoperiod (due to the reduction of solar elevation, to atmospheric factors, or to very distant neighbours) would be less significant than those effects caused by a similar drop near to midday caused by closer neighbours, i.e. providing a more relevant signal for the shade avoiding strategy.

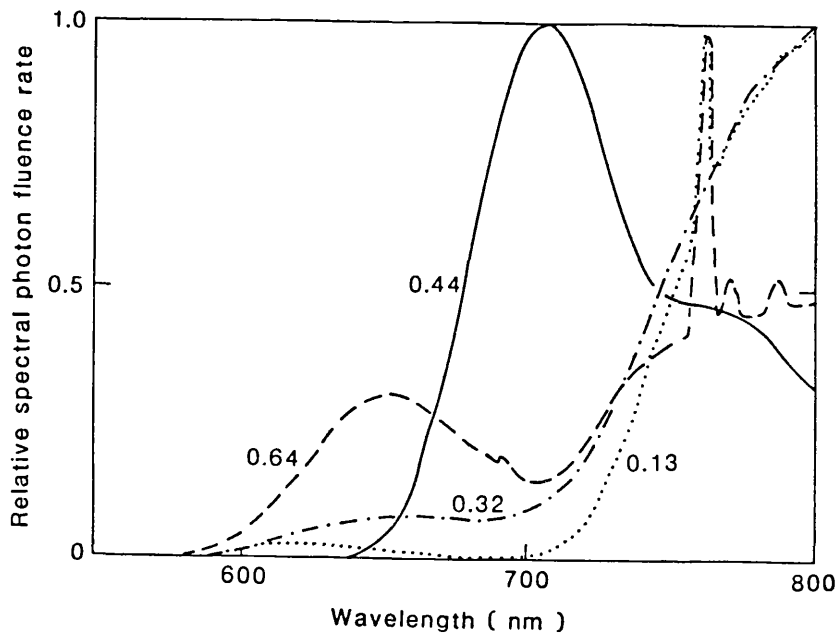


Fig. 4.1. The relative spectral photon fluence rates of the sources used for the light pulses in the transducer- (solid line) and ruler-measurement (dotted and/or broken lines) experiments. The numbers indicate the calculated  $P_{fr}/P$ . The spectral scans of the sources providing  $P_{fr}/P = 0.01$  (FR); 0.54; and 0.85 (R) are shown in Fig 2.1.

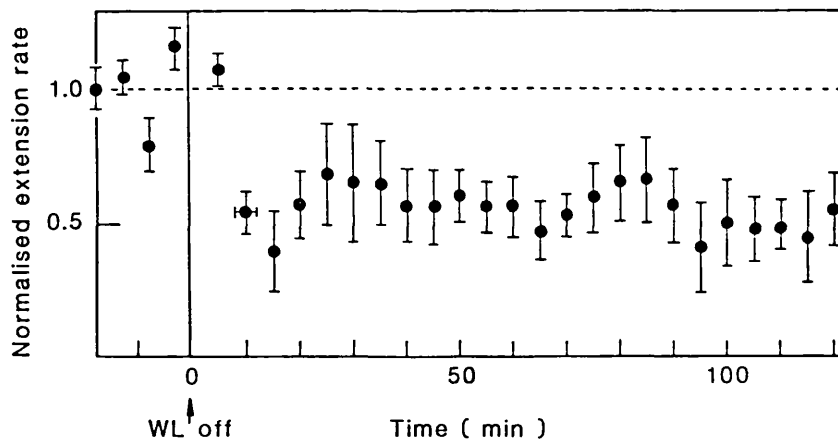


Fig. 4.2. Time course of extension growth as affected by the transition from continuous WL to D. Growth rate was individually normalised against the growth rate in the last 20 min under WL set at unity. Data are means of 9 plant replicates  $\pm$  sem.

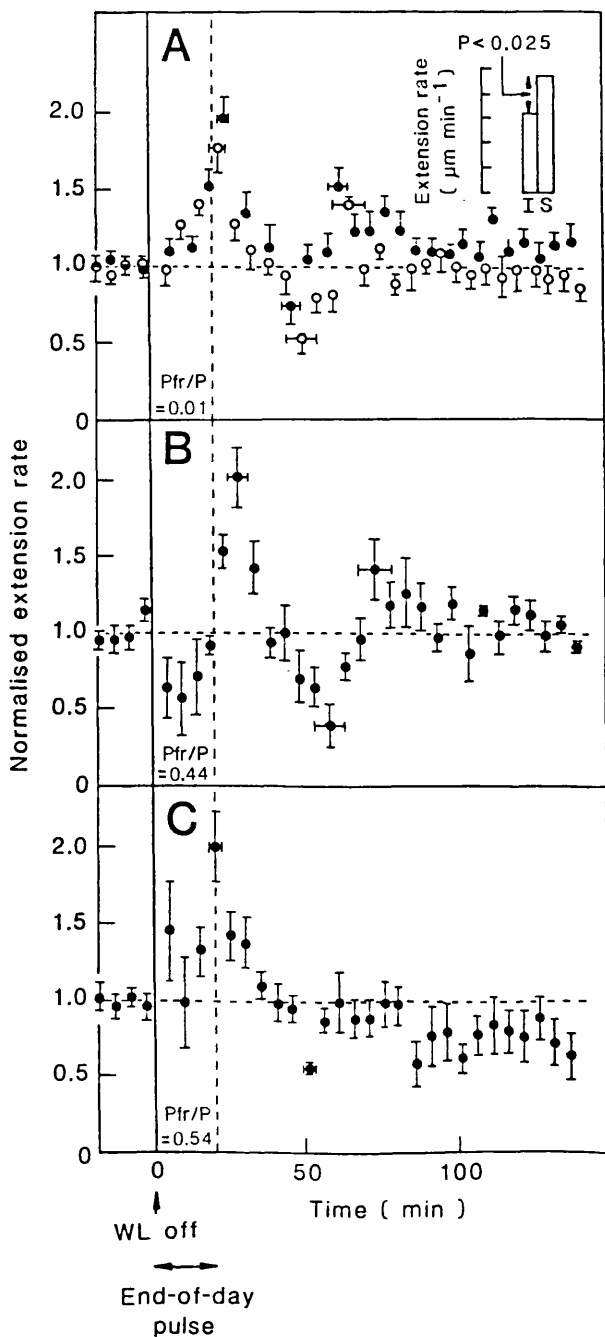
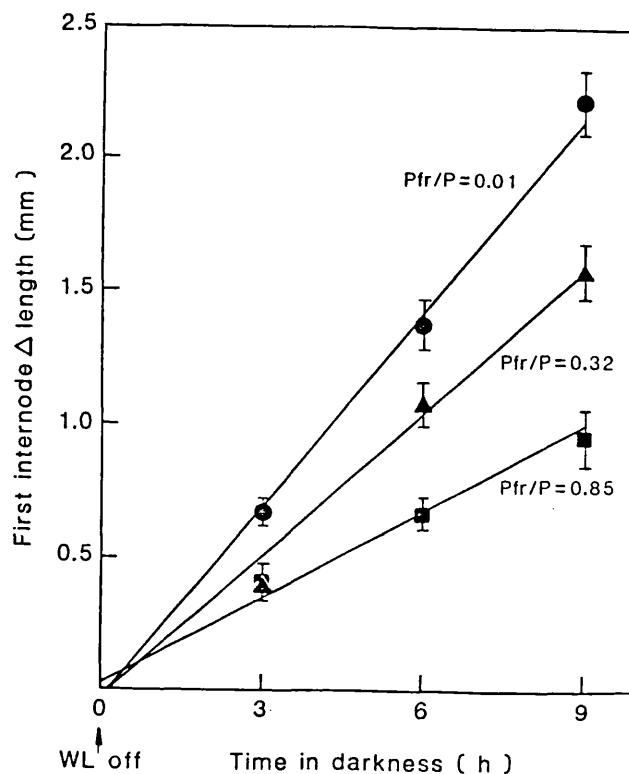


Fig. 4.3. Time course of extension growth as affected by 20 min light pulses providing  $Pfr/P = 0.01$  (=FR) (A), 0.44 (B), or 0.54 (C), given to the whole shoot (closed circles) before D at the end of continuous WL. Growth rate was individually normalised against the growth rate in the last 20 min under WL set at unity. In (A), the open circles show the effect of a FR pulse directed to the internode and the inset shows the average growth rate between 2.5 and 5 h in whole-shoot (S), and internode-treated (I) plants (analysed using ANCOVA, with the preceding growth rate under WL as concomitant variable). Data are means of 14 (A), 9 (internode, A), 5 (B) or 6 (C) plant replicates  $\pm$ sem.

Fig. 4.4. Time course of first internode extension growth (ruler measurements) as affected by 20 min light pulses providing  $Pfr/P = 0.01$  (FR) (circles); 0.32 (triangles); or 0.85 (R) (squares) given before D at the end of continuous WL. Lines were drawn after linear regression analysis. Each datum point (light treatment or time) is a mean of 70-79 different plant replicates  $\pm$ sem.



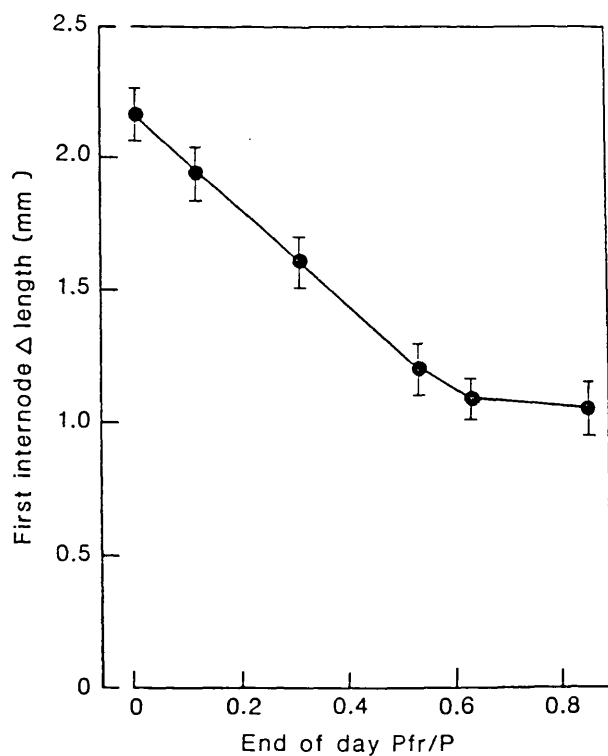
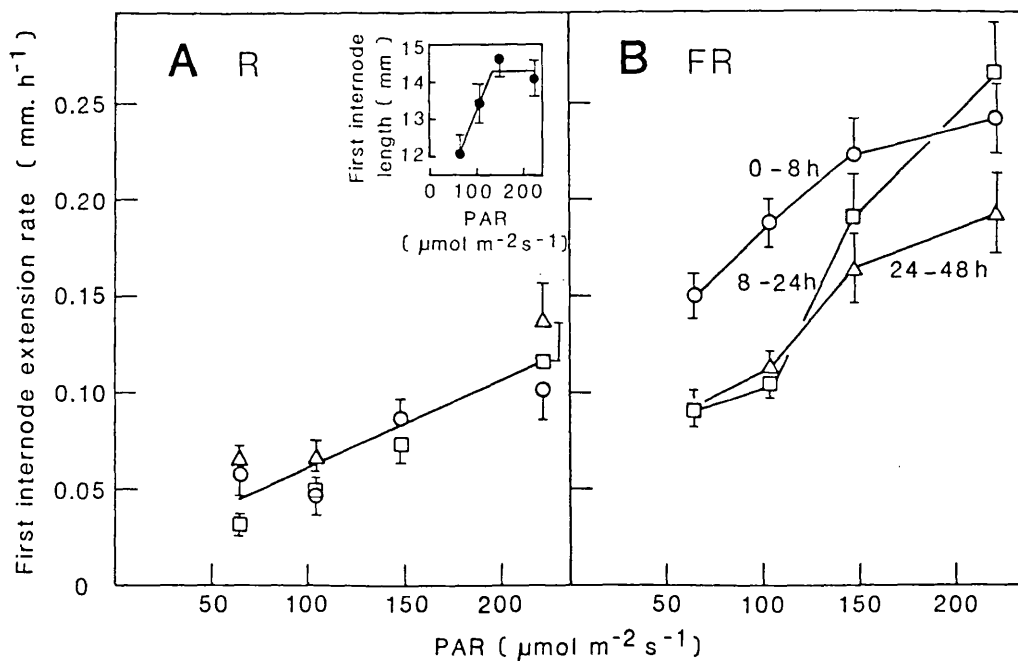


Fig. 4.5. The relationship between the elongation of the first internode between 0 and 8 h after the end of continuous WL and the calculated Pfr/P provided by 20 min light pulses immediately before D. Data are means of 49-53 plant replicates  $\pm$  sem.

Fig. 4.6. The relationship between the first internode elongation rate in D and the PAR of continuous WL under which the plants were grown for 13 d before the treatments. The plants received a R (A) or a FR (B) pulse before D. Mean elongation rate between 0 and 8 h (circles); between 8 and 24 h (squares); between 24 and 48 h (triangles), (end of WL = 0 h). The inset in (A) shows the relationship between the length of the internode at 0 h and the previous PAR. Temperature under WL was 21.5-24.5 °C and in D 25 °C. Data are means of 45-53 or 93-104 (inset) plant replicates  $\pm$  sem.





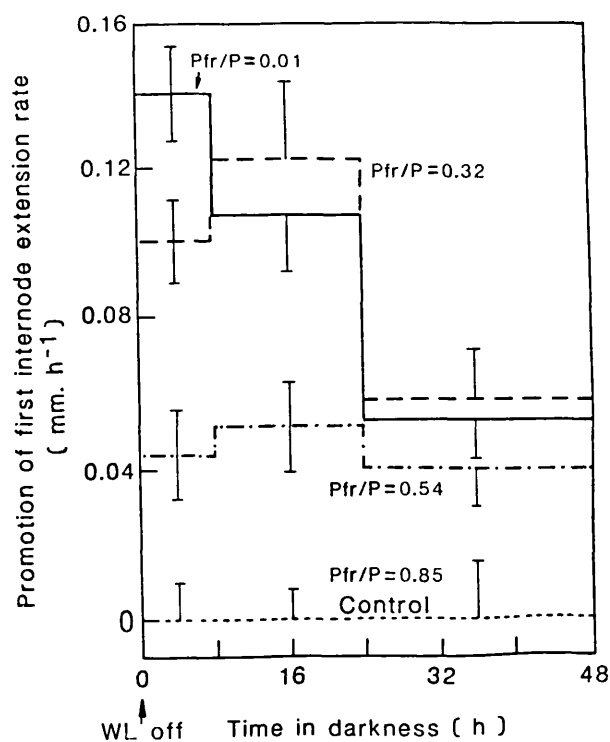


Fig. 4.7. Time course of the average promotion caused by 20 min light pulses providing  $Pfr/P = 0.01$  (FR) (solid line); 0.32 (broken line); and 0.54 (broken-dotted line) compared to 0.85 (R) (dotted line) before D at the end of WL. Data are means of 67-100 plant replicates  $\pm$  sem.

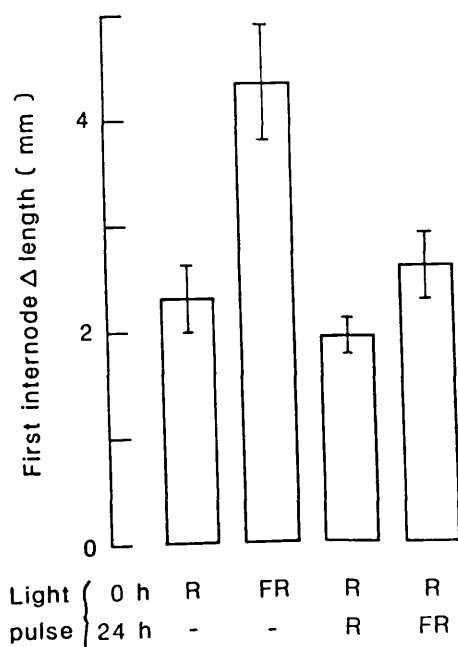


Fig. 4.8. Internode elongation between 24 and 48 h after the beginning of D as affected by R or FR pulses given at 0 and 24 h. Data are means of 40-53 plant replicates  $\pm$  sem.

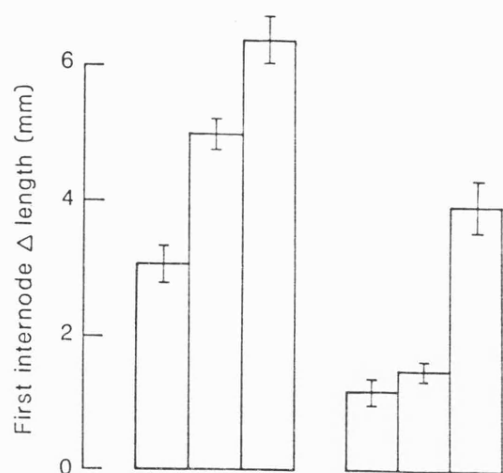


Fig. 4.9. Internode elongation as affected by light conditions during the growth measurement period (i.e. 0-18 h) (continuous WL: calculated  $P_{fr}/P = 0.8$ ; continuous WL+FR: calculated  $P_{fr}/P = 0.5$ ; or D) and during the preceding 7 h (continuous WL, WL+FR or WL terminated with a 10 min duration W+FR pulse). Data are means of 22 plant replicates  $\pm$  sem.

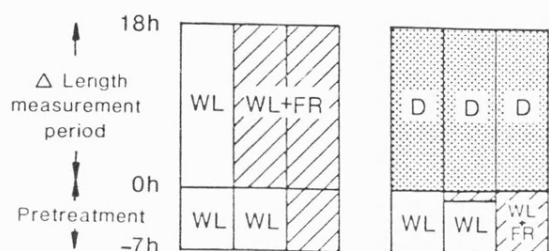
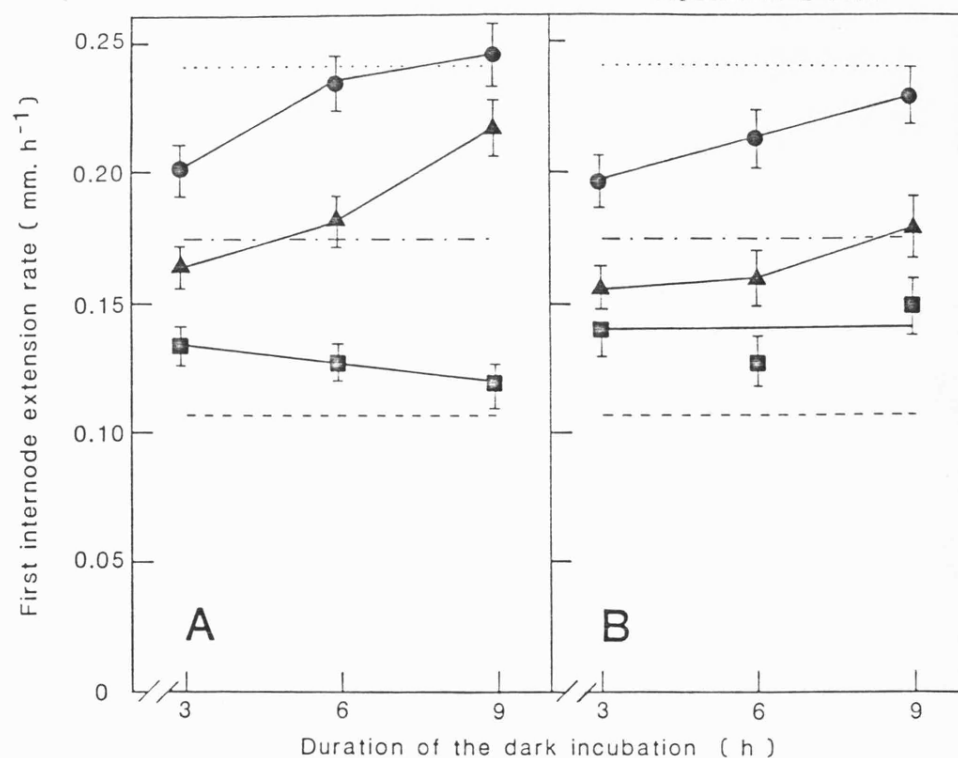


Fig. 4.10. Effect of 20 min light pulses providing  $P_{fr}/P = 0.85$  (R) (squares);  $0.32$  (triangles); or  $0.01$  (FR) (circles) before dark incubations of different durations (abscissa) on the extension growth of the first internode between 0 and 14 h (A), or 14 and 22 h (B) after the time at which the plants were returned to continuous WL. The dotted, dotted-broken, and broken lines represent the average growth rate during the preceding period in D for  $P_{fr}/P = 0.01$ ;  $0.32$  and  $0.85$  respectively (from Fig. 4.4). Each datum point is a mean of 62-72 plant replicates  $\pm$  sem.



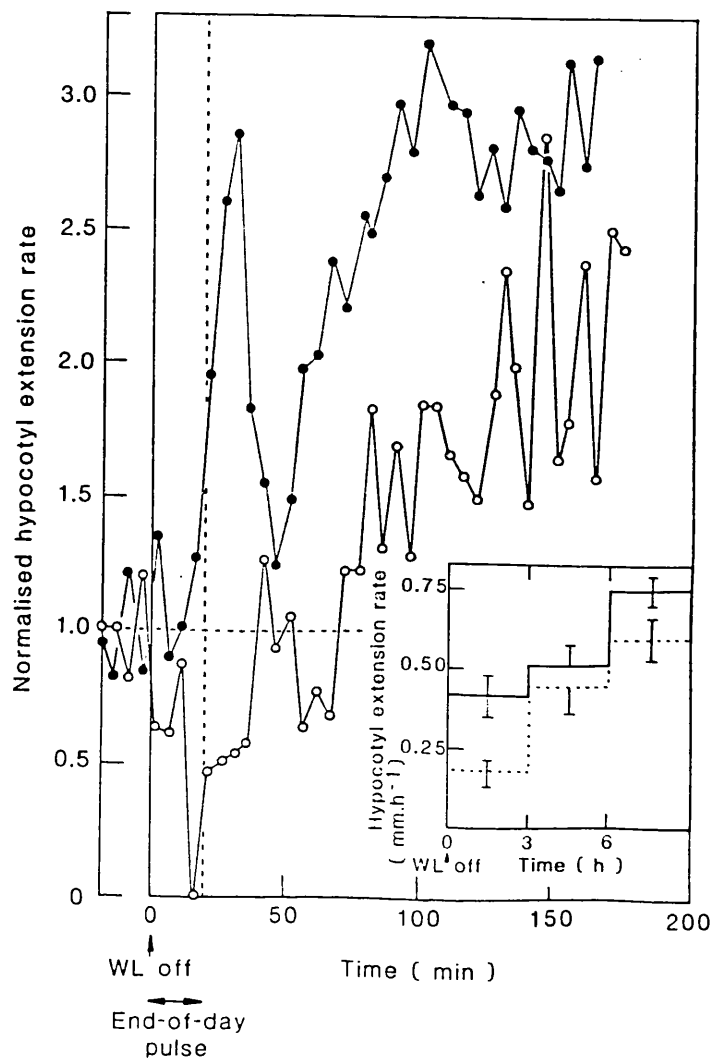


Fig. 4.11. Time course of hypocotyl extension growth as affected by the transition between continuous WL and D with (closed symbols) or without (open symbols) a 20 min FR pulse. Growth rate was normalised with the growth rate during the last 20 min under WL set at unity. The data presented are those of two single plants. Inset, time course of hypocotyl extension growth (ruler measurements) after the end of continuous WL as affected by a 20 min R (dotted line) or FR (solid line) pulse before D. Data are means of 16 plant replicates  $\pm$  sem.

## CAPTER 5

EFFECTS OF BLUE LIGHT PRETREATMENTS ON INTERNODE  
EXTENSION GROWTH IN MUSTARD SEEDLINGS AFTER THE  
TRANSITION TO DARKNESS. ANALYSIS OF THE INTERACTION  
WITH PHYTOCHROME\*.

\*Casal and Smith (1989) Journal of Experimental Botany  
(in press)

## INTRODUCTION

At least two photoreceptors, phytochrome and a specific B-absorbing photoreceptor, are involved in the photomorphogenic control of first internode growth in mustard. Low Pfr/P established in the growing internode cause a rapid promotion of extension growth which reaches a more-or-less steady level after 60 min of continuous supplementary FR directed to the internode (Child and Smith, 1987). Under continuous WL given to the whole seedling, the elevated growth rate persists as long as FR is given to the internode, and returns to the pre-stimulation value ca. 16 min after the end of FR (Child and Smith, 1987). Low Pfr/P established in the leaves cause a less-rapid growth rate promotion that persists even for 24 h after the high Pfr/P are re-established in the leaves (Chapter 2). In other words, at least two different growth responses of the first internode to Pfr/P can be identified in mustard seedlings. One of these responses is known to interact with a specific B effect (i.e. not perceived by phytochrome). The promotion caused by low Pfr/P provided to the internode alone is enhanced by B given to the leaves (Chapter 3). As a consequence of this B requirement, the promotion of internode growth rate caused by low Pfr/P provided by "end-of-day" FR in mustard is triggered mainly in the leaves, while FR given to the internode alone causes only a transient increase in growth rate in subsequent darkness (D) (Chapters 3 and 4).

The growth promotion triggered by end-of-day FR (perceived mainly by the leaves), is increased by WL pretreatments of increasing fluence rates (Figs 4.6 and 4.8). Whether this effect of WL is mediated by photosynthesis, by a B-absorbing photoreceptor or by some fluence rate dependent parameter of phytochrome is not known. Therefore, in this paper we examine whether the effect of different Pfr/P at the end of continuous

SOX ("end-of-day") is affected by different B pretreatments. In addition, a novel effect of B pretreatments on the subsequent growth in D, found in the course of this investigation, is described.

#### MATERIALS AND METHODS

Mustard (Sinapis alba L., Asmer seeds, Leicester, U.K.) seedlings were sown in Petersfield (Leicester, U.K.) No 2 commercial-range potting compost in 120 cm<sup>3</sup> pots, watered with tap water and grown under SOX (280  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) for 11 d. Temperature was 25-27 °C. Some groups of seedlings received supplementary B (16  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , unless indicated otherwise) either during the whole 11 d or during restricted parts of the SOX pretreatment period. After 11 d, when the first internode was in extension, the seedlings received a 10 min red light (R) (4  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) or FR (102  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) before being transferred to D. Calculated Pfr/P (Hayward, 1984) were 0.85 for R and 0.01 for FR. In some experiments, the seedlings were grown under continuous SOX for only 4 or 9 d in order to investigate B effects on hypocotyl growth or internode growth in younger seedlings.

The SOX was provided by 135 W Thorn EMI (Birmingham, U.K.) lamps and B was provided by Deluxe natural fluorescent tubes (Thorn EMI) filtered through one layer of No 32 medium-blue "Masterline Cinemoid" (Rank Strand, Middlesex, UK). A detailed description of the R and FR sources and of all the spectra have been shown elsewhere (Figs 2.1 and 3.2, and Rich, Whitelam and Smith, 1985). In some experiments the internodes were covered by two parallel sheaths (1x1 cm<sup>2</sup>) of No 5 orange "Professional Cinemoid". This filter transmitted ca 80 per cent of the SOX and less than 10 per cent of the light provided by the B source. Localised internode irradiations were obtained by means of fibre optics and internode covers previously described (see Chapter 3). In the present case, however, the covers were made of orange Cinemoid

rather than black cardboard.

Each experiment was conducted on 2 to 4 different occasions, each plant being assigned at random to the different light regimes in every case. Data were pooled for the statistical analysis.

## RESULTS

Mustard seedlings were grown for 11 d under SOX with or without the addition of B. At the end of this period, the seedlings received a R or a FR pulse in factorial combination with the pretreatment and were transferred to D. Under continuous light, the growth of the first internode was not significantly affected by the supplementary B (Table 5.1). In D, however, internode growth rate was higher in FR than R "end-of-day" treated plants and was reduced by the addition of B to the SOX during the pretreatment (Fig. 5.1). The effect of the previous exposure to B was stronger during the second than the first 24 h in D. The effects of the R vs FR "end-of-day" pulses and of the addition of B to continuous SOX were additive (Fig. 5.1).

In other experiments, the seedlings were grown for 11 d under SOX and B was added either continuously during the 11 d or only during the days 0-9, 0-10, 10-11 or 9-11 (Fig. 5.2 bottom). All the seedlings received FR before being transferred to D. Supplementary B provided during restricted parts of the SOX pretreatment period was as effective as B during the whole 11 d (Fig. 5.2). In further experiments, the effects of shorter periods with supplementary B before D were investigated. The inhibition of internode growth during the subsequent D was saturated by 18 h of supplementary B, and 1 h caused more than 50 per cent of the maximum effect (Fig. 5.3). In contrast, low fluence rates of B (i.e. 4 or 7  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) applied for 24 h caused no detectable response (Fig. 5.4), indicating the requirement of a minimum threshold fluence rate of B.

The addition, for 24 h before D, of  $16 \mu\text{mol m}^{-2} \text{s}^{-1}$  of SOX (instead of a similar fluence rate of B) to the background SOX caused no inhibition of growth in subsequent D (i.e. [means $\pm$ sem; n=20]  $\Delta$  length 0-24 h: SOX=  $13.9\pm0.9$ ; SOX+SOX=  $13.9\pm0.9$ ;  $\Delta$  length 24-48 h: SOX=  $14.3\pm1.8$ ; SOX+SOX=  $14.8\pm1.4$ ).

In other experiments the seedlings received 10 d of SOX followed by a further day with SOX or SOX+B before being transferred to D. In order to reduce the amount of B reaching the internode below the threshold level, this organ was covered with an orange filter during the supplementary B period. Covering the internodes did not significantly affect either growth in control plants or the response to supplementary B (Fig. 5.5). If B ( $2 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was added to the internode alone, rather than to the whole shoot, by means of fibre optics, no inhibition of internode growth was observed (i.e. growth for 48 h in D [mean $\pm$  sem]: SOX=  $29\pm3$  ; SOX+B internode=  $32\pm4$ ). The fluence rate of B given to the internode was presumably within the range of B reaching that organ when an effective amount of B was provided from above to the whole shoot.

Mustard seedlings were also pretreated with continuous SOX for only 9 d before D and were exposed to supplementary B for the last 24 h of this pretreatment. These younger individuals showed a similar behaviour to the 11 d old seedlings, as internode growth in D was reduced by B added to the SOX (Table 5.2). However, if the seedlings continued under SOX (without B) instead of being transferred to D, the previous addition of B was not effective (Table 5.2). This indicates that D enhanced the expression of the inhibition of internode growth rate caused by a previous exposure to B. In contrast, in even younger seedlings, hypocotyl growth was inhibited by supplementary B both during continuous SOX (Table 5.1b) and during the subsequent D (Fig. 5.6). In D, the effect of previous B on hypocotyl growth was only observed after a FR "end-of-day" pulse (Fig. 5.6).



The effect of low Pfr/P during (rather than after) the B pretreatments was also investigated. The pretreatments were 1 h of B under a background of either SOX (Pfr/P= 0.8) or FR (Pfr/P= 0.1), and a third group of plants received no B. Following a saturating FR pulse all the plants were transferred to D. Low Pfr/P during the irradiation with B reduced the inhibitory effect of the latter in subsequent D (Fig. 5.7).

## DISCUSSION

### Inhibitory effect of B expressed in subsequent D.

Present results agree with previous reports in that, under continuous irradiation, low fluence rates of supplementary B inhibit hypocotyl growth of mustard seedlings (Table 5.1b) (Cosgrove, 1982; Drumm-Herrel and Mohr, 1985; Kristie and Jolife, 1987), but do not significantly inhibit internode growth in more mature seedlings (Tables 5.1a, 5.2) (see also Chapter 3). Furthermore, present results show a novel effect as, after the transition to D, both internode (Fig. 5.1) and hypocotyl growth rates (Fig. 5.6) were reduced by B added during the SOX pretreatment. The effect of B on internode growth reported here is characterised as follows:

a) The inhibition is the result of B being absorbed by a specific B-absorbing photoreceptor (i.e. different from phytochrome) as, in agreement with previous studies using the SOX+B technique (Thomas and Dickinson, 1979; Rich, Whitelam and Smith, 1985; Chapter 3), no differences in calculated Pfr/P, were detected and increasing the fluence rate of SOX, rather than adding B, caused no inhibition of internode extension growth.

b) The B pretreatment is effective only above a threshold fluence rate. The lack of B effect below a certain threshold fluence has been reported for the inhibition of hypocotyl growth by B under continuous irradiation in Cucumis sativus (Attridge, Black and

Gaba, 1984), Sesamum indicum (Drumm-Herrel and Mohr, 1984), and mustard (Drumm-Herrel and Mohr, 1985).

c) The response does not follow the reciprocity law. Short durations of supplementary B above the threshold fluence rate, given before the end of the 11 d SOX pretreatment, were very effective. The reduction of internode growth in D was saturated by 18 h of supplementary B ( $16 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and 1 h (i.e. total fluence  $\sim 0.06 \text{ mol m}^{-2}$ ) caused more than 50 per cent of the maximum effect (Figs 5.2 and 5.3). However, 24 h of 4 or  $7 \mu\text{mol m}^{-2} \text{s}^{-1}$  (i.e. total fluence  $\sim 0.35$  or  $\sim 0.60 \text{ mol m}^{-2}$ ) showed no effect.

d) The light signal is perceived mainly in the leaves and/or cotyledons, since covering the internode during the B irradiation did not prevent the response.

e) The inhibition of growth is mainly found after the transition to D, as continuous SOX is able to prevent the expression of the effect of previous B for at least 48 h (Table 5.2) without obviously affecting the expression of this effect once D has started (Fig. 5.2). This also indicates that the B effect can be stored for a long period of time.

These characteristics are consistent with a hypothetical control by B of the translocation from the leaves of some substance that limits growth in D but not under high fluence rate SOX. It is important to note that the effect of B pretreatments, and the effect of different Pfr/P established in the leaves appear to be at least partially different responses, since the expression of the latter is not prevented by continuous light (see Chapters 2, 3 and 4).

#### Interaction with phytochrome status

Pretreatments of the whole seedling with high fluence rates of WL increase the extent of promotion of internode growth caused by an "end-of-day" FR pulse (Figs 4.6 and 4.8). This effect is not mediated by a B-absorbing photoreceptor, since the response to the Pfr/P established before D was not increased by the

addition of B to SOX. However, low Pfr/P provided during the pretreatment with B reduced the inhibitory effect of the latter (Attridge, Black and Gaba, 1984; Mohr, 1986). In other words, the inhibition of internode growth by B requires high Pfr/P in order to be triggered but, once triggered, shows no significant interaction with the Pfr/P established immediately prior D. Figure 5.8 shows a descriptive scheme which summarises the interactions between the processes triggered by B, and by phytochrome absorbable radiation in the control of internode extension growth in mustard.

Table 5.1. Length of the first internode (a) or of the hypocotyl (b) in mustard seedlings grown under continuous SOX or SOX+B.

Light conditions after sowing	Length (mm)		
<hr/>			
(a) <u>First internode growth</u> <sup>1</sup>			
11 d SOX	14.9	}	NS
11 d SOX+B	14.1		
<hr/>			
(b) <u>Hypocotyl growth</u>			
3 d SOX	7.0	}	P<0.05
4 d SOX	12.2		
3 d SOX + 1 d SOX+B	10.5		

<sup>1</sup>Internode extension became detectable ca 7 d after sowing

NS= not significant. Data are means of 124-132 (a) or 48-50 (b) plant replicates.

Table 5.2. Effect of 24 h supplementary B provided during the SOX pretreatment as affected by the age of the seedlings at the end of the pretreatment period (11 or 9 d) and by the light or dark condition during the  $\Delta$  length measurement period (i.e. 0-48 h).

Light conditions		First internode $\Delta$ length (mm)	
Pretreatment <sup>1</sup>	0-48 h	0-24 h	24-48 h
11 d SOX	D <sup>2</sup>	10.1	10.9
10 d SOX + 1 d SOX+B	D	6.8 ***	6.6 ***
9 d SOX	D	10.3	9.8
8 d SOX + 1 d SOX+B	D	7.6 ***	7.0 *
11 d SOX	SOX	5.2	5.3
10 d SOX + 1 d SOX+B	SOX	5.5 NS	4.7 NS

<sup>1</sup>all pretreatments started after sowing (d=0).

<sup>2</sup>a 10 min FR pulse was given immediately prior to D.

Data are means of 34-38 plant replicates. \*\*\*= P<0.001;

\*= P<0.05; NS= not significant.

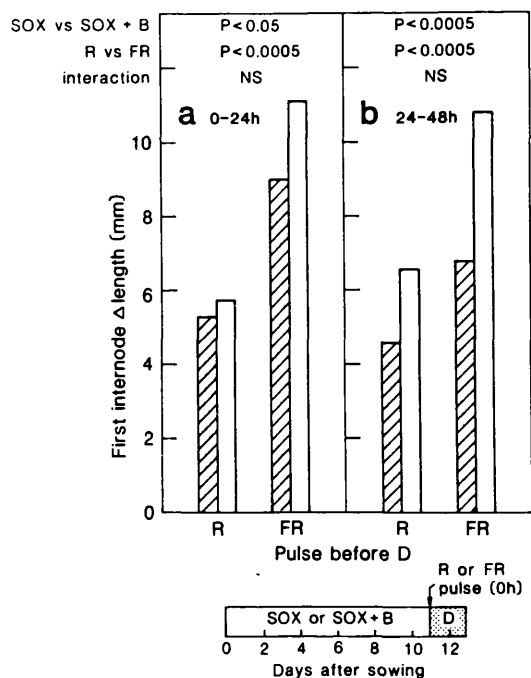


Fig. 5.1. Growth of the first internode between 0-24 h (a) and 24-48 h (b) after the beginning of D as affected by continuous SOX (open columns) or SOX+B (dashed columns) given for 11 d after sowing and a R or a FR saturating pulse given before D. The protocol is indicated at the bottom of the figure. Data are means of 35-39 plant replicates.

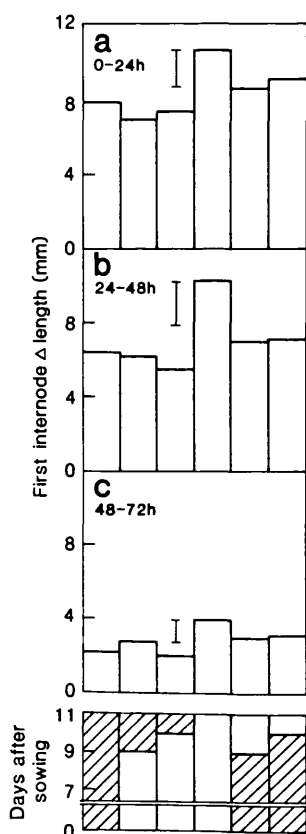


Fig. 5.2. Growth of the first internode between 0-24 h (a), 24-48 h (b) and 48-72 h (c) after the beginning of D. The protocol is indicated at the bottom of the figure: SOX was given for 11 d after sowing either alone (open areas) or with supplementary B (dashed areas) provided during different parts of the 11 d pretreatment period (i.e. days 0-11; 9-11; 10-11; 0-9 or 0-10). Immediately before D all groups received a saturating FR pulse. Data are means of 30-45 plant replicates and the thin bars indicate the LSD ( $P < 0.05$ ).

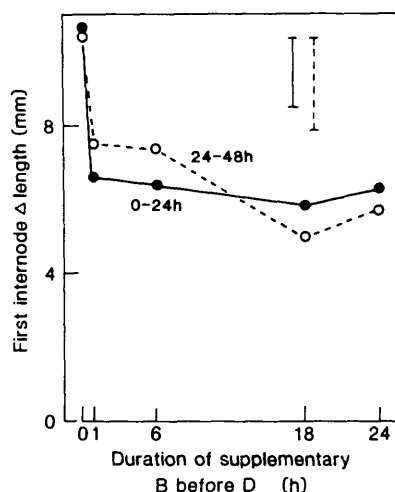


Fig. 5.3. Growth of the first internode between 0-24 h and 24-48 h after the beginning of D plotted against the duration of the period for which B was added to SOX. The seedlings were exposed to continuous SOX for 11 d and the different durations of supplementary B were achieved by changing the time at which B was switched-on while keeping constant the time at which B was switched-off (the latter coincided with the termination of SOX). Immediately before D all groups received a saturating FR pulse. Data are means of 22-31 plant replicates and the solid and broken bars indicate the LSD ( $P < 0.05$ ) for the 0-24 h and 24-48 h periods respectively.

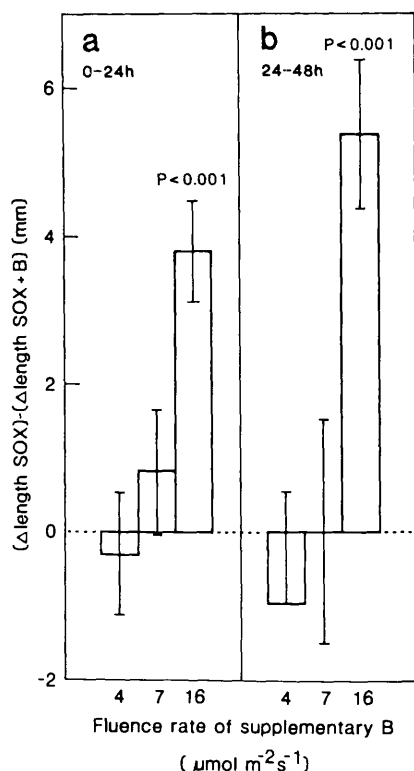


Fig. 5.4. Absolute inhibition of the first internode growth between 0-24 h (a) and 24-48 h (b) after the beginning of D caused by supplementing the continuous SOX with 4; 7 or 16  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of B during the 24 h preceeding the transition to D. Data are mean differences  $\pm$  the standard errors of the differences calculated after groups of 46-50 plant replicates each.

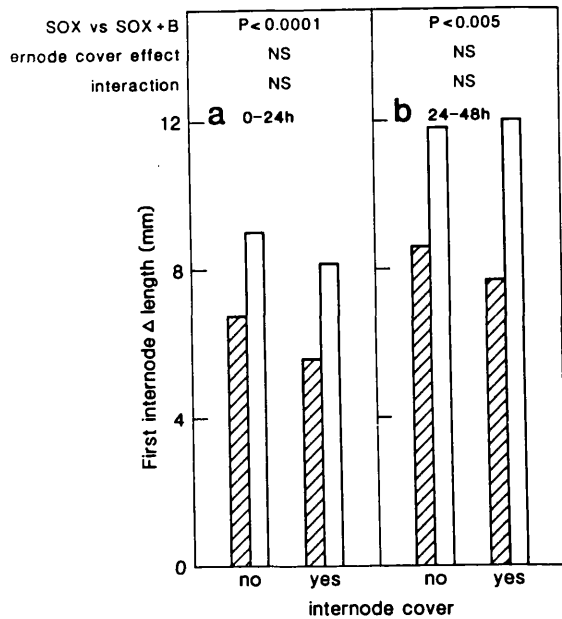


Fig. 5.5. Growth of the first internode between 0-24 h (a) and 24-48 h (b) after the beginning of D. The plants were exposed for 10 d to SOX and during the 11th d they received SOX or SOX+B with or without the growing internode being covered by an orange filter (in order to reduce the amount of B reaching this organ). Immediately before D all the groups received a saturating FR pulse. Data are means of 22-24 plant replicates. NS= not significant.

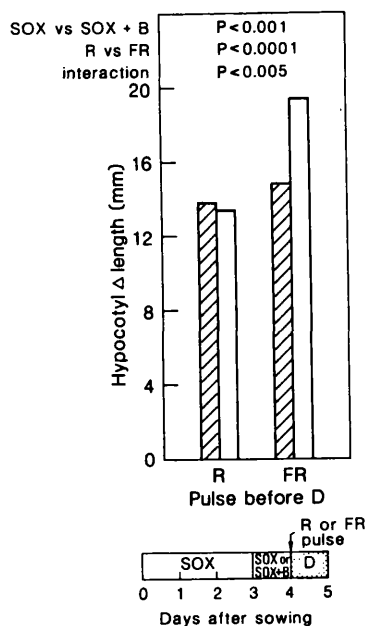


Fig. 5.6. Hypocotyl growth between 0-24 h after the beginning of D as affected by a SOX pretreatment with (dashed columns) or without (open columns) supplementary B and a R or a FR saturating pulse before D. The protocol is indicated at the bottom of the figure. Data are means of 24-25 plant replicates.



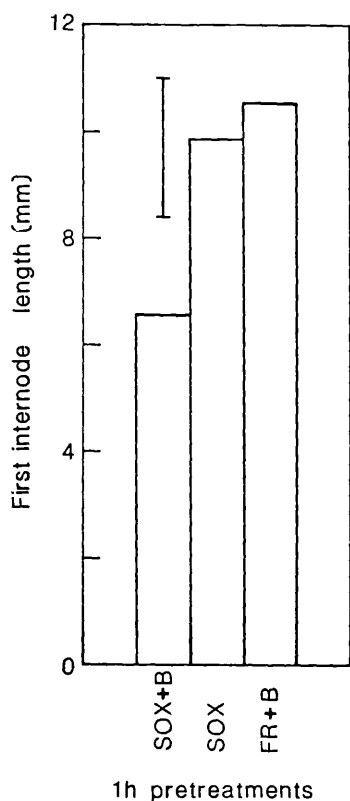


Fig. 5.7. Growth of the first internode between 24-48 h after the beginning of D. After 11 d continuous SOX, the seedlings received 1 h of SOX+B, FR+B or SOX. Immediately before D all the groups received a saturating FR pulse. Data are means of 37-40 plant replicates and the bar indicates the LSD ( $P<0.05$ ).

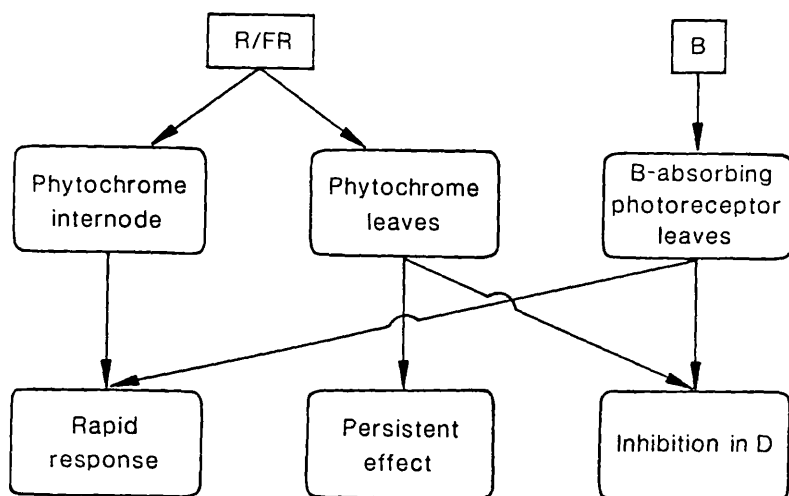


Fig. 5.8. Descriptive model showing the interaction between the processes triggered by B and phytochrome absorbable radiation in the control of internode growth in mustard. The arrows represent flux of information, the squares show the sources of information and the round edge squares the processes described in the present and previous papers (see Child and Smith, 1987; Chapters 2, 3 and 4).

## CHAPTER 6

PHYTOCHROME CONTROL OF EXTRACELLULAR PEROXIDASE  
ACTIVITY IN MUSTARD INTERNODES: CORRELATION WITH  
GROWTH, AND COMPARISON WITH THE EFFECT OF WOUNDING

## INTRODUCTION

Since the early work of Downs, Hendrick and Borthwick (1957) showing the promotion of internode growth by FR compared to R pulses given at the end of the photoperiod, similar responses to changes in phytochrome status at the end of the photoperiod have been found in a large number of species, including mustard (Wildermaun et al., 1978a, Chapters 2, 3, 4, 5). However, the molecular basis of the control of stem extension growth by phytochrome, in either etiolated or WL- grown seedlings, is not well understood (Cosgrove, 1986).

A possible role for extracellular peroxidases in the regulation of extension growth has recently been proposed (Fry, 1979, 1986; Taiz, 1984; Cassab and Varner, 1988; Everdeen et al., 1988). This hypothesis is based on the following observations: (a), Changes in growth and peroxidase activity are often correlated (see Fry, 1986). (b), In vitro, and very likely in muro, peroxidase activity is able to catalyse the formation of cross-links between cell-wall components (Cassab and Varner, 1988). (c), Increasing number of cross-links would increase cell wall rigidity and hence reduce growth (Fry, 1979; 1986).

We have recently characterised the effects of changes in Pfr/P provided at the end of continuous WL on the subsequent growth of the first internode in D in mustard seedlings (see Chapters 2, 3, 4, 5). The aim of this present paper is to investigate whether phytochrome can modulate extracellular peroxidase activity in mustard and, if so, whether these changes could be involved in the control of stem growth by phytochrome. Wounding is known to increase extracellular peroxidase activity (eg Birecka and Miller, 1974; Espelie, Franceschi and Kolattukudy, 1986) and, under given experimental conditions, B is known to inhibit growth (Chapter 5). Therefore, the effects of internode wounding and B pretreatments were also investigated in order to

evaluate the extent of correlation between peroxidase activity and growth.

## MATERIALS AND METHODS

Plant material. Mustard (Sinapis alba L.; Asmer seeds, Leicester, U.K.) were sown in Petersfield (Leicester, U.K.) No 2 commercial range potting compost in 120-cm<sup>3</sup> pots (one seedling per pot) and grown under continuous fluorescent WL for 12 d. In one group of experiments the plants were grown under orange light (i.e. light from low pressure sodium lamps), instead of WL.

Light sources. The scans and details of construction of sources of the WL (PAR= 126  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; Pfr/P= 0.86), R (4  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; Pfr/P= 0.85), FR (102  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; Pfr/P= 0.01), orange light (280  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; Pfr/P= 0.84) and B (16  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; Pfr/P= 0.84 for orange plus B), instruments for light measurement and Pfr/P calculations were as described previously (Chapters 2 and 3).

Growth measurements. Incremental length measurements were carried out with a ruler or with a linear-voltage-displacement transducer as described previously (Chapters 2 and 4). For some incremental length measurements, the upper and lower halves, or different 0.5 cm sections of the internodes, were identified immediately prior to the treatments (i.e. time= 0), by a slight ink marks. The incremental dry weight of the internodes was calculated as the difference between the dry weight at a given time and the estimated dry weight at time= 0. The dry weight at time= 0 was estimated by using a relation between internode length and internode dry weight: dry weight (mg)= -0.185 +0.151 length (mm);  $r= 0.85$ ;  $n= 199$   $P<<0.01$ . The plants used for the construction of the calibration curve were grown under the same conditions as the plants used for the treatments. Internode dry weight was measured after drying the material for 3 d at

80 °C.

Extraction of cell wall protein. The first internode of mustard seedlings (i.e. the internode between the cotyledonary node and the first leaf pair node) was harvested at different times after treatment. The upper and lower halves of the internodes were separated, immediately placed in ice-cooled extraction solution (50 mM Ca Cl<sub>2</sub>), and infiltrated in vacuo (still on ice) for 10 min. The internode sections were then externally dried by gently rolling them on absorbent paper.

Six sections (i.e. one replicate) were placed in a 1.5 cm length, 0.5 cm diameter cylindrical plastic tube. Each cylindrical tube was placed in a 1.5 ml microcentrifuge tube with its lower tip covered by a layer of muslin pressed between the walls of the cylindrical tube and the walls of the microcentrifuge tube. The samples were centrifuged for 10 min (4 °C) at 650g. All these steps were done under dim green light. The samples were either analysed immediately or stored at -20 °C.

Extraction of intracellular plus extracellular peroxidase. Upper halves of mustard internodes were ground in 0.1 M phosphate buffer, pH 7.0 with insoluble polyvinyl polypyrrolidone (25 per cent of the tissue fresh weight) in an ice-cooled mortar at 4 °C. The homogenate was centrifugated at 26 000 g for 20 min at 4 °C and the supernatant was freeze-dried. The dried powder was resuspended in distilled water, centrifuged (13 000 g, 5 min) and used immediately for peroxidase assays.

Protein determination. The protein concentration in the extracts was determined by the method of Ghosh et al (13). One µl samples were spotted onto Whatman 3 mm paper, and after air drying, Coomassie Brilliant Blue R 250, was used as stain. The spots were scanned in a GS 300 Hoefer densitometer (Hoefer Scientific Instruments, San Francisco, CA). Protein concentration was related to the area of the peaks (height x width/2). Bovine serum

albumin was used as standard.

SDS-PAGE. SDS-PAGE was carried out according to Laemmli (1970) in 12.5 per cent, 1.5 mm thick, gels using Mini-Protean II Dual Slab cells (Bio-Rad Laboratories, Richmond, CA). Low molecular weight standards from Bio-Rad were used. Gels were stained with 0.025 % (w/v) Coomassie Blue R in 50 % (v/v) ethanol, 5 % (v/v) acetic acid (60 min at 37 °C), and de-stained with 7.5 % (v/v) acetic acid, 5 % (v/v) methanol.

Peroxidase assay in aqueous solution. Two ul of each extract were diluted in 500 ul of ice-cooled distilled water or, alternatively, the whole volume of the centrifugate was measured, diluted in 1000 ul, and the results corrected using volume as concomitant variable (both procedures yielded similar results). Ten ul of the dilution were added to 200 µl of assay mixture. Assay mixture contained 0.1 mg ml<sup>-1</sup> 3,3',5,5' tetramethyl benzidine in 0.1 M citrate/acetate buffer, pH 5, containing 0.002 % (v/v) hydrogen peroxide. Peroxidase activity was determined at room temperature by recording the changes in absorbance at 450 nm after 14 min (the reaction was stopped by adding 50 µl of 2.5 M H<sub>2</sub>SO<sub>4</sub>). An arbitrary unit of peroxidase activity was chosen as the change in absorbance produced per µl of extract from control plants (i.e. the plants receiving a R pulse before D) as this was stable through the period of experimentation.

Isoelectrofocusing gels. Data in Figures 6.6, 6.7 and 6.10 are from polyacrylamide gels run in Model 111 Mini IEF Cells (Bio-Rad) according to the instruction manual. Data in Figure 6.5 are from vertical gels run in Mini-Protean II Dual Slab Cells according to Robertson et al (1987). Ampholites were 1809-001 Ampholine pH 3.5-10 (Pharmacia LKB Biotechnology AB, Bromma, Sweden) or Bio-Lyte pH 3.85-5.1 (Bio-Rad Laboratories, Richmond, CA). Isoelectric point markers were from the Electran Kits, range 4.7-10.6 or range 2.4-5.65 (BDH Limited, Atherstone, Warwickshire, UK). Gels were washed for 15

min in distilled water, stained for peroxidase activity with 40 ml of 40 mg ml<sup>-1</sup> 4 chloronaphtol and 0.002 % (v/v) hydrogen peroxide, and fixed with 7.5 % (v/v) acetic acid, 5 % (v/v) ethanol. The dots were scanned (transmittance) and an arbitrary unit of peroxidase activity was chosen as the average area (height x width / 2) of the peaks obtained by scanning the dots corresponding to the isoform A3, for R treated (phytochrome experiments) or control plants (wounding experiments).

## RESULTS

Phytochrome mediated effects on internode growth and extracellular peroxidase activity. Mustard plants were grown under continuous WL for 12 d and transferred to D after a R or a FR pulse. FR treated plants showed a higher rate of extension growth of the first internode than R treated plants (Fig. 6.1A). This effect was paralleled by a proportionate increase in the rate of dry matter accumulation by the internode as a result of the FR pulse (Fig. 6.1B). Light treatment effects on extension growth were virtually restricted to the upper half of the growing internode (Fig. 6.1A, inset).

Although the treatments caused large differences in growth rate, the increases in internode biomass during the sample period were small compared to the initial internode biomass. Consequently, there were no observed effects of the light treatments on the volume of extracellular fluid removed from the upper halves of the internodes by the infiltration and low speed centrifugation technique (5.5 h after the beginning of D: R= 5.8 µl; FR= 6.1 µl; n= 42; P>0.1).

The proteins found in the centrifugates showed a distinctive pattern in SDS gels when compared to the proteins found in internode homogenates (i.e. including intracellular proteins) (Fig. 6.2). The concentration of extracellular proteins in the extracts from the upper

halves of the internodes was  $0.9 \pm 0.07 \mu\text{g}/\mu\text{l}$  ( $n = 73$ ) for both R and FR treated plants. In the lower halves, the concentration was ca 30 per cent lower than that for the upper halves under both light treatments (Fig. 6.3). Separation of these proteins by SDS-PAGE showed no obvious qualitative differences between R and FR treated plants or between the upper and lower internode sections (Fig. 6.3).

Peroxidase activity was lower in internode centrifugates from FR than from R treated plants (Fig. 6.4). This effect was virtually restricted to the upper half of the internode (Fig. 6.4A). Immediately after the pulses (i.e. 10 min after the transition from WL to D) no significant effect was found, but the differences were present in the plants harvested 45 min after the beginning of D and persisted without significant changes throughout the period of experimentation (Fig. 6.4B). In order to test whether the earliest differences were already present at the time of harvest, or developed between the harvest and the centrifugation (intermediate steps took in some cases 45 min), groups of plants were harvested immediately after a R or a FR pulse (i.e. at 10 min, when the differences were still not present, Fig. 6.4B) but they were incubated in D in the extraction solution for 1.5-2 h before the subsequent extraction steps continued. Since no differences between R and FR treated plants were found in these control experiments (i.e.  $R = 1.00$ ;  $FR = 1.06$ ;  $\text{sem} = 0.058$ ;  $n = 18$ ), we conclude that the true lag for the expression of differences in extracellular peroxidase activity lies between 10 and 45 min.

Isoelectrofocusing gels revealed the presence in the extracellular internode extract of four peroxidase isoforms with pI values between 3.95 and 4.8, in the extracellular internode extract (Fig. 6.5). In concentrated extracts from homogenised internodes, two additional isoforms were observed, one basic and one acidic (Fig. 6.5). Subsequent experiments were



concentrated on the two more abundant extracellular isoforms: A3 and A4. FR, compared to R, treatments reduced the activity of A4, whilst A3 was unaffected (Figs 6.6 and 6.7A). Both A3 and A4 were more active in the upper than the lower half of the internode, and the effects of the light treatments were restricted to the upper half (Fig. 6.7B).

The effects of the light treatments on growth and extracellular peroxidase activity were mediated by the perception of the light stimuli by phytochrome as revealed by reversion experiments (Fig. 6.8).

Effects of wounding on internode growth and peroxidase activity. The first internode of WL-grown mustard plants was cut at the point of insertion of the first pair of leaves. This treatment reduced growth rate with a lag of ca 10 min, and the lower growth rate persisted during the period of experimentation (Fig. 6.9). The activity of A3 was increased by wounding (Fig. 6.10). The differences were already present 45 min after the treatment (Fig. 6.10). In contrast, A4 showed no significant changes in activity (Fig. 6.10). The proteins separated by SDS-PAGE and stained with Coomassie showed a small increase in concentration, but only 90 min after wounding (Fig. 6.11).

Effect of B pretreatments on internode growth and peroxidase activity. Mustard plants were also grown under background orange light from low pressure sodium lamps. Supplementing this blue-deficient light with a small amount of B reduced the growth of the internode in subsequent D (Table 6.1). No differences in extracellular peroxidase activity were found correlated to the effect of B on growth (Table 6.1).

## DISCUSSION

A FR, compared to a R, pulse provided immediately prior to D promoted the extension growth of the first internode in WL-grown mustard (see also Chapters 2, 3,

4, 5). These phytochrome mediated changes in the rate of extension growth were correlated to rapid (lag <3h) changes of similar magnitude in the rate of dry matter accumulation by the internode (Fig 6.1).

The extracellular peroxidase activity centrifuged from the upper half of mustard internodes was reduced by a FR, compared to a R, pulse perceived by phytochrome. Four anionic or moderately anionic peroxidases (isoelectric points between 3.95 and 4.85) were found in the centrifugates after separation by isoelectrofocusing PAGE. At least some anionic and moderately anionic peroxidase isoforms are known to be extracellular (Birecka and Miller, 1974; Cassab and Varner, 1988; Espelie et al., 1986). Only the more active isoforms, A3 and A4 (both having a molecular weight ca 60 kdalton, data not shown), were investigated in further detail. Low Pfr/P reduced the activity of A4 centrifuged from the upper half of the internodes, whilst A4 activity from the lower internode half, and the activity of A3 were unaffected (Figs 6.4, 6.6, 6.7). Sharma, Sopory and Guha-Mukherjee (1976) reported differences in peroxidase activity in homogenates from etiolated maize shoots that were subjected to different phytochrome status. However, no distinction was made between extracellular and intracellular peroxidase isoforms.

It is noteworthy that the pattern of extracellular peroxidase response to wounding was opposite to that found after R or FR treatments. Wounding increased A3 activity with a lag of less than 45 min but caused no significant effects on A4 (Fig. 6.10). The activity of A3 did not increase further between 45 and 90 min. A rise in anionic peroxidase activity as a result of wounding has been reported for potato tuber tissue (Espelie et al., 1986) and tobacco pith tissue (Lagrimini and Rothstein, 1987), but in both studies the time course showed maximum activity several days after wounding. In potato, the anionic peroxidase was immunologically localized in the inner side of

suberizing walls (Espelie et al., 1986).

Infiltration of intact stems with  $\text{CaCl}_2$ , followed by low speed centrifugation, allows the extraction of ionically bound extracellular proteins with a small degree of contamination from cytoplasmic contents (e.g. Morrow and Jones, 1986; Ros Barcelo et al., 1988). The differences between intact internode centrifugates and internode homogenates observed in the patterns of proteins stained with Coomassie (Fig 6.2), and in the relative activity of peroxidase isoforms (Fig 6.5), are consistent with this assumption. The proteins centrifuged from mustard internodes showed no obvious quantitative or qualitative differences as a result of the R or FR pulses. This observation is consistent with previous work in peas, which showed that infiltration of stem sections with antibodies against the bulk of the ionically-extractable proteins had no significant effects on growth (Morrow and Jones; Melan and Cosgrove, 1988).

Present results allow three possible interpretations: internode growth and A4 responses to Pfr/P are not causally related; changes in A4 cause changes in growth rate; changes in growth rate cause changes in A4. Several pieces of circumstantial evidence are in favour of the second hypothesis:

(a). Independent evidence suggests that extracellular peroxidases could catalyse the formation of cross-links between cell wall matrix components, and that these cross-links could modulate cell wall extensibility and extension growth (Fry, 1979; Fry, 1986; Taiz, 1984; Cassab and Varner, 1988; Everdeen et al, 1988).

(b). There is a good correlation (within the level of resolution achieved here) in timing and localization of the changes in growth and A4 activity. After a FR pulse given immediately prior to D, internode growth rate is promoted with a lag of ca 10 min, reaches a maximum 20-30 min after the beginning of D, followed by a transient decrease in growth rate that leads to a

minimum at 40-60 min, and by a final recovery of growth rate beyond 60 min (Fig. 4.3). The early events are induced in the growing internode itself, whilst a contribution of the leaves to the growth promotion becomes apparent beyond 60 min (Fig. 4.3). The lag for phytochrome mediated changes in the activity of A4 lies between 10 and 45 min. Therefore, the effects on A4 appear to overlap the first growth rate acceleration, and anticipate the final growth recovery and the promotion initiated in the leaves. In addition, after the differences in growth rate and peroxidase activity are established by the R or FR pulses, these differences remain relatively constant during the period of experimentation. Finally, phytochrome mediated changes in growth rate and peroxidase activity are both virtually restricted to the upper half of the internode.

(c). B pretreatments and internode wounding reduced internode extension growth rate but did not affect A4 activity significantly. Cosgrove (1988) showed biophysical evidence against the involvement of changes in peroxidase cross-linking in the control of extension growth by B in Cucumis sativus.

(d). Extracellular peroxidases could be involved in defence mechanisms (see Cassab and Varner, 1988), but this does not appear to be the case for the isoform A4, whose activity was not significantly affected by wounding, whilst this treatment increased the activity of A3.

Present results are consistent with the hypothesis that, in mustard, phytochrome mediated changes in growth rate are at least partially mediated by changes in extracellular peroxidase activity. However, an element of caution is required as a more firm conclusion will only be possible after the results of further investigations on the biochemistry and biophysics of the internode responses to Pfr/P become available.

Table 6.1. Effects of supplementing blue-deficient light ( $280 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) with  $16 \mu\text{mol m}^{-2} \text{s}^{-1}$  of B, on the subsequent growth of the first internode, and on extracellular peroxidase activity after the transition to continuous D (= 0 h). Data are means of 46-47 plant replicates (a,b) or 26-27 independent centrifugates (c),  $\pm$  sem.

	Control	+ B
a) $\Delta$ length of the upper 0.5 mm section (mm).		
0-24 h	$5.0 \pm 0.26$	$3.6 \pm 0.26$
24-48 h	$5.3 \pm 0.41$	$3.7 \pm 0.40$
b) $\Delta$ length of the sub-apical 0.5 mm section (mm).		
0-24 h	$4.1 \pm 0.22$	$3.3 \pm 0.22$
24-48 h	$4.2 \pm 0.36$	$3.0 \pm 0.35$
c) Extracellular peroxidase activity (upper 0.5 mm 24 h)	$1.0 \pm 0.08$	$1.0 \pm 0.06$

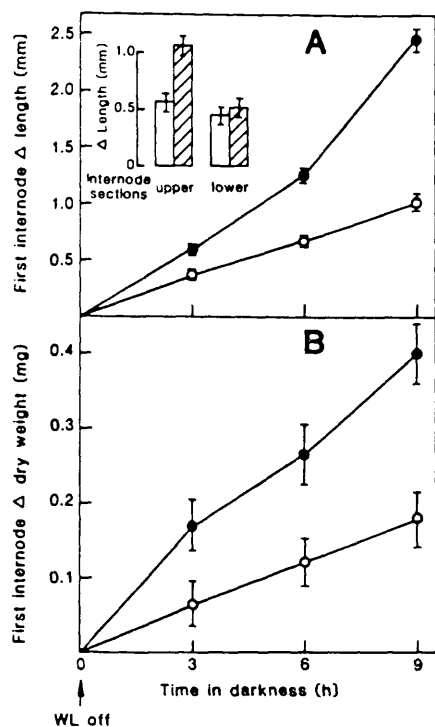


Fig 6.1. First internode  $\Delta$  length (A) and  $\Delta$  dry weight (B) in WL-grown mustard plants transferred to D after a R (open circles) or a FR pulse (closed circles). The inset in (A) shows the  $\Delta$  length of the upper and lower halves of the internodes (6 h) in plants treated with a R (open columns) or a FR pulse (dashed columns). Data are means of 99-104 (A, B) or 28 (A inset) plant replicates  $\pm$  sem.

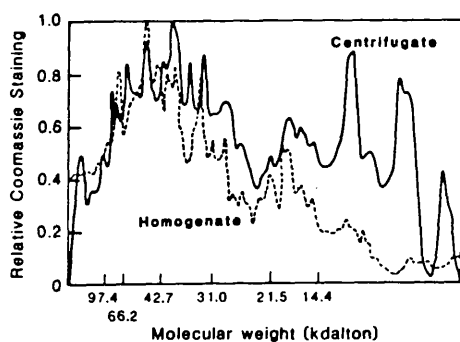


Fig. 6.2. Scans of the proteins centrifuged from intact mustard internodes after infiltration with  $\text{CaCl}_2$  (ie extracellular proteins) (solid line), and the proteins obtained by homogenization of the internodes (ie including intracellular proteins) (dotted line), after separation by SDS-PAGE followed by staining with Coomassie.

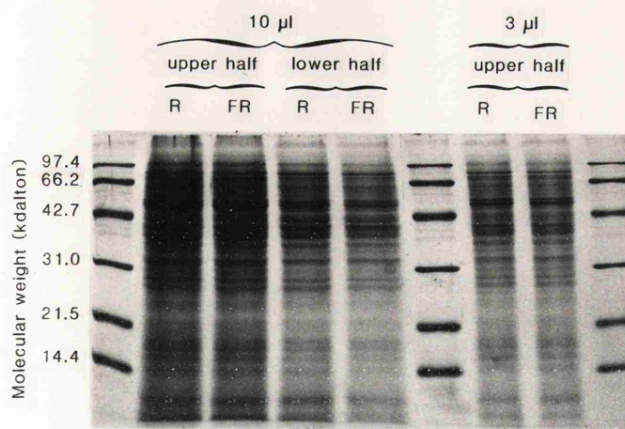


Fig. 6.3. SDS-PAGE of the proteins centrifuged from intact internodes (upper or lower half) from plants treated with a R or a FR pulse followed by D. Equal volumes of centrifugate (either 10 or 3 µl) were loaded per well.

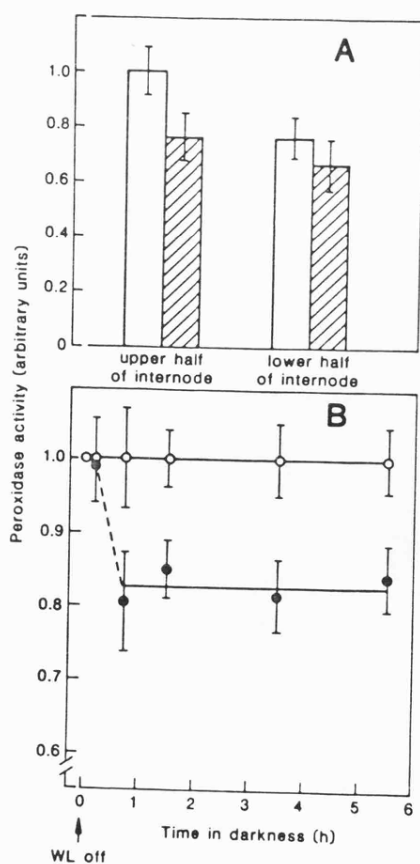


Fig. 6.4. Peroxidase activity in internode centrifugates from WL-grown mustard plants transferred to D after a R (open columns and circles) or a FR pulse (dashed columns, closed circles). (A), Activity in the centrifugates from the upper and lower halves 5 h after the beginning of D. (B), (different experiments) time course of changes in activity in centrifugates from the upper halves (times of internode harvest are indicated). Data are means of 23 (A) or 14-21 (B) independent centrifugates  $\pm$  sem.

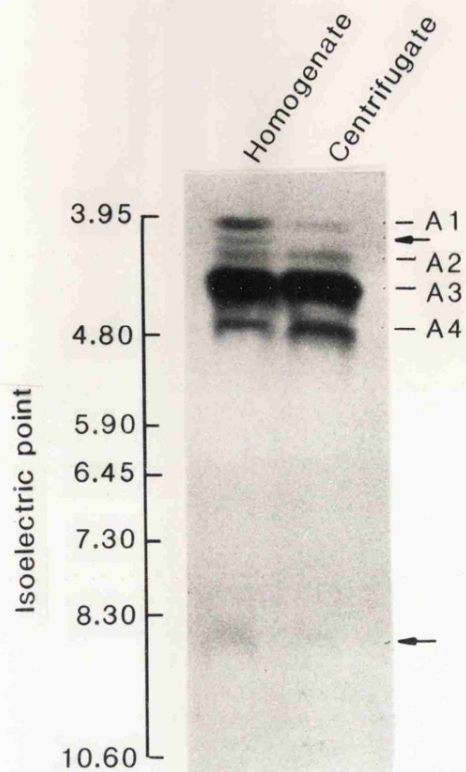


Fig. 6.5. Peroxidase isoforms isolated from mustard internodes, separated by isoelectrofocusing and stained for peroxidase activity. Samples were obtained either by homogenization and concentration (lyophilization) of the extract or by centrifugation of intact internodes infiltrated with  $\text{CaCl}_2$ . Equal peroxidase activities were loaded. A1, A2, A3 and A4, indicate the isoforms found in the centrifugate and the arrows point to additional isoforms in the homogenate.

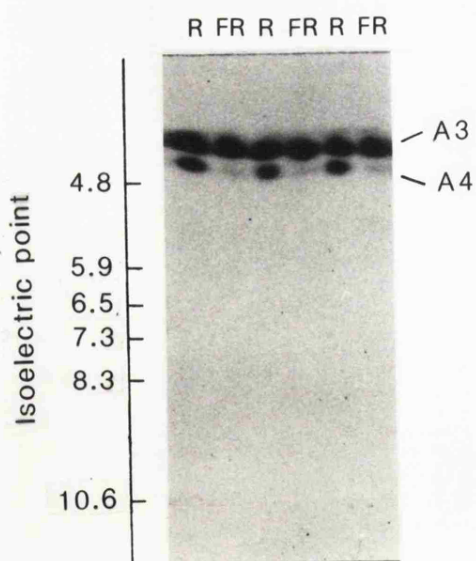


Fig. 6.6. Isoelectrofocusing gel showing peroxidase activity (isoforms A3 and A4) centrifuged from the upper half of intact internodes from mustard plants that received a R or a FR pulse followed by 4 h D. One microlitre centrifugate samples were loaded.



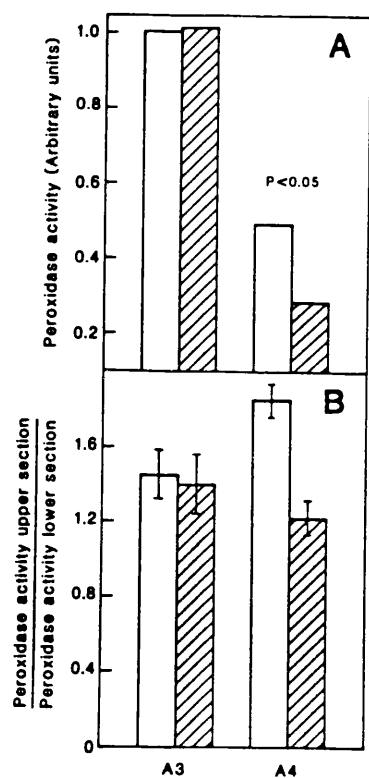


Fig. 6.7. Peroxidase activity (isoforms A3 and A4) centrifuged from the upper half of intact internodes from mustard plants that received a R (open columns) or a FR (dashed columns) pulse followed by D. Data for 1 and 4 h dark incubation showed the same characteristics and were averaged. (A), activity centrifuged from the upper halves. (B) (different experiments) ratio between the activities of the upper and lower halves. Data are means of 10 (A) and 18 (B), independent centrifugates. The  $\ln(x+1)$  transformation was used for the statistic analysis in (A), and means were back transformed to be shown. Bars in (B) are + sem.

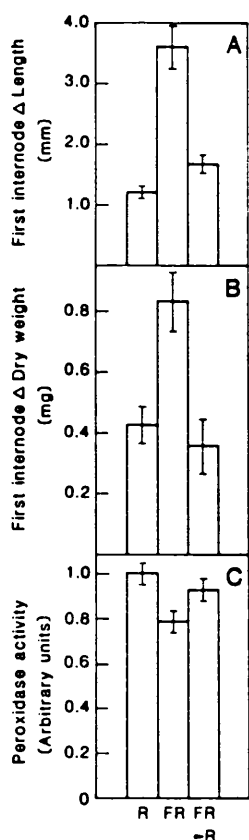


Fig. 6.8. Photoreversibility of the effects of FR pulses on internode  $\Delta$  length (24 h) (A), internode  $\Delta$  dry weight (24 h) (B), and extracellular peroxidase activity centrifuged from intact internodes (1 or 2 h)(C). Data are means of 58-61 plant replicates (A,B), or 29-31 independent centrifugates (C),  $\pm$  sem.

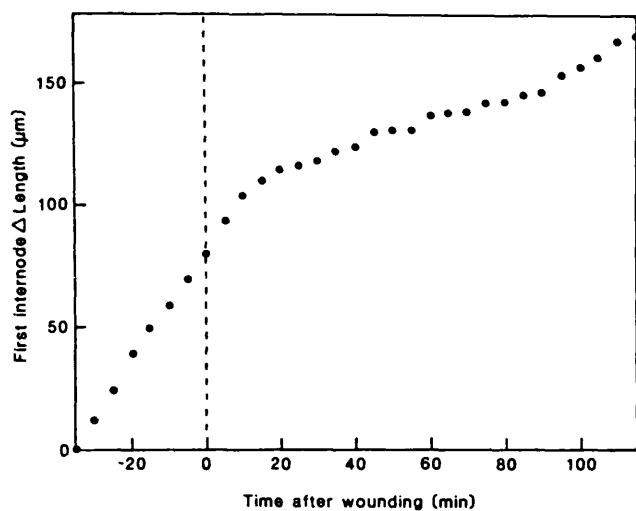


Fig. 6.9. Effects of internode wounding (cut at the point of insertion of the first pair of leaves) on internode extension growth recorded by means of a linear voltage displacement transducer (data from a representative trace).

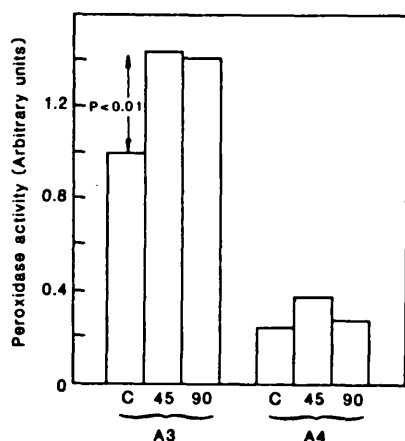


Fig. 6.10. Effects of internode wounding (cut at the point of insertion of the first pair of leaves) on peroxidase activity (isoforms A3 and A4) centrifuged from the internodes. C= control plants; 45= 45 min after wounding; 90= 90 min after wounding. The  $\ln(x+1)$  transformation was used for the statistical analysis and means were back transformed to be shown. Data are means of 23-24 independent centrifugates.

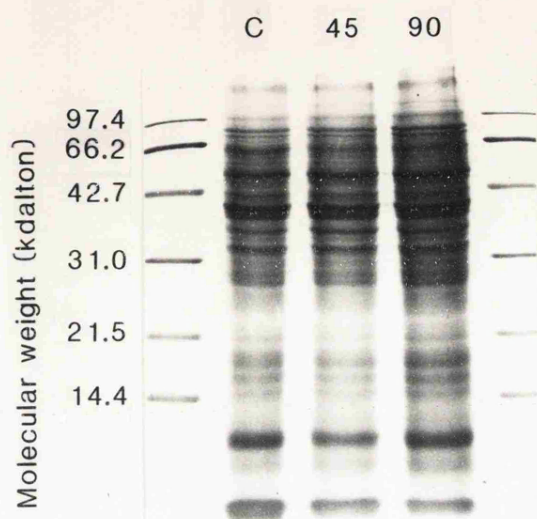


Fig. 6.11 Effects of internode wounding (cut at the point of insertion of the first pair of leaves) on extracellular proteins centrifuged from the internodes. Proteins were separated by SDS-PAGE and stained with Coomassie. C= control plants; 45= 45 min after wounding; 90= 90 min after wounding. The centrifugates were the same ones used for peroxidase activity measurements (Fig. 6.10).

## CHAPTER 7

### THE ECOLOGICAL SIGNIFICANCE OF PHOTOMORPHOGENIC VEGETATIVE RESPONSES IN FULLY DE-ETIOLATED PLANTS

## THE HYPOTHESIS

It has been proposed that photomorphogenic responses mediated by phytochrome provide green plants grown under natural conditions with a tool for adapting properly to the changing light environment in plant stands (Kasperbauer, 1971; Smith, 1973, 1982). This hypothesis implies (Smith, 1982): (a), that under natural conditions, changes in the light environment associated with canopy density provide the plant with precise signals, unaffected by other variables; (b), that plants are able to perceive these light signals, and (c), that signal perception leads to responses which increase the probability of survival and reproductive success. The aim of this chapter is to summarise the current state of the evidence in relation to the above hypothesis, particularly in relation to the latest findings included in this thesis. For reviews of the earlier work, and for further references, see Morgan and Smith (1981), Smith (1982) and Smith and Morgan (1982).

## LIGHT SIGNALS RELATED TO CANOPY ARCHITECTURE

When sunlight reaches green plant organs, a substantial proportion is absorbed, the rest being transmitted or reflected. This is a selective process determined by the leaf pigments (mainly chlorophyll) and the optical characteristics of the leaf (Knipling, 1970; Woolley, 1971; Monteith, 1976; Holmes and Fukshansky, 1979). Consequently, increasing canopy density may bring about not only a reduction in the amount of light, but also a marked change in the spectral distribution, since B and R are depleted much more than green light and FR. In herbaceous stands, the ratio of the photon fluence rate of R, to that of FR, (R:FR) received by a given plant organ can be affected by its height within the canopy (Holmes and Smith, 1977b), the number of individuals

that surround it (Holmes and Smith, 1977b; Casal, Sanchez and Deregibus, 1986; Ballare et al 1987), the extent to which the canopy is defoliated (Deregibus, Casal, Sanchez, 1985), and the orientation of the rows within crops (Kasperbauer, 1987).

The changes in R:FR, and the changes in PAR, which occur as a result of canopy density are not completely correlated. On the one hand, it is obvious that senescing or dead organs, with a low chlorophyll content, will have, in relative terms, a larger impact on the PAR than on the R:FR ratio when compared with green organs. However, although senescent organs per se produce only small changes in the R:FR ratio, within a canopy they can modify differentially those components of the light environment which have, or have not, been already modified by transmission through, or reflection from green organs. In other words, senescing organs can act as "neutral density filters" intercepting light with a high or a low R:FR according to their position within the canopy; hence, they can change (increase or decrease) the R:FR ratio of the light reaching the lower strata of the canopy (Deregibus, Casal and Sanchez, 1985). On the other hand, there are cases in which the R:FR ratio can be affected without the involvement of serious changes in PAR available per plant. For instance, in sparse, even, canopies the FR reflected by neighbours can reach photoreceptive organs, such as the internodes of dicotyledonous seedlings, well before the leaves (the main photosynthetic organs) become shaded (Ballaré et al 1987). An example of this is shown in Figure 7.1. A small green stand of mustard, orientated east-west, altered the R:FR on the south (sunlit) side when the sensor faced the stand. A gradual reduction in R:FR was found when the sensor was moved towards the stand. This reduction resulted mainly from an increase in the amount of FR reaching the sensor due to selective reflection of sunlight from the leaves, and also from a

small reduction of R due to the partial obstruction of diffuse light from the north hemisphere of the sky by the stand.

Early descriptions of R:FR within plant canopies were focused mainly on changes occurring along a vertical axis within the canopy. These changes could provide the target plant with quantitative information about its height in relation to that of surrounding plants. The more recent studies of the changes along the horizontal dimension suggest that R:FR may provide information on the vicinity of neighbouring plants.

The question is, do changes in the light environment associated with canopy density comply with the first condition of the above hypothesis?; i.e. are these changes precise signals of the status of the canopy or are they disturbed by modifications caused by other factors? The fluence rate received by a plant is strongly affected not only by the density of the canopy but also by solar elevation (i.e. time of day, time of year, latitude, etc.) and cloudiness. In contrast, R:FR is much more stable (ca 1.1-1.2 for sunlight, Holmes and Smith, 1977a). It is strongly correlated with canopy density and less affected by cloudiness and solar elevation. Therefore, in principle, the R:FR appears as a more reliable signal of canopy density than photon fluence rate.

Nevertheless, however small in magnitude the atmospheric perturbations of R:FR may be, they lie within the range to which plants are able to respond (eg Fig 2. 6). For instance, R:FR is lower during twilight (solar elevation  $<20^{\circ}$ ) than during daytime (solar elevation  $>20^{\circ}$ ), although the magnitude of this reduction changes according to cloudiness, to the latitude and to the time of year (Shropshire, 1973; Holmes and Mc Cartney, 1976; Goldberg and Klein, 1977; Holmes and Smith, 1977a; Hughes et al., 1984). There are ways in which these perturbations could be avoided since certain combinations of R:FR and fluence rate are

characteristic signals of the presence of neighbours. A relatively low R:FR reaching the internodes together with a high fluence rate reaching the leaves will be experienced at midday by a plant with nearby neighbours reflecting FR (Ballare et al., 1987; Fig. 7.1), whereas, a reduction in R:FR at the end of the day, caused by atmospheric factors or very distant plants would not be accompanied by a high fluence rate. This means that if the plants are able to weigh the response they exert to a reduction in R:FR according to the fluence rate they receive, the perturbations that take place at the extremes of the photoperiod would be much less important. Recent physiological investigations in relation to this issue discussed below suggest that such fine tuning of R:FR perception does occur (see also Chapters 3 and 4).

#### PERCEPTION OF CANOPY DENSITY LIGHT SIGNALS

Light signals can only give place to responses if they are perceived by appropriate photoreceptors. Phytochrome is a chromoprotein capable of perceiving R:FR signals. It possesses two relatively stable, photoconvertible forms, Pr and Pfr and, measured spectrophotometrically, the proportion of total phytochrome in the Pfr form (i.e.  $Pfr/P$ ) is, at equilibrium, a direct function (approximately a rectangular hyperbola) of the R:FR of the incident radiation (Smith and Holmes, 1977). Even the changes in R:FR caused by the FR reflected in neighbour plants can modify  $Pfr/P$  measured in vitro (Table 7.1). There is good evidence in favour of phytochrome perception of R:FR as plants of many species show strong responses to a depression of R:FR caused by supplementing artificial or natural WL with different fluence rates of FR (Morgan and Smith, 1976; 1978; Morgan, O'Brien and Smith, 1980; Casal, Sanchez and Deregibus, 1987a, b). These responses are the result of a depression of R:FR and not the result of





place, however, the transformation from Meta Rb to Pfr occurs at a specific rate that is virtually independent from the fluence rate of the incident radiation. As a result, at high fluence rates the transformations between Pfr and Pr and between Pr and Meta Rb occur very quickly and a large proportion of the total phytochrome is accumulated as Meta Rb while the concentration of Pr and Pfr decrease (Spruit, 1982; Gardner and Gracefo, 1982; Kendrick, Kome and Jaspers, 1985). At equilibrium, the ratio between Pfr and Pr, and hence  $Pfr/Pfr+Pr$ , is not affected by fluence rate as:

$$\Delta [Pfr] = 0 \quad \therefore \quad [Pfr].v_3.I = [Pr].v_1.I.v_2 \quad \therefore$$

$$Pfr/Pr = v_3/v_1.v_2$$

(where I represents fluence rate and  $v_1$ ,  $v_2$ , and  $v_3$ , the rate constants for the  $Pfr \rightarrow Pr$ ,  $Pr \rightarrow Meta\ Rb$  and  $Meta\ Rb$  transformations respectively). However, [Pfr] or  $Pfr/P$  would be lower at higher fluence rates, since at high fluence rates P includes [Meta Rb] as an important component. These considerations mean that if plants respond to  $Pfr/Pr+Pfr$  or to the ratio of Pfr to Pr, the perception of R:FR would not be affected by high fluence rates. However, if plants respond to [Pfr] or  $Pfr/P$  alone, high fluence rates could give a signal equivalent to that of low R:FR ratios. In other words, a fully isolated plant exposed to midday sunshine could produce shade avoiding reactions.

There is an increasing body of evidence in favour of the occurrence of responses to R:FR under natural conditions (discussed below), yet perturbations caused by high fluence rates have not become apparent. There are several possibilities. First, the accumulation of Meta Rb, for the pool of phytochrome controlling shade avoidance responses, is much lower than that predicted by means of spectrophotometry (Kendrick, Kone and Jaspers, 1985) or by measuring the amount of Pfr available for destruction (Smith, Jackson and Whitelam, 1988). This could be accounted for by the a different

environment surrounding the relevant pool of phytochrome (Spruit, 1982; Kendrick, Kone and Jaspers, 1985). Secondly, plants may measure  $Pfr/Pr+Pfr$ . Thirdly, plants may measure  $[Pfr]$  or  $Pfr/P$ , but at high fluence rates the responses caused by a reduction in the values of these parameters are compensated by responses of opposite direction dependent on fluence rate. Such responses are well documented in etiolated seedlings (Mohr, 1972; Johnson and Tasker, 1979), but the picture is far from clear for de-etiolated seedlings. Nevertheless, recent data obtained with fully de-etiolated seedlings, indicate that extension growth can be reduced by increasing fluence rates of B (Casal and Alvarez, 1988; Chapter 5) or blue-deficient light (Table 7.2).

Bearing in mind the above issue, we will continue referring to plant responses to the calculated  $Pfr/P$  (or simply  $Pfr/P$ ) because this parameter is used to describe the light treatments in a way that, for many purposes, is physiologically meaningful.

It has been shown in etiolated plants that at low fluence rates the  $Pfr/P$  values may be below those expected from the spectral composition of the light due to selective degradation of phytochrome in the Pfr form (Jabben, Beggs and Schäfer, 1982). This reaction occurs at a specific rate that is independent of the light conditions and therefore, the lower the fluence rate, the larger the relative incidence of degradation. However, there is evidence from both immunological and spectrophotometric studies (Brockmann and Schäfer, 1982; Abe et al., 1985; Tokuhisa, Daniels and Quail, 1985) that at least two types of phytochrome exist. The "etiolated", or type I, phytochrome is the predominant component in dark-grown seedlings and is subject to rapid degradation, particularly in the Pfr form (Quail et al., 1973). The "green", or type II, phytochrome is the predominant form in light-treated seedlings and is more stable. It is likely that in green seedlings

responses such as stem extension are mainly under the control of type II phytochrome. In fact, the effect of high Pfr/P at the beginning of darkness can be reversed by a FR pulse (reversion of the response display) even after several hours in darkness (Downs, Hendricks and Borthwick, 1957; Schafer, Ebert and Scheitzer, 1984; Chapter 4, Results section). Furthermore, type II phytochrome mutants, having a type I phytochrome level similar to the wild genotype, lack at least some of the typical growth responses to phytochrome (Adamse et al., 1988). This indicates that a large distortion of the perception of R:FR by phytochrome at low fluence rates would not be expected.

#### THE RESPONSES OF FULLY DE-ETIOLATED SEEDLINGS TO PHYTOCHROME STATUS

There are several aspects of vegetative growth in green plants that are strongly influenced by the status of phytochrome. The increase of stem growth associated with a depression of Pfr/P is one of the more dramatic and more intensively-investigated responses. The reduction in Pfr/P can be achieved by lowering the R:FR ratio of the light by addition of FR in varying amounts to a constant background source of PAR, either under controlled conditions (e.g. using fluorescent tubes, Heathcote, Bambridge and McLaren, 1979; Morgan and Smith, 1976) or under natural radiation (Casal, Sanchez and Deregibus, 1987a, b). Less realistic in ecological terms, but still a very useful tool to uncover physiological responses are the so-called "end-of-day" R or FR pulses, i.e. short irradiations given at the end of the period during which PAR is provided (Downs, Hendricks and Borthwick, 1957). The response of stem growth to Pfr/P has been reported for many dicotyledonous species, including Phaseolus vulgaris (Downs, Hendrick and Borthwick, 1957), Lycopersicon esculentum (Selman and Ahmed, 1962), Nicotiana tabacum

(Kasperbauer, 1971), Chenopodium album (Morgan and Smith, 1976) and Fuschia hybrida (Vince Prue, 1977). In addition, in culmless grass plants, the leaf sheath responds in a way comparable to the stem in dicotyledonous plants (Smith, 1981; Casal, Sanchez and Deregibus, 1987a). In mustard there are two at least partially different pathways of internode-growth-rate response to a reduction in Pfr/P that have been characterised under controlled conditions. Low Pfr/P established in the growing internode itself of plants grown under continuous WL triggers a rapid (lag ca 10 min) growth promotion, but once high Pfr/P is re-established in the internode, the growth rate returns to the pre-stimulation rate within ca 16 min (Child and Smith, 1987). In contrast, low Pfr/P established in the leaves triggers a less rapid promotion (Morgan, O'Brien and Smith, 1980) that persists for at least 24 h after high Pfr/P is re-established (Figs 2.2-2.7, 4.10). Higher fluence rates of B reaching the leaves enhance the promotion of internode growth triggered in the internode itself (i.e. the "rapid" response) (Fig 3.7). As a consequence of this, low Pfr/P established in the growing internode appears to be not very efficient in promoting internode growth during dark periods (Figs 3.3, 3.4 and 4.3). In contrast, low Pfr/P established in the leaves is effective in promoting growth in subsequent darkness (Figs 2.2, 3.3, 4.3, 4.4, 4.5, 4.6, 4.7, 5.1, 6.1). However, when compared over the same period in darkness, growth rate is higher if the Pfr/P is reduced during, rather than at the end of the photoperiod (Fig 4.9). This is not accounted for by a response lag, since the latter is not measurable with the ruler (Fig. 4.4). Rapid responses of stem growth to a reduction in Pfr/P have also been observed in Vigna sinensis (Lecharny and Jacques, 1982) and Cucumis sativus; (Gaba and Black, 1985), but a full characterisation of these responses is not available at present.

Apical dominance in dicotyledonous (Bogorad and McIlrath, 1960; Kasperbauer, 1971; Tucker and Mandsfield, 1972; Vince-Prue, 1977) and grass plants (Casal, Sanchez & Deregibus, 1987 b) is also increased by a reduction in Pfr/P. In dicotyledonous plants, leaf area may be reduced (Satter and Wetherell, 1968; Kasperbauer, 1971; Frankland and Letendre, 1978; McLaren and Smith, 1978; Inada and Matsuno, 1985), increased (Cogliatti and Sanchez, 1982; Thomas and Raper, 1985; Casal, Aphalo and Sanchez, 1987), or remain virtually unchanged (Casal and Sadras, 1987). Responses of different direction may occur in different leaves of the same plant (Child, Morgan and Smith, 1981) or even in different stages of the same leaf (Casal and Sadras, 1987). In some species chlorophyll content may be reduced by low Pfr/P (Satter and Wetherell, 1968; Kasperbauer, 1971; Casal, Aphalo and Sanchez, 1987) even when the leaves had reached full expansion before the beginning of treatments (Casal and Aphalo, 1989).

Strong evidence in favour of the occurrence of these responses in the field as a result of different R:FR caused by canopy density is only available for apical dominance in grasses (tillering) and shoot extension growth. This evidence was gathered (a), by simulating the natural changes in R:FR ratio under controlled or semi-controlled conditions using the WL+FR technique (e.g. Morgan and Smith, 1978; Casal, Sanchez and Deregibus, 1987a,b); (b), by growing plants outdoors at different densities, i.e. exposed to different R:FR and partially reverting the low R:FR by means of localized irradiations with low fluence rate R (Deregibus, Sanchez, Casal and Trlica, 1985; Casal, Sanchez and Deregibus, 1986; Ballare, Scopel and Sanchez, 1989); or (c) by growing plants outdoors at different densities, i.e. different R:FR ratios caused by FR reflection, before the PAR available per plant is significantly reduced as a result of mutual shading (Casal, Sanchez

and Deregibus, 1986; Ballare, Sanchez, Scopel and Ghera, 1988).

#### INTERACTION BETWEEN LIGHT SIGNALS AND PLANT RESPONSES

The first signals of impending competition by light can anticipate competition itself in growing canopies. As mentioned above, the R:FR ratio of the light parallel to the soil surface is reduced by selective reflection from neighbour plants, and this signal can reach the internode of dicotyledonous plants before their leaves became shaded (Ballare et al., 1987; Fig. 6.1). Subsequently, along with the development of the canopy, individual leaves are likely to become shaded by neighbouring foliage for short periods of time (e.g. 2-3 h per d). Laboratory and field experiments indicate that dicotyledonous seedlings are capable of exhibiting rapid responses to these signals (e.g. Ballare et al., 1987; Child and Smith, 1987; Chapter 2). These data also suggest that the magnitude of the response would be correlated to the extent of the likelihood of future shading, since the lower the R:FR and the longer the duration of the R:FR depression, the larger the response and, when the leaves became temporarily shaded the response is more persistent. Similarly, grass plants show large responses (e.g. tillering) to small reductions in the R:FR ratio of the incident radiation below the values typical of sunlight, even if these changes in R:FR are restricted to the base of the tillers (Casal, Sanchez and Deregibus, 1987b) that, in many cases, is the part of the shoot more likely to become early shaded by neighbours. A situation where the lower leaves and internodes are shaded, and hence receive low R:FR, but the upper leaves are almost fully exposed to sunlight, could be very prolonged in time if the canopy is even. Shade-avoiding reactions would be expected to continue under those circumstances. However, would a fully shaded plant of a shade-avoiding

species continue responding to the low R:FR it receives? A definite answer cannot be given at present. However, the evidence to hand suggests that the responses would be poor compared to that of a plant more exposed to sunlight (Smith and Hayward, 1985; Casal, Sanchez and Deregibus, 1986; Figs 3.6, 3.7 and 4.6).

Green plants can show relatively large responses to early signals of competition. Can they distinguish between these signals and the perturbations of R:FR found at the extremes of the photoperiods? Field evidence is still needed in order to obtain a definite answer; nevertheless, the observation that under controlled conditions low PAR levels, or darkness, severely reduce the ability of mustard seedlings to respond to a reduction in R:FR (Figs 3.6, 3.7, 4.6, 4.7, 4.8, 4.9) strongly suggest this possibility.

#### ADAPTIVE VALUE OF PHYTOCHROME MEDIATED RESPONSES TO CANOPY DENSITY LIGHT SIGNALS

Sensu stricto, the demonstration that phytochrome mediated responses to canopy density light signals have adaptive value in nature requires evidence that such responses increase the probability of passing genes to the next generation. Such evidence is not available since, to the best of our knowledge, the relevant experiments have not been conducted. However, there is an increasing body of circumstantial evidence. This issue can be divided into two main questions: (a), are longer shoots or an increased apical dominance an advantage for plants at risk to becoming heavily shaded in the near future? (b), are plants with shorter shoots and more profusely branched more successful in open habitats? If the answer were negative to any of these questions, then the plasticity provided by phytochrome mediated responses would be useless to the plant. However, this does not appear to be the case.



Longer shoots reduce the chance of a plant becoming heavily-shaded by its neighbours (Ludlow, 1978). Similarly, a reduced tillering rate, particularly in those grasses where branches arise at the base of parent shoots, reduces the investment of resources in organs placed in the lower strata of the canopy and this could increase the resources available for existing shoots (Smith and Rogan, 1980; Casal, Sanchez and Deregibus, 1987b). In wheat, for instance, the inhibition of tillering is stronger for the higher order tillers i.e. those tillers that appear later and have less chance to survive (Casal, 1988). Some degree of anticipation to real shade in these responses would appear advantageous since "when plants are competing for light, a slight difference in tallness can give one plant a significant advantage, which with time "snowballs" until the shorter plant is totally suppressed" (Newman, 1973; see also Black, 1960, and Ross & Harper, 1972).

High rates of tillering would favour plants growing in the absence of other nearby plants, since growth rate is theoretically exponential if resources are not seriously limiting and every branch is able to give place to new branches (the  $\Delta$  tiller per tiller rate remains constant). Shorter shoots could allow a larger allocation of resources into photosynthetic organs and reduce the impact of wind and the accessibility to large herbivores. It is interesting to note that when different genotypes of grasses are compared, larger yields are correlated in open habitats with the ability to produce new tillers, and, in closed stands, with the growth capacity of individual tillers (Nelson and Zarroug, 1981; Sugiyama *et al.*, 1985). Since high R:FR increase tillering, and low R:FR increases tiller extension growth, the suggestion that photomorphogenic responses help grass plants adjust to canopy density appears to be justified (Casal, Sanchez and Deregibus, 1987).

## CONCLUSIONS

1) R:FR provides a reliable signal of canopy density as it is only slightly affected by factors other than the presence of neighbours, even before these neighbours shade the plant under consideration. The reductions of R:FR during twilight are accompanied by low fluence rates, hence, low R:FR together with high fluence rates is a signal apparently unique to impending competition.

2) Plants of many species are able to perceive and respond to these R:FR signals, and are also able to regulate (at least in some cases) their responsiveness according to the fluence rate they receive. The latter makes more unlikely large responses to a reduction in R:FR not related to the presence of neighbours.

3) Circumstantial evidence suggests that these responses would increase the probability of survival under natural conditions.

4) In addition to the regulation of the responsiveness to R:FR, other fluence rate dependent responses occur at least in de-etiolated plants of some species, but these responses are not necessarily shade-avoidance reactions. Some fluence rate dependent processes, presumably mediated through photosynthesis, and/or a reduction in [Pfr] or Pfr/P, promote extension growth, others mediated by a B-absorbing photoreceptor and also perhaps by phytochrome cycling, inhibit growth. The balance among these opposite reactions will have to be studied under a range of conditions before the function of fluence rate dependent responses of de-etiolated seedlings in nature is understood.

Table 7.1. Pfr/P measured in highly purified samples of oat phytochrome placed in a cuvette within a black, ice cooled box provided with a window facing a stand of mustard seedlings. Data are means of 3 determinations  $\pm$  standard deviation.

Sample	measured Pfr/P
Facing a stand of mustard	0.594 $\pm$ 0.022
In the same position, but after removing the stand	0.673 $\pm$ 0.006
Fully exposed to sunlight (i.e. out of the black box)	0.663 $\pm$ 0.030

Table 7.2. First internode  $\Delta$  length in 12-d-old mustard seedlings transferred from  $90 \mu\text{mol m}^{-2} \text{s}^{-1}$  of continuous WL to different fluence rates of SOX for a 24 h period. Data are means of 4 independent sets of 9 plants each  $\pm$  standard deviation.

Fluence rate of SOX ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	First internode $\Delta$ length (mm)
57	5.22 $\pm$ 0.96
83	4.28 $\pm$ 0.46
179	3.62 $\pm$ 0.58

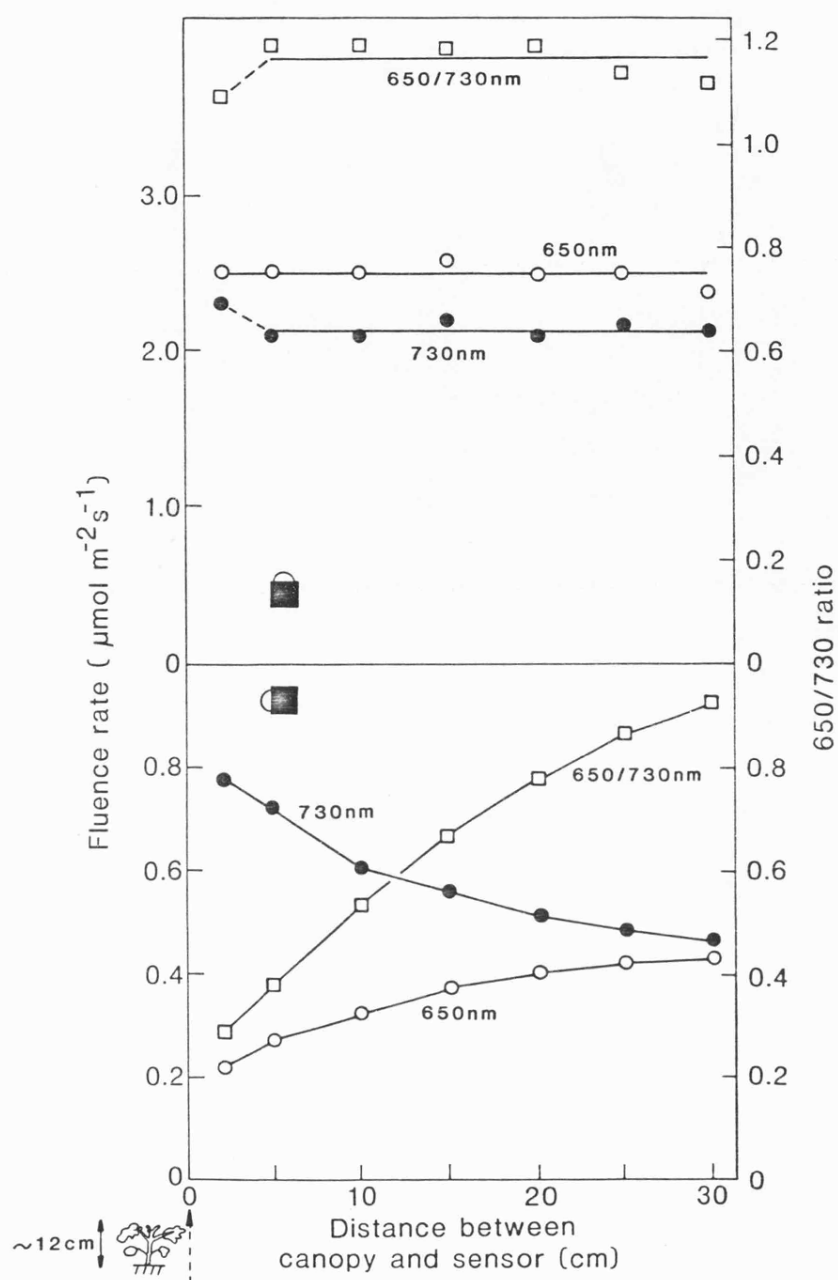


Fig. 7.1. Fluence rate at 650 and 730 nm and 650:730 quantum flux ratio of the light reaching a cosine-corrected planar sensor placed parallel to the soil surface (top) or facing a green mustard fence (bottom) plotted against the distance between the sensor and the fence. Data were taken in Leicester on 7 September 1988 between 9:00 and 9:30 a.m. The stand was orientated east-west and measurements were taken at the sunlit side of it.

## CHAPTER 8

THE PHYSIOLOGY OF THE PHOTOMORPHOGENIC CONTROL OF  
INTERNODE EXTENSION GROWTH IN MUSTARD.

## INTRODUCTION

The aim of this last chapter is to highlight the significance of the observations reported in this thesis in relation to the search for clues about the mechanisms involved in the control of extension growth by light in the mustard seedling.

### GROWTH RESPONSES TO THE FLUENCE RATE OF THE LIGHT

In a relatively recent review, Mohr (1984) mentioned the lack of evidence for the existence of responses to the fluence rate of the light in fully de-etiolated seedlings (eg mustard seedlings in which the first or subsequent internodes are in expansion, and the hypocotyl is fully or almost fully expanded). Several such responses were revealed during the present investigations. Some of them involved a promotion of internode extension growth, others a reduction of internode extension rate. Apparently, the manifestation of these responses depends on the particular experimental conditions.

First, growth was promoted by increasing the fluence rate of WL. This promotion became evident as follows: (a), after 13 d under continuous WL, internodes were longer with increasing fluence rates of WL (Fig. 4.6, inset); (b), when these seedlings were transferred from continuous WL to D (after a R or a FR pulse), internode growth continued to be faster in plants pretreated with high fluence rate WL (Fig. 4.6) ; (c), the transition from continuous WL to D caused a rapid (lag <10 min) decrease in growth rate (Fig. 4.2).

Secondly, the promotion of internode extension rate by low Pfr/P established in the internode alone was enhanced by the fluence rate of B received by the leaves, as long as the fluence rate of B was above a threshold level (Fig. 3.7).

Thirdly, pretreatments with higher fluence rates of B

reduced the extension growth of the internode in subsequent D (after either a R or a FR pulse) (Figs 5.1, 5.5).

Fourthly, the end-of-day response to a reduction in Pfr/P (a promotion induced mainly in the leaves) was increased by increasing fluence rates of WL pretreatments (Fig. 4.6). In addition, for a given period in D, growth rate was higher if Pfr/P was reduced during, rather than at the end of continuous WL (Fig. 4.9), and at the end, of continuous WL rather than later (Fig. 4.8).

Fifthly, increasing the fluence rate of B-deficient light reduced internode extension in 24 h periods (Table 7.2). Very little detail is available about this response at present.

The photoreceptors of some of these responses were partially identified. The enhancement by B of the promotion of internode growth caused by low Pfr/P provided to the internode alone, and the inhibition by B pretreatments of internode growth in subsequent D, are due to a specific B-absorbing photoreceptor (ie neither phytochrome nor the photosynthetic pigments) located in the leaves (see Chapters 3 and 5). This is important since it indicates that specific B-absorbing photoreceptor(s) are active in mustard not only during the processes of de-etiolation but also once the seedling is de-etiolated. Photosynthetic pigments could be responsible for the promotion of internode extension growth by WL (Fig. 4.2 and 4.6 inset). At least this is very likely the case for long term experiments involving different fluence rates of WL (Fig. 4.6 inset). The modulation by WL of the responsiveness to Pfr/P in subsequent D appears not to be mediated by a B-absorbing photoreceptor (Figs 5.1 and 5.2). It could be either a regulatory effect of photosynthesis, for which there is very little evidence in photomorphogenesis so far (Mohr, 1984) or processes mediated by a parameter of phytochrome that is dependent on fluence rate (eg



"cycling", Johnson and Tasker, 1979). Fluence rate dependent, phytochrome-mediated modulation of the responsiveness towards phytochrome occurs in mustard seedlings during de-etiolation (Mohr and Schäfer, 1983; Johnson and Whitelam, 1983). A similar sort of phytochrome-mediated response could explain the inhibition of growth by increasing fluence rates of B-deficient light during a 24 h period (Table 7.2).

#### THE IMPORTANCE OF THE LIGHT REACHING THE LEAVES

Some of the responses described in this thesis highlight the importance of the leaves in the control of internode growth. In the leaves, a reduction of Pfr/P triggers a persistent promotion of internode growth (Fig. 2.7). Blue light reaching the leaves modulates the extent of response to the Pfr/P established in the growing internode (Fig. 3.7 Table 3.1). B reaching the leaves during light pretreatments inhibits the subsequent growth in D (Fig. 5.5). Other responses reported here could be induced in the leaves, such as the promotion of internode growth by WL (cf the kinetics of the growth reduction after switching-off WL [Fig. 4.2] and after cutting the internode at the point of insertion of the leaves [Fig. 6.9]).

#### THE INVOLVEMENT OF CHANGES IN PEROXIDASE ACTIVITY IN THE CONTROL OF INTERNODE GROWTH

In Chapter 6, the correlation (organ localisation and kinetics of the changes) between phytochrome effects on internode extension growth and extracellular peroxidase activity is described (Figs 6.1, 6.3). Wounding the internode affected the rate of extension growth but modified the activity of a different peroxidase isoform than the one affected by phytochrome (Figs 6.9, 6.11). In contrast, B pretreatments affected the subsequent internode growth in D, but caused no obvious effects on

extracellular peroxidase activity (Chapter 5, Table 6.1). These results are consistent with the hypothesis that changes in extracellular peroxidase activity are involved in the control of internode growth by phytochrome in mustard. They could also be useful steps in order to understand the function of different peroxidase isoforms, and the ways in which phytochrome works in light-grown plants.

### CONCLUSIONS

Several responses were revealed and characterised in terms of kinetics and localisation of the organs where they are induced. In some cases the photoreceptor was also identified. These results underline: (a), the importance of the light reaching the leaves in the control of internode growth; (b), the ability of mustard plants to continue responding to previous light signals, probably because these signals can be accumulated in some step(s) of the transduction chains; and (c), that photoreceptors capable of measuring light quantity (including specific B-absorbing photoreceptor[s]) are involved in the photomorphogenic control of internode growth in light-grown mustard. In addition, a hypothesis at a more biochemical level is proposed, on the basis of experimental results, to explain at least partially the responses to Pfr/P. It must be admitted, much work remains to be conducted. Nevertheless, in my opinion, the experiments reported here contribute to the formation of a more solid basis on which subsequent attempts to investigate physiological aspects of the control of stem growth by light could step on.

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PHOTOCONTROL OF INTERNODE EXTENSION GROWTH IN  
Sinapis alba L.

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This study is concerned with the responses of internode extension rate in white-light-grown Sinapis alba seedlings to light: the kinetics, nature and organ localization of the photoreceptors and possible transduction chains. Phytochrome status was modified either by means of red, or far-red, light pulses given at the end of the photoperiod, or by supplementing white fluorescent light with different fluence rates of far-red light during the photoperiod. The status of specific blue light-absorbing photoreceptor(s) was modified by supplementing a background of blue-deficient light with different fluence rates of blue light.

Low Pfr/P established in the leaves induced a promotion of internode extension rate that persisted for ca 24 h after the plant returned to high Pfr/P. The effect of Pfr/P during dark periods is due mainly to this persistent response. Several fluence-rate-dependent responses were revealed: (a), blue light reaching the leaves modulates the responsiveness of the internode to a reduction of Pfr/P restricted to the internode itself; (b), blue light reaching the leaves inhibits internode extension in subsequent darkness; (c), for a given period in darkness, internode growth rate is higher if Pfr/P is reduced before, rather than at the beginning of darkness, or at the beginning of darkness, rather than later; (d), the transition from light to darkness causes a rapid reduction (lag < 10 min) of internode extension rate.

A correlation (kinetics and localization) between the extracellular activity of one moderately acidic peroxidase isoform extracted from the internodes, and the effect of Pfr/P on internode extension rate is presented. The activity of another extracellular peroxidase isoform was unaffected by Pfr/P, but showed a rapid increase after wounding. This treatment caused no significant effects on the isoform controlled by Pfr/P.

Present findings are discussed in relation to the ecological significance and the physiological basis of the control of internode extension growth by light.