

The Photocontrol of Stem Elongation
in Light-Grown Sinapis alba L.

by

Richard Child.

Abstract

An investigation has been made into the mechanisms involved in the photocontrol of stem elongation in the ruderal species, Sinapis alba L.; particularly those mechanisms concerned with the changes in stem elongation mediated by variations in the degree of vegetational shade. Throughout the investigation an attempt was made to grow plants in conditions which were as natural as possible; i.e., in white light at a suitable fluence rate and with a 16h photoperiod.

The effects of white light fluence rate and red:far-red ratio (R:FR), on hypocotyl and internode elongation are compared and contrasted; but the main thrust of the thesis is an examination of the rapid increase in internode elongation rate which occurs in response to reductions in R:FR perceived by the internode itself. The R:FR was reduced by giving supplementary far-red light directly to the internodes of plants in background white light, and linear displacement transducers were used for the high-resolution, continuous monitoring of elongation.

The elongation rate was inversely and linearly related to the phytochrome photoequilibrium, set up by the light treatment, over a wide range of values. Moreover, phytochrome cycling rate is not involved in modulating internode elongation, and elongation rates can only be related to Pfr concentration if the total amount of phytochrome is constant.

A similar rapid response to supplementary far-red has been demonstrated in isolated internode segments, and this has allowed a study of the effect of R:FR on cell enlargement. From this study an hypothesis is proposed that suggests phytochrome modulates cell enlargement by regulating the secretion of protons into the cell wall. Supplementary far-red could cause a lowering of cell wall pH, which may bring about the displacement of Ca^{2+} from molecules involved in maintaining the rigidity of the wall.

2007/20

THE PHOTOCONTROL OF STEM ELONGATION IN

LIGHT-GROWN SINAPIS ALBA L.

by

Richard Child

**Thesis submitted to the University of Leicester
for the degree of Doctor of Philosophy**

1984

**Department of Botany
University of Leicester
University Road
Leicester, U.K.**

UMI Number: U350039

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



UMI U350039

Published by ProQuest LLC 2015. Copyright in the Dissertation held by the Author.
Microform Edition © ProQuest LLC.

All rights reserved. This work is protected against
unauthorized copying under Title 17, United States Code.



ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

THE PHOTOCOPY OF STEIN ELIASSON IN

LIGHT-GROWN SINAPIS ALBA L.



Richard Child

Thesis

24.2.1985

This is submitted to the University of Leicester
for the degree of Doctor of Philosophy

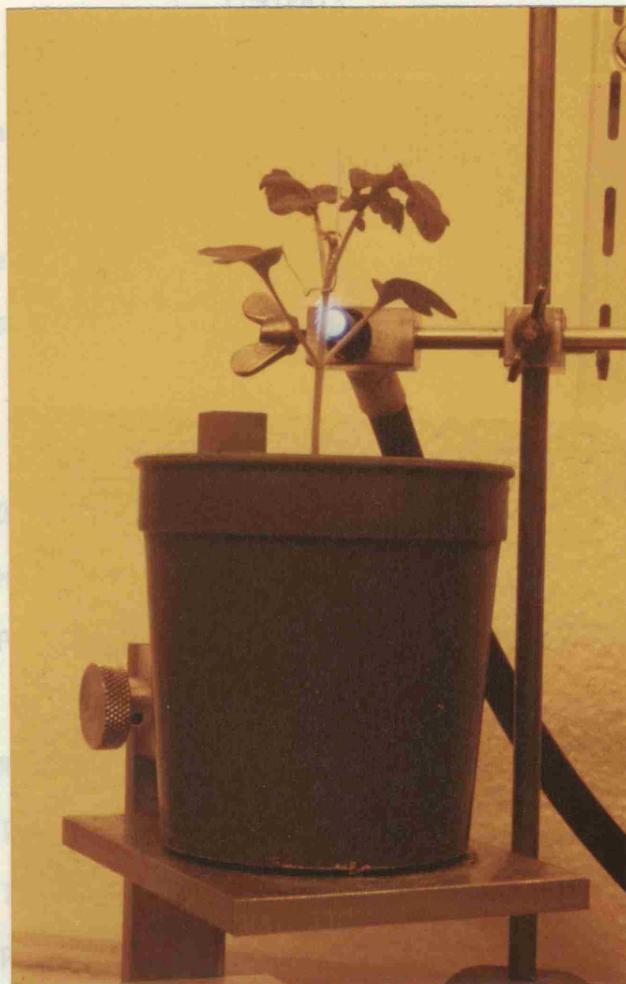
1985

Department of Biology
University of Leicester
University Road
Leicester, U.K.

CONTENTS

Acknowledgements
 Abbreviations
 List of figures
 List of Tables
 Abstract
 Quotation
 GENERAL INTRODU
 SECTION I. Th
 Introduction

Page No
 vi
 vii
 x
 xiii
 xiv
 xv
 1
 4
 4
 4
 5
 8
 12
 12



(1) The Blue Light Photoreceptors (B-Photoreceptors)

The irradiation of the first internode of a 14 day old Sinapis alba plant with supplementary blue light (λ max 452 nm) in background light from a low pressure sodium discharge lamp (λ max 585 nm). The background light, λ max 585 nm, has, essentially, the same effect as red light, with regard to the photoconversion of phytochrome, and is considered as such in the text.

The photograph illustrates the basic technique for analysing the photocontrol of internode elongation in intact plants in this thesis: the plant is mounted in transducer apparatus 2; normally there were two fibre-optic probes, mounted 10 mm from the internode and at 180° to each other.

CONTENTS

	<u>Page No</u>
Acknowledgements	vi
Abbreviations and Definitions	vii
List of Figures	x
List of Tables	xiii
Abstract	xiv
Quotation	xv
GENERAL INTRODUCTION	1
SECTION 1 THE PHOTOCONTROL OF STEM ELONGATION IN INTACT PLANTS	4
<u>Introduction</u>	4
R:FR as an Index of Vegetational Shade	4
The Effects of Fluence Rate on Development	5
The Effects of R:FR on Development	8
Photoreceptor Systems	12
(i) The Blue Light Photoreceptors (B-Photoreceptors)	12
(ii) The Photosynthetic Photosystems	14
(iii) Phytochrome	17
The Physiologically Active Form of Phytochrome	21
Experimental Rationale	27
<u>Materials and Methods</u>	
Plant Material	33
Stem Elongation Measurements	33
(i) Long-Term Measurements	33

(ii) Short-Term High Resolution Continuous Recording	33
Apparatus 1	34
Apparatus 2	37
Light Measurement	39
Light Sources	40
(i) Background Sources	40
(ii) Supplementary Sources	42
Phytochrome Measurement	42
Experimental Procedures	44
The Effect of WL Fluence Rate on Stem Elongation	44
The Effect of Added B on Stem Elongation	44
The Effect of R:FR on Stem Elongation	45
The Effect of Different FR Wavelengths on Stem Elongation	46
The Effects of End-of-Day Treatments on Stem Elongation	47
Statistical Treatment of Results	49
<u>Results and Discussion</u>	49
The Effect of WL Fluence Rate on Stem Elongation	49
The Effect of B on Stem Elongation	62
The Effect of R:FR on Stem Elongation	65
The Effect of Different FR Wavelengths on Stem Elongation	83
The Effects of End-of-Day Treatments on Stem Elongation	85

SECTION 2	THE PHOTOCONTROL OF ELONGATION IN ISOLATED	
	INTERNODE SEGMENTS	94
<u>Introduction</u>		94
	Cell Enlargement	94
	The Effect of Light on Cell Enlargement	95
	The Control of Cell Enlargement	96
	Proton Efflux	99
	Cell Wall Loosening	101
	Experimental Rationale	105
<u>Materials and Methods</u>		
	Plant Material	109
	The Transducer Apparatus	109
	The Light Sources	111
	Light Measurement	113
	Phytochrome Measurement	113
	Statistical Treatment of Results	114
<u>Results and Discussion</u>		
	Spontaneous Changes in Elongation Rate	115
	The Effect of R:FR on Elongation Rate	121
	The Effect of Medium pH on Elongation Rate	134
	The Interaction Between pH and FR	139
	The Effect of Ca ²⁺ and EDTA on Elongation	142
	The Role of Growth Regulators in the Response to Lowered R:FR	145
CONCLUSIONS		148
A WORKING HYPOTHESIS FOR THE PHOTOCONTROL OF STEM ELONGATION		156
Bibliography		157

Acknowledgements

I am grateful for the help and support of my Supervisor, Professor Harry Smith, throughout this project and I am indebted to the Science and Engineering Research council for granting me an "Instant Award".

I wish to thank Dr David Morgan, for his guidance during the early stages of the project, and Drs Bill Cockburn, Garry Whitelam and Mike Malone for valuable discussion.

Dave Halsall, Mike Jackson and Neal Harvey gave me excellent technical assistance.

Abbreviations and Definitions

ATP	: adenosine triphosphate.
B	: blue light.
bar	: unit of pressure, 10^5 newton per square metre.
°C	: degree Celsius.
cm	: centimetre.
D	: darkness.
d	: day.
DC	: direct current.
d.f.	: degrees of freedom.
Δ ER	: delta ER; difference in elongation rate.
$\Delta\pi$: delta pi; difference in osmotic potential.
EDTA	: ethylenediamine tetraacetic acid.
F	: value of the F-statistic.
FR	: far-red light.
g	: gram.
GA	: gibberellic acid; gibberellin.
h	: hour.
HIR	: high irradiance responses.
hv	: energy of a single quantum; h is Planck's constant, v is the frequency.
IR	: infra-red radiation.
KD	: kilodalton.
L	: hydraulic conductivity.
λ max	: lambda max; peak wavelength.
LVDT	: linear voltage displacement transducer.
\log_{10}	: logarithm to the base 10.
M	: cell wall extensibility.
m.s.	: mean square.

mRNA	: messenger ribonucleic acid.
μm	: micrometre.
$\mu\text{m}/\text{min}$: micrometre per minute.
$\mu\text{mol}/\text{m}^2 \text{ s}$: micromole per metre squared per second.
μM	: micromolar.
mm	: millimetre.
mg	: milligram.
mM	: millimolar.
min	: minute.
nm	: nanometre.
NADPH	: the reduced form of nicotinamide adenine dinucleotide phosphate.
N.S.	: not statistically significantly different.
PAR	: photosynthetically active radiation; fluence rate 400-700 nm waveband defined by McCree (1981).
Pr	: red absorbing isomer of phytochrome.
Pfr	: far-red absorbing isomer of phytochrome.
Ptot	: total phytochrome.
ϕ	: phi; phytochrome photoequilibrium (Pfr/Ptot).
ϕ_e	: estimated phytochrome photoequilibrium; estimated from the plot of ϕ v R:FR (see, Smith, 1982).
ϕ_m	: measured phytochrome photoequilibrium; measured directly in achlorophyllous tissue.
R	: red light.
R:FR	: red to far-red ratio; defined as: $\frac{\text{photon fluence rate in 10nm band centred on 660nm}}{\text{photon fluence rate in 10nm band centred on 730nm}}$
RNA	: ribonucleic acid.
S	: second.
S^2	: variance.

S.E. : standard error.
s.s. : sum of squares.
SPFR : spectral photon fluence rate.
t : value of the t statistic.
W : watt.
WL : white light.
Y : wall yield stress threshold.
* : statistically significant at the 5% level.
** : statistically significant at the 1% level.
*** : statistically significant at the 0.1% level.

List of Figures

1. A current view of the phytochrome system.	18
2. Diagram of transducer apparatus 1.	35
3. Diagram of transducer apparatus 2.	38
4. Relative spectral photon fluence rates for light sources.	41
5. Relative spectral photon fluence rates for light sources.	43
6. Scheme for the end-of-day treatment of <u>Sinapis alba</u> plants.	48
7A. The effect of fluence rate on stem elongation in <u>Sinapis alba</u> .	51
7B. The effect of fluence rate on stem elongation in <u>Sinapis alba</u> .	52
8. The effect of fluence rate on hypocotyl elongation in <u>Sinapis alba</u> .	53
9. The relationship between hypocotyl elongation and WL fluence rate in <u>Sinapis alba</u> .	54
10. The effect of fluence rate on first internode elongation in <u>Sinapis alba</u> .	55
11. The relationship between specific length and WL fluence rate for the first internode of <u>Sinapis alba</u> .	57
12. The short-term effect of a reduction in fluence rate on stem elongation in <u>Sinapis alba</u> .	60
13. The effect of supplementary B on stem elongation in <u>Sinapis alba</u> .	64
14. The effect of R:FR on hypocotyl elongation in <u>Sinapis alba</u> .	66
15. The relationship between hypocotyl elongation and ϕ_e in <u>Sinapis alba</u> .	67
16. The effect of R:FR on first internode elongation in <u>Sinapis alba</u> .	69
17. The relationship between specific length and ϕ_e for the first internode of <u>Sinapis alba</u> .	70
18A. The effect of R:FR on stem elongation in <u>Sinapis alba</u> .	71

18B. The effect of R:FR on stem elongation in <u>Sinapis alba</u> .	72
19. The rapid response of first internode elongation to supplementary FR in <u>Sinapis alba</u> .	73
20. The rapid response of first internode elongation to supplementary FR in <u>Sinapis alba</u> .	77
21. The rapid response of first internode elongation to supplementary FR in <u>Sinapis alba</u> .	78
22. The relationship between elongation rate and ϕ_m for the rapid response to supplementary FR in <u>Sinapis alba</u> .	80
23. The relationship between elongation rate and ϕ_m for the rapid response to supplementary FR in <u>Sinapis alba</u> .	81
24. The time course for the achievement of photoequilibrium.	82
25. The effect of supplementary FR of different wavelengths on elongation rate in <u>Sinapis alba</u> .	84
26. The effect of darkness on stem elongation in <u>Sinapis alba</u> .	86
27. The effect of end-of-day treatments on stem elongation in a subsequent photoperiod in <u>Sinapis alba</u> .	88
28. A comparison between the response of the intact <u>Sinapis alba</u> plant to supplementary FR and the response of excised segments to auxin.	106
29. The transducer apparatus for internode segments.	110
30. Relative spectral photon fluence rate for light sources.	112
31. Spontaneous changes in elongation rate in internode segments from <u>Sinapis alba</u> .	116
32. The effect of supplementary FR on elongation in excised internode segments from <u>Sinapis alba</u> .	117
33. The effect of medium composition on elongation in internode segments from <u>Sinapis alba</u> .	118

34. The effect of supplementary light on elongation in internode segments from Sinapis alba. 123
35. The effect of supplementary FR on elongation in internode segments from Sinapis alba. 124
36. The effect of supplementary FR on elongation in internode segments from Sinapis alba. 125
37. The effect of supplementary R on elongation in internode segments from Sinapis alba. 129
38. The relationship between elongation rate and ϕ_m in internode segments from Sinapis alba. 131
39. The effect of IR on elongation in internode segments from Sinapis alba. 133
40. The effect of a decrease in pH on elongation in internode segments from Sinapis alba. 135
41. The effect of a decrease in pH on elongation in internode segments from Sinapis alba. 136
42. The effect of pH on elongation rate in internode segments from Sinapis alba. 138
43. The interaction between supplementary FR and medium pH in internode segments from Sinapis alba. 140
44. The effect of CaCl_2 and EDTA on elongation in internode segments from Sinapis alba. 143
45. The interaction between supplementary FR and CaCl_2 in internode segments from Sinapis alba. 144
46. The effect of GA_3 on elongation in internode segments from Sinapis alba. 146

List of Tables

1. The short-term effect of changes in fluence rate on stem elongation in <u>Sinapis alba</u> .	58
2. The effect of supplementary B on stem elongation in <u>Sinapis alba</u> .	63
3. The decay kinetics for the rapid response of first internode elongation to supplementary FR in <u>Sinapis alba</u> .	75
4. The effect of end-of-day treatments on stem elongation in <u>Sinapis alba</u> .	90
5. A comparison between the kinetics for the response of the intact <u>Sinapis alba</u> plant to supplementary FR and the response of excised segments to auxin.	107
6. The effect of medium composition on the response to supplementary FR in internode segments from <u>Sinapis alba</u> .	122
7. The effect of supplementary light on elongation in internode segments from <u>Sinapis alba</u> .	126
8. Details of the light treatments for the experiments with internode segments from <u>Sinapis alba</u> .	130
9. The effect of IR on elongation rate in internode segments from <u>Sinapis alba</u> .	132

Abstract

An investigation has been made into the mechanisms involved in the photocontrol of stem elongation in the ruderal species, Sinapis alba L; particularly those mechanisms concerned with the changes in stem elongation mediated by variations in the degree of vegetational shade. Throughout the investigation an attempt was made to grow plants in conditions which were as natural as possible; i.e., in white light at a suitable fluence rate and with a 16h photoperiod.

The effects of white light fluence rate and red:far-red ratio (R:FR), on hypocotyl and internode elongation are compared and contrasted; but the main thrust of the thesis is an examination of the rapid increase in internode elongation rate which occurs in response to reductions in R:FR perceived by the internode itself. The R:FR was reduced by giving supplementary far-red light directly to the internodes of plants in background white light, and linear displacement transducers were used for the high-resolution, continuous monitoring of elongation.

The elongation rate was inversely and linearly related to the phytochrome photoequilibrium, set up by the light treatment, over a wide range of values. Moreover, phytochrome cycling rate is not involved in modulating internode elongation, and elongation rates can only be related to Pfr concentration if the total amount of phytochrome is constant.

A similar rapid response to supplementary far-red has been demonstrated in isolated internode segments, and this has allowed a study of the effect of R:FR on cell enlargement. From this study an hypothesis is proposed that suggests phytochrome modulates cell enlargement by regulating the secretion of protons into the cell wall. Supplementary far-red could cause a lowering of cell wall pH, which may bring about the displacement of Ca^{2+} from molecules involved in maintaining the rigidity of the wall.

"The erroneous belief that science eventually leads to the certainty of a definitive explanation carries with it the implication that it is a grave scientific misdemeanour to have published some hypothesis that eventually is falsified. As a consequence scientists have often been loath to admit the falsification of such an hypothesis, and their lives may be wasted in defending the no longer defensible. Whereas according to Popper, falsification in whole or in part is the anticipated fate of all hypotheses, and we should even rejoice in the falsification of an hypothesis that we have cherished as our brain-child".

J.C. Eccles: Facing Reality, p. 107.

GENERAL INTRODUCTION

S. alba L (white mustard) is a ruderal. According to Grime (1979), the ruderals are plants which have evolved a strategy that enables them successfully to colonise environments of low stress, but high disturbance (such as arable fields and pastures). Typically, they are annuals capable of rapid growth, and when they grow in close proximity the competition for resources may be intense. Unlike woodland herbs, which tolerate shading by other plants, the ruderals are shade-avoiders and rely on increased stem elongation to carry their leaves into superior positions in a canopy. Because of this, dramatic changes in stem elongation rate are a striking feature of a series of co-ordinated responses shown by ruderals (e.g., S. alba and Chenopodium album) in simulated vegetational shade (Morgan & Smith, 1976; 1979; Morgan et al, 1980; Child et al, 1981a). The aim of the experiments described in this thesis was to investigate the mechanisms involved in these shadelight-mediated changes in stem elongation rate in S. alba.

S. alba was originally introduced to Britain from the Mediterranean region as a crop plant, but is now a frequent weed of field borders. It has been the subject of a considerable amount of photomorphogenic research by H. Mohr and colleagues at Freiburg University, and H. Smith and his co-workers at Leicester University. In the experiments reported here, fully light-grown plants were used; and the light treatments were designed to represent natural conditions as far as possible. The first internode was chosen for examination, because although the hypocotyl is often used as an

experimental organ in research of this kind, its behaviour in emergent seedlings is not representative of subsequent stem elongation (see discussions of the HIR).

The Measurement of Stem Elongation

The study of stem elongation depends upon the accuracy and resolution of the measuring technique. In the nineteenth century Julius Sachs recognised that a high-resolution method of continuously monitoring stem growth may answer the often raised question of whether plants grow faster during the daytime or at night, and he built an "auxanometer" capable of continuously recording plant growth to within 10 μm (Sachs, 1882). With this he followed the diurnal growth fluctuations of Dahlia shoots, and found a daily maximum soon after sunrise, and a minimum just before sunset. Baranetzky (1897) modified this apparatus and described circadian rhythms in the stem elongation of Brassica rapa.

Throughout the twentieth century there have been many instruments designed to improve upon Sachs' efforts (e.g., Bose, 1929). Most of these have used a lever system (as in the Sachs auxanometer) which is subject to limitations, because of the inherent mechanical resistance (see, Idle, 1956). Other methods include the "contact auxanometers", where the plant closes a switch as it grows. The sensitivity of the "contact auxanometers" ranges from 20 μm (e.g., Ranson & Harrison, 1955) down to 0.5 μm (Idle, 1956). The advent of linear displacement transducers has, however, allowed even greater resolution, combined with convenience of use. Basically, a linear displacement transducer consists of a ferromagnetic rod, or armature, which moves through a series of wire coils in the outer

barrel of the transducer. The plant is attached to one end of the rod, so that as the stem elongates a DC signal is generated which is proportional to the distance grown. The signal may be amplified and fed into a recording device. Originally, transducers were used to measure elongation in rapidly growing organs with rates between 15-60 $\mu\text{m}/\text{min}$, for example: gherkin hypocotyls (Meijer, 1968; Gaba & Black, 1979), and maize mesocotyls (Vanderhoef et al, 1979). More recently measurements have been made on internodes of light-grown plants with growth rates of less than 10 $\mu\text{m}/\text{min}$ (Morgan & Smith, 1978a; Lecharny & Jacques, 1980; Morgan et al, 1980; 1981).

Here transducer systems have been developed which permit elongation rates of about 0.2 $\mu\text{m}/\text{min}$ to be continuously monitored in whole plants and isolated internode segments.

SECTION 1

THE PHOTOCONTROL OF STEM ELONGATION IN INTACT PLANTS

Introduction

For ruderals growing in an herbaceous stand, selective advantage will be conferred on those that are able to detect shading by others and rapidly to modify their stem elongation rate. To accomplish this, a plant must possess a photoreceptor system capable of discriminating between vegetational shade and other forms of shade, and of translating this information into the re-direction of cellular processes. A prerequisite of this is the existence of a parameter which is indicative of vegetational shade.

R:FR as an Index of Vegetational Shade

In vegetational shade a reduction in the fluence rate of photosynthetically active radiation (PAR) is accompanied by a relative increase in far-red (FR), because of the selective absorption of blue (B) and red (R) wavelengths by the photosynthetic pigments of canopy leaves (Vezina & Boulter, 1966; Woolley, 1971; Holmes & Smith, 1977b; Tasker & Smith, 1977). These are the main features of vegetational shade, but the situation is often complicated by contributions from reflected and scattered light, and sunflecks.

Only the reduction in R:FR is specifically reliable as an index of vegetational shade; reductions in PAR and B also occur in neutral shade, at night, and with cloud cover. A fully overcast sky can reduce fluence rate by more than 90% (Smith, 1982), but the R:FR of daylight is only slightly affected by cloud cover and averages about 1.15 throughout the year (Holmes & Smith, 1977a).

Studies on the variation of R:FR within a wheat canopy, in which arable weeds like S. alba may grow, show that small increases in the depth of the canopy are associated with relatively large decreases in R:FR. For example, the R:FR can drop from 1.15 above the canopy to 0.4 at 60 cm beneath the top (Holmes & Smith, 1977b, Holmes, 1981). The R:FR may continually be altered as a result of wind moving the leaves and allowing sunflecks of unfiltered light to enter the canopy. These transient sunflecks, and more permanent gaps in the canopy, predominantly are unfiltered sunlight and increase the R:FR (Yocum et al, 1964; Vezina & Boulter, 1966; Holmes & Smith, 1977b). Overcast conditions also increase the R:FR under canopies by augmenting the proportion of diffuse daylight entering through gaps.

The Effects of Fluence Rate on Development

The observations described above were made possible by technical advances in the measurement of light, but the impulse to make the measurements required a change in the theory of the effects of light on plant development. Despite the predictions of Sachs (1882) - that leaves may alter the spectrum of incident light, and that this could be important for plants growing in shade - the prevailing opinion

was, until recently, that plants responded to the reduced fluence rates found in shadelight. This was not without foundation, as in low light levels the rate of photosynthesis is reduced together with the amount of B, and B was considered most important in controlling stem elongation (see below). Consequently, the investigations into the effects of shading on plant growth carried out before 1970 often were concerned with changes in fluence rate.

Bjorkman (1981) describes plants as being "sun" or "shade" species. These are not two clearly delimited categories, but represent the opposite ends of a spectrum. The "sun" plants are species from open habitats and have the capacity for high rates of photosynthesis at high fluence rates, but at low fluence rates they are much less efficient. The level of photosynthetic saturation is, however, determined by the fluence rate in which the plants have grown. For example, Atriplex triangularis plants grown in fluence rates of 92,290 and 920 $\mu\text{mol}/\text{m}^2\text{s}$ PAR had corresponding saturation levels of 250,500 and 1500 $\mu\text{mol}/\text{m}^2\text{s}$ (Bjorkman et al, 1972), and similar results have been reported for S. alba (Boardman, 1977). In contrast to the "sun" plants, the "shade" species, which generally inhabit woodlands, are able to photosynthesise effectively at low light levels, but are incapable of high photosynthetic rates in full sunlight.

When "sun" and "shade" species are grown in low fluence rates both show acclimative responses, but the "shade" plants are able to adapt more fully. These responses include the maintenance of leaf area at the expense of leaf thickness, (e.g., Blackman & Wilson, 1951; Hughes & Evans, 1962; Morgan & Smith, 1981). "Shade" species are capable of directing a greater proportion of their photosynthate

into the formation of leaf material than are "sun" species. At low light levels "sun" plants are also handicapped by having higher dark respiration rates than "shade" plants and are, therefore, less able to conserve their resources (Mahmoud & Grime, 1974).

Research on the effects of fluence rate on stem elongation has yielded different results. Grime & Jeffrey (1965) grew a range of species in plastic cylinders designed to give different degrees of neutral shade; they found that plant height was dependent upon the species, and the stage of development. In general, plants frequenting grassland were found to grow tallest in low light levels. Grime & Jeffrey described the response as consisting of two phases: an initial phase of rapid hypocotyl elongation, largely dependent on seed storage capacity; and subsequent internode elongation relying on photosynthesis. In comparison with these results, a range of species from open, hedgerow and woodland habitats, transferred from high WL to a range of WL fluence rates, exhibited only small changes in elongation rate, but marked increases in specific stem length (length per unit weight); the plants were channelling their resources into stem elongation at the expense of stem diameter (Morgan & Smith, 1981).

The disparity between these results might be a consequence of the different methods used to reduce the fluence rate. Morgan & Smith measured the fluence rate and the spectral properties of their light sources, and maintained the same high R:FR in all treatments. Grime & Jeffrey did not provide any spectral data, and presumably assumed that the plastic cylinders only reduced the fluence rate. The plastic they used is not specified, but some neutral density filters (e.g., Kodak Wratten) transmit relatively more FR than visible

wavelengths, thereby decreasing the R:FR; so Grime & Jeffrey may have also modified light quality in their experiments. Another explanation is that the height of the plants in the experiments of Grime & Jeffrey was determined by hypocotyl and internode elongation, while Morgan & Smith only followed internode elongation. It is possible that fluence rate has different effects on hypocotyl and internode development.

A number of workers using linear displacement transducers have described rapid responses to changes in the fluence rate of WL. In de-etiolated gherkin, an increase in WL caused a rapid decrease in hypocotyl elongation rate, because of the increase in B fluence rate (Gaba & Black, 1979). Lecharny & Jacques (1980) demonstrated a progressive inhibition of internode elongation in light-grown Vigna sinensis by an increase in WL fluence rate, but could not attribute the response to any specific wavelength. Similarly, Morgan & Smith (1978a) found that an increase in WL from 25-119 $\mu\text{mol}/\text{m}^2$ led to a 25% decrease in the internode elongation rate of C. album, and a reversal of the treatment produced a corresponding increase. The lag times for these changes in growth rate in C. album were about 12 min, but no photoreceptor was suggested.

The Effects of R:FR on Development

The realisation that vegetational shade involves a decrease in R:FR has initiated the recent upsurge in research into the effects of light quality on morphogenesis. R:FR has comparatively less influence on leaf development than fluence rate, but substantially modifies stem elongation.

Leaf area was reduced at low R:FR values in Rumex obtusifolius (McLaren & Smith, 1978), and slightly reduced in C. album (Morgan & Smith, 1981). Specific leaf area, however, is unaffected by R:FR in C. album (Morgan & Smith, 1981; Child et al, 1981b).

In plants from open habitats a reduction in R:FR causes a dramatic increase in stem elongation rate, but in shade species (e.g., Mercurialis perennis and Teucrium scorodonia), the response is very much smaller (Morgan & Smith, 1978b; 1979; Child et al, 1981b). This stimulation of stem elongation by low R:FR has been observed when a few minutes of supplementary FR is given at the end of the photoperiod (Downs et al, 1957), but is greatest when the low R:FR is sustained throughout the light period (Morgan & Smith, 1978b).

Using linear displacement transducers, Morgan & Smith (1978a) demonstrated that the addition of FR to background WL elicited a rapid increase in stem elongation rate, with a lag of 7 min in C. album, and 13.8 min in S. alba (Morgan et al, 1980). These rapid responses to changes in R:FR are cogent evidence for light quality affecting cell enlargement, rather than cell division, at least in the initial stages of the response. When fibre-optic probes were used to deliver supplementary FR to individual organs of S. alba plants growing in background WL, Morgan et al (1980) discovered that FR given to the first internode of 14 day old plants caused an increase in elongation rate with a lag of 10-15 min. This increase involved an initial large, transient increase in elongation rate, followed by a rapid decline to a steady-state level, about double the original WL rate. If FR was given to the internode for 4 h or more there was a third, more gradual increase in elongation rate. On switching off the FR, the rate declined over about 15 min to a

constant level, still higher than the original WL rate. Additional R gave only a transient increase in elongation rate after a lag of 10-15 min and this persisted for only 25-30 min, after which the elongation rate returned to the rate observed in WL. If, however, R was given simultaneously with FR, the FR effect was negated; indeed the elongation rate could be modulated by adding or removing R in continuous WL + FR.

The irradiation of leaves with FR showed no short-term response, but after 3h of continuous irradiation an increase in elongation rate of the internode was detected. When the FR was switched off there was a gradual decline in elongation rate.

These results establish that there are two separate effects of R:FR on internode elongation. First, a rapid response to low R:FR, detected by the internode itself, which can continually be modulated. Second, a slower change brought about by photoperception in the leaves and transmitted to the internode. There are, however, a number of problems with the responses described by Morgan et al. The first is that following FR given to the stem, a return to WL resulted in a rapid decline in elongation rate, but to a level still higher than the original WL rate. There will be little selective advantage conferred by a response to a reduction in R:FR, if it cannot rapidly be "switched off" once the R:FR has increased. Moreover, the results with simultaneous additional R and FR suggest that rapid modulation does occur. A possible explanation is that in positioning the fibre-optic probes 20 mm from the internode the leaves were also irradiated. This would explain the second increase described for the internode response after 4h, and the persistence of a higher elongation rate when the FR was removed. A second

problem is the response to R alone, which was not explained by Morgan et al.

Part of the aim of the experiments described in this section was to re-examine the nature of the rapid response of stem elongation to FR given to the internode.

Photoreceptor Systems

Green plants possess at least three photoreceptor systems, each with different qualifications to be suitable as a photoreceptor for the light quantity and light quality effects described above.

(i) The Blue Light Photoreceptors (B-Photoreceptors)

Many plants exhibit responses to B, and the function of B in phototropism is well-documented (e.g., Presti & Delbruck, 1978), but the evidence for a distinct B effect on non-directional stem elongation is also substantial. Characteristically, B responses have an action maximum in the region of 350-500 nm and there are three candidates for the role of photoreceptor: carotenoids, flavins and a flavin-cytochrome complex localised in the plasma-membrane (see Presti & Delbruck, 1978; Briggs & Iino, 1983). The ability of a plant to perceive the fluence rate of B will not allow vegetational shade to be distinguished from neutral shade (e.g., cloud cover), as reductions in B fluence rate are common to most forms of shade.

In the latter years of the nineteenth century B was shown to inhibit stem elongation. This inhibition could not be attributed to any photosynthetic effect, because in R, where photosynthesis is most efficient, plants were found to become very elongated (Sachs, 1882; Palladin, 1914). These results generally were confirmed by a number of large-scale experiments in which cultivated plant species were

grown under glass transmitting narrow wavebands of light (e.g., Popp, 1926).

More recently, B inhibition of stem elongation has been described in a number of plants (see, Meijer, 1968; Black & Shuttleworth, 1974; Gaba & Black, 1979; Cosgrove, 1981; 1982; Thomas, 1981). The lag times for these responses are rapid (i.e., 10 seconds to a few minutes) and much shorter than responses to other wavelengths (Meijer, 1968; Gaba & Black, 1979; Cosgrove, 1982). Furthermore, Black and Shuttleworth (1974) demonstrated that in cucumber the site of perception for B resides in the hypocotyl itself, whereas R and FR are perceived by the cotyledons. Although this is compelling evidence for a specific B- photoreceptor, most of the results were obtained using dark-grown, or recently de-etiolated, seedlings, and the position in fully light-grown plants is less clear. This is especially true for S. alba. Wildermann et al (1978) found that in light-grown S. alba seedlings, hypocotyl inhibition only occurred in R, even though B inhibited elongation in dark-grown plants. The change was attributed to the loss of the HIR and they concluded that there is no need to implicate a B-photoreceptor controlling hypocotyl elongation in this species. Using a linear displacement transducer system to analyse the responses of dark-grown S. alba to B, R and FR, Cosgrove (1982) has shown that a specific B-photoreceptor is involved in controlling hypocotyl elongation, but that inhibition requires the B to be continuous. On the basis of this Cosgrove suggests that the experimental procedure followed by Wildermann et al may have been responsible for them missing the specific B response (plants were given brief end-of-day treatments with monochromatic light, and growth measurements were made, at 3h intervals with a ruler).

(ii) The Photosynthetic Photosystems

Chlorophyll-absorbed light may influence plant development by governing the rate of carbon dioxide fixation and the formation of ATP and NADPH.

The amount of assimilate formed by photosynthesis limits growth directly and photosynthesis may also affect the rate of translocation of assimilates. A number of studies have shown that the export of sugars from source leaves declines when the fluence rate is reduced. Moorby et al (1963) found that the rate of sugar export from soyabean leaves doubled when they were illuminated, and decreased immediately in darkness. In prolonged darkness the initial decrease was followed by a further progressive decrease. Also in soyabean, the ratio of assimilate transported upwards to that moved downwards is affected by fluence rate (Thrower, 1962). At low light levels basipetal translocation is increased. These effects may be dependent on the rate of carbohydrate production (Servaites & Geiger, 1974), or, alternatively, on the synthesis of ATP. Hartt (1965) observed that the basipetal translocation of sugars from maize leaves was saturated at lower light levels than saturated photosynthesis, and she proposed that translocation was dependent on the synthesis of ATP from cyclic photophosphorylation. Hartt (1966) also showed that this basipetal transport was stimulated by B and R wavelengths more than WL, and that FR and darkness reduced the rate. Because of the wide emission characteristics of the lamps used, Hartt could not predict which pigment was involved.

Plaut and Reinhold (1969) have proposed that ATP from non-cyclic photophosphorylation may promote phloem loading; and reports are accumulating that suggest phloem loading involves a carrier which co-transporters protons and a neutral solute (e.g., sucrose) (Hutchings, 1978a, b; Delrot, 1981). Light-activated, plasma-membrane proton pumps have been demonstrated in Nitella (Spanswick, 1974) and Atriplex (Luttge & Higinbotham, 1979) and there is evidence that the solute transport is related to the rate of ATP generation in the chloroplast (Raven, 1976).

The formation of ATP for these processes may only be a function of fluence rate, but it is feasible that light quality could also be involved. In Nitella, MacRobbie (1965) recorded different responses of Cl^- and K^+ uptake to FR, and proposed that Cl^- uptake was driven by non-cyclic photophosphorylation, whilst K^+ uptake relied on the amount of ATP available.

If light quality can modify the uptake of ions via photosynthesis, it may be a consequence of differential excitation of the two photosystems. At wavelengths upto 705 nm both photosystems are active, but between 705-730 nm it is largely photosystem I that is stimulated, and beyond 730 nm photosynthetic electron flow is negligible (Luttge & Higinbotham, 1979).

Non-cyclic electron flow involves the photosystems acting in series, and for maximum electron flow to occur there must be an equal supply of quanta to photosystems I and II. Carbon dioxide fixation requires ATP and NADPH in the ratio of 1:5, but it is unclear whether sufficient ATP can be formed from non-cyclic electron flow.

Whenever this ratio falls below 1:5 auxiliary reactions (e.g., cyclic photophosphorylation) are necessary. Cyclic photophosphorylation generates only ATP and may act as a "safety-valve" to avoid an excess of reducing agents. It is an efficient producer of ATP and is controlled by photosystem I; so it is sensitive to FR (see, Arnon, 1977). When R:FR is low, and the NADPH requirement of carbon fixation is satisfied, it is feasible that cyclic electron flow could provide ATP for solute transport. If the R:FR were subsequently to increase, then the ATP may be channelled into carbon reduction and the amount available for solute transport would be reduced. This effect would probably be transient, however, as the phenomenon known as the "carburetter effect", which has been described in algae and pea leaves, enables the photosystems to adapt to changes in spectral quality and to optimise the electron flow within a few minutes (see, Barber et al, 1981). MacRobbie (1965) did not indicate the kinetics of the response of ion exchange to different wavelengths. If the "carburetter effect" is widespread, then the statement of McCree (1981), to the effect that in most ecophysiological studies, PAR can be defined without considering the spectral sensitivity of the photosynthetic apparatus, is justified.

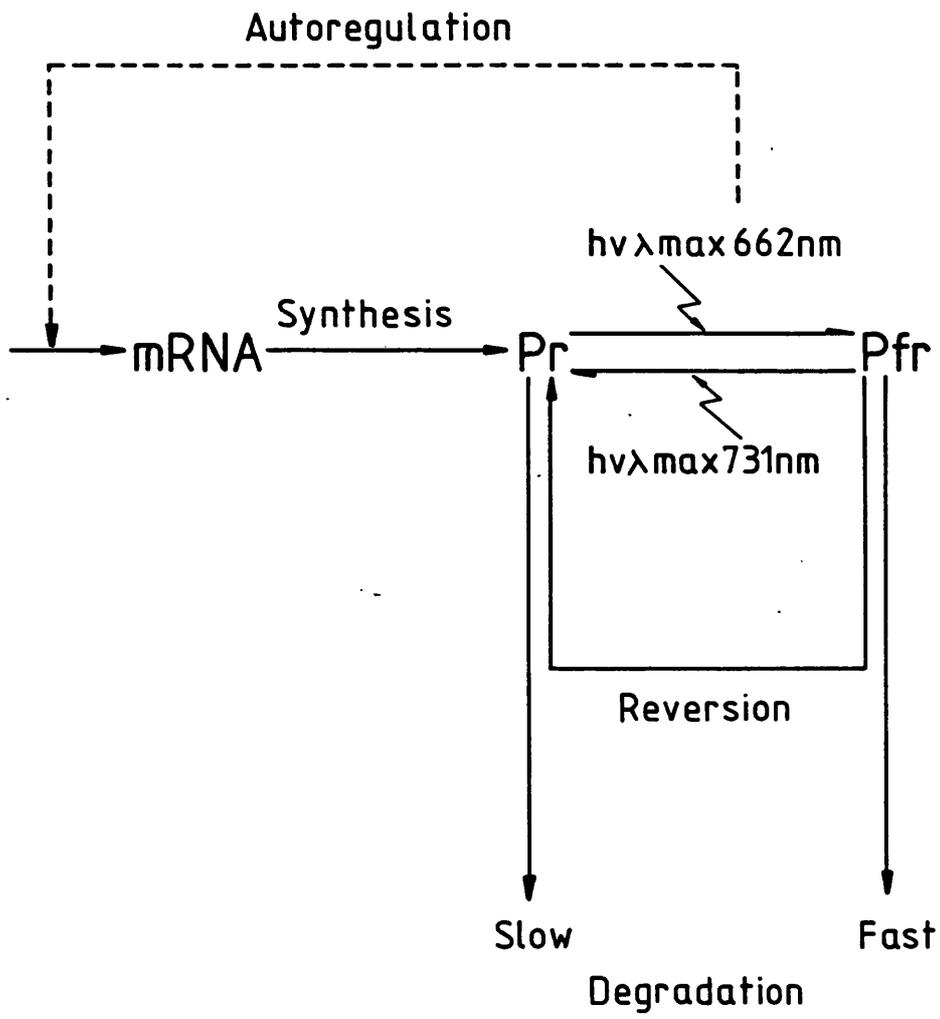
(iii) Phytochrome

Phytochrome is a soluble chromoprotein that has been detected in all taxonomic groups of green plants (Borthwick, 1972). The molecule is a linear tetrapyrrole chromophore, covalently linked to a polypeptide, and it varies in weight from 120-124 KD, depending upon the size of the polypeptide in different species (see, Pratt, 1982). Phytochrome exists as two stable isomers: Pr and Pfr. Pr absorbs light mainly in the R region of the spectrum (λ_{\max} 662 nm) and is converted to the Pfr form, which absorbs light in the FR region (λ_{\max} 731 nm) and is converted back to Pr. These absorption characteristics make phytochrome ideally suited to monitor the R:FR of incident radiation. The action spectra for the conversion of one form to the other overlap below about 730 nm, so that at any wavelength below 730 nm, or in broadband irradiation, the two forms are continuously interconverted and in dynamic equilibrium. This photoequilibrium is expressed as Pfr/P_{tot} (ϕ) and has a maximum value of about 0.80 (Butler et al, 1964). At photoequilibrium the relative proportions of Pr and Pfr are determined by the spectral distribution of incident radiation, the extinction coefficients of Pr and Pfr, the quantum efficiencies of the photoconversions, and also the thermal reactions. The thermal reactions include the synthesis and destruction of phytochrome, reversion reactions, and intermediate reactions between Pr and Pfr.

The basic scheme of the phytochrome system is shown in Figure 1. Phytochrome is synthesised de novo in the Pr form (Quail et al, 1973) and accumulates in the dark until a steady-state between Pr

Figure 1

A current view of the phytochrome system. This is a composite description of the system and certain components only have been demonstrated in particular groups of plants. For example, thermal reversion of Pfr to Pr has not been found in Monocotyledons and the Centrospermae, and the autoregulation of mRNA levels has been shown in oats only.



synthesis and degradation is reached. When plants are transferred to the light, a new level of phytochrome is established, which is only 1-3% that of the dark level; this is because Pfr is degraded about 100 times more rapidly than Pr (Quail et al, 1973). Kinetic studies of phytochrome destruction in Amaranthus caudatus and S. alba, suggest that there are two pools of phytochrome with different rates of degradation (Heim et al, 1981; Schafer, 1981). Approximately 90% of the phytochrome undergoes fast destruction, with a half-life of 2-5s at 25°C; the remaining 10% has a half-life of 20-60 min. Because of these destruction kinetics, the transition from darkness to light results in an 80% reduction in total phytochrome within 3h.

The return of plants to darkness would be expected to bring about an increase in the level of phytochrome, because Pr will accumulate, rather than being converted to Pfr and becoming subject to rapid degradation. This accumulation should be further enhanced by photoconverting Pfr to Pr at the onset of a dark period. Actual measurements have, however, revealed discrepancies. In light-grown oats, the phytochrome content increased to 6 times the original light level during a 24h dark period, and was 50 times higher after 48h (Hunt & Pratt, 1980). In plants given cycles of 12h photoperiods the increase was 3 times; or 4 times in plants given FR at the onset of darkness. These results were obtained using radio-immunoassay (the overriding absorption of light by chlorophyll in green plants precludes the use of spectrophotometry). Phytochrome levels have been measured by spectrophotometry in fully light-grown plants treated with the herbicide norflurazon, which allows chlorophyll to be bleached by light (see, Jabben & Holmes, 1983). In these plants, no increase in spectrophotometrically detectable phytochrome was

measured following 24h darkness, and there was only a small increase after 48h (see, Jabben & Holmes, 1983; Kilsby & Johnson, 1981; 1982). These differences originally were thought to be related to the sensitivities of the assay techniques and the different properties of the molecule which they measure. Radioimmunoassay has a much higher sensitivity than spectrophotometry, but it measures the amount of soluble phytochrome protein and not the chromophore. Using an enzyme-linked immunosorbant assay on crude extracts of oats, Shimazaki et al (1983) have shown that norflurazon inhibits phytochrome accumulation and have vindicated the measurements made by Hunt & Pratt. They have also demonstrated that in prolonged irradiation an increasingly smaller proportion of spectrally detectable phytochrome can be measured by the immunoassay. After 16h the spectrally detectable phytochrome reached a plateau at 2-3% the level observed in dark-grown tissue prior to irradiation. By 24h, however, the immunoassayed phytochrome had not reached a plateau, and the level was down to 0.1% of the dark-grown value. Shimazaki et al propose that there are two pools of phytochrome representing the products of different genes: one responsible for the synthesis of phytochrome in the dark; and the other determining phytochrome production in the light.

In addition to the synthesis and degradation reactions described above, there is in oats a feed-back control of phytochrome synthesis. The concentration of mRNA for phytochrome declines when dark-grown oat tissue is given R (Colbert et al, 1983), with a reduction of 20% within 2h. The effect can be reversed by FR and, therefore, phytochrome synthesis is itself under phytochrome control; and high R:FR will decrease phytochrome levels by increasing the amount of Pfr available for destruction and reducing

phytochrome synthesis. In extended darkness the depletion of Pfr allows a reversal of inhibition (Gottmann & Schafer, 1982). The level of regulation is at the steady-state concentration of mRNA rather than translation (Hershey et al, 1984).

Phytochrome photoequilibria depend upon an interplay between the photochemical and thermal reactions. At temperatures above 0°C, wavelengths of light which are comparatively inefficient at photoconverting phytochrome may not establish a true photoequilibrium. Under most natural conditions there are sufficient photons to drive phytochrome to photoequilibrium (Holmes & Wagner, 1980), but the extent to which this may represent a truly stable photoequilibrium is not known. With etiolated tissue exposed to natural radiation under canopies, and at twilight, photoequilibrium is reached between 10-15s, but values of phytochrome-absorbable radiation below $10 \mu\text{mol}/\text{m}^2\text{s}$ may not give a photoequilibrium (Schafer, 1981; Jabben et al, 1982). Also, at low temperatures, fluence rates above $1000 \mu\text{mol}/\text{m}^2\text{s}$ maintain high levels of intermediates between Pr and Pfr, and Pfr concentration will be irradiance dependent and lower than anticipated from the R:FR (Kendrick & Spruit, 1972).

The Physiologically Active Form of Phytochrome

Unlike other photoreceptors, phytochrome does not photosensitize molecules, nor is there any form of energy transduction involved in photoconversion; so the physiological activity must be a property of the isomers, the interconversion reactions, or possibly a relationship between the two.

The most popular view is that the Pfr isomer is the physiologically active form. This is based on correlations between spectrophotometrically measured Pfr concentrations and physiological responses - e.g., lipoxygenase activity (Oelze-Karow & Mohr, 1973) and anthocyanin synthesis (Drumm & Mohr, 1974; Schmidt & Mohr, 1982 in the cotyledons of dark-grown S. alba seedlings - and upon the differences in chemical reactivity of the two isomers (Hahn et al, 1984).

The physiological responses in dark-grown plants which correlate with Pfr concentration are typical of "induction responses". These are characterised by being R/FR reversible with low doses of actinic light and show reciprocity between the fluence rate and duration of the light. Although the induction responses have widely been studied in etiolated plants, parallel responses in light-grown plants have been reported for end-of-day R/FR treatments (e.g., Downs et al, 1957). In the induction responses a change in ϕ in the region of 0.01 to 0.05 is associated with a large response (the corresponding change in ϕ from 0.79 to 0.75 is, proportionately, very small and it is difficult to envisage such a relatively small change in the amount of Pfr being responsible for large changes in physiological activity). In maize Briggs & Chon (1966) observed that the sensitivity to B of the "first-positive" phototropic curvature of coleoptiles was decreased by a pre-treatment with R, which saturated at very low energies, so low in fact that the amounts of Pfr formed could not be detected by spectrophotometry.

A second group of responses is recognised in emergent seedlings, and although they have no counterpart in fully light-grown plants they are important, because they are difficult to explain solely in terms

of Pfr levels. These are the "high irradiance responses" (HIR) which have been demonstrated in, for example, the inhibition of hypocotyl elongation (see, Mohr, 1972; Hartmann, 1966; Wildermann et al, 1978). Typically the HIR lack photoreversibility and have action maxima in the B, R and FR regions of the spectrum. They also correlate with both the duration and fluence rate of light.

There are two models for phytochrome action in HIR. The first was suggested by Hartmann (1967) to explain the photoinhibition of lettuce hypocotyls and it depends upon a low concentration of Pfr being maintained in the light. Others have interpreted the fluence rate dependency in terms of the cycling rate between the Pr and Pfr forms of phytochrome and have proposed models based on a co-action between the amount of Pfr and a product of cycling rate (e.g., Schafer, 1975; Johnson & Tasker, 1979). In Sinapis arvensis seeds, Bartley & Frankland (1982) have correlated the photoinhibition of germination with cycling rates calculated for the different light treatments. Similarly, Wall & Johnson (1983), using data from a number of sources for the HIR of S. alba, have shown that although many of the main features of the HIR can be related to Pfr concentration, there is also a requirement for a second function which corresponds with cycling rate.

In de-etiolated plants the HIR to B and FR are lost (Jose & Vince-Prue, 1977; Wildermann et al, 1978; Holmes et al, 1982) and they may only be important in emergent seedlings, where they could enable development to be governed by fluence rate and light quality, via phytochrome, before photosynthesis is instituted. The eventual loss of the HIR might be associated with the fall in phytochrome levels in the light (Beggs et al, 1980). Cycling rate should be

considered in studies of phytochrome action in light-grown plants, however.

Most examinations of phytochrome action in light-grown plants have concentrated on stem elongation. Usually this is inversely related to ϕ (e.g., Morgan & Smith, 1976; 1978b; 1979; 1981; Morgan et al, 1980; Wall & Johnson, 1981), but in some cases maximum elongation occurs at R:FR which give intermediate values of ϕ (Satter & Wetherell, 1968; Vince-Prue, 1977; Holmes & Wagner, 1981). In Chenopodium rubrum, hypocotyl elongation was maximal at $\phi = 0.35$ in low fluence rates, but at higher light levels it was inversely related to ϕ between 0.05 and 0.75 (Holmes & Wagner, 1981). Similar relationships have been found in S. alba and were attributed to the relative contributions to ϕ of the photochemical and thermal reactions of the phytochrome system at different fluence rates (Holmes et al, 1982). Where stem elongation is inversely related to ϕ , the relationship may be linear or biphasic (Morgan & Smith, 1979; Wall & Johnson, 1981). In the experiments of Morgan & Smith (1979) the response of most species was found to be linear (exceptions were Senecio vulgaris and Circaea lutetiana). They grew plants in background WL ($100 \mu\text{mol/m}^2\text{s}$) to which FR was added. At these fluence rates, ϕ will essentially be determined by the photochemical reactions of phytochrome, but for technical reasons values of ϕ below 0.30 could not be obtained. Johnson (1981) has argued that the light regimes used by Morgan & Smith resemble neither shadelight nor sunlight in either quality or fluence rate, and has grown plants in low fluence rate green light ($13 \mu\text{mol/m}^2\text{s}$) with added FR. This, he maintains, is a better representation of shadelight. In norflurazon-treated plants of S. alba, transferred from WL to these simulated shade conditions, hypocotyl elongation was linearly related

to ϕ in one batch, but a biphasic correlation was found in a second batch (Wall & Johnson, 1981). The biphasic relationship was also found for hypocotyl and internode elongation in green plants from both batches of seeds. This relationship consisted of steep gradient between $\phi = 0.7$ and $\phi = 0.6$ and shallow gradient below $\phi = 0.6$. Wall & Johnson (1982) also found that the plot of ϕ /elongation is affected by temperature. At 25°C the graph was biphasic, but at 15°C it was linear. In addition, stem elongation was fluence rate dependent at 25°C, but not at 10°C. These observations can be interpreted in terms of the thermal and photochemical reactions of phytochrome: at high temperatures and low fluence rates, Pfr will be formed slowly, and subject to rapid destruction; so that as R:FR is reduced less Pfr is formed and ϕ approached a minimum value. An increase in fluence rate will increase the rate of Pfr formation and, therefore, the ϕ .

A second explanation is that ϕ may be affected by the internal light environment of the phytochrome molecules. The differences in ϕ /elongation for bleached and green plants implies that chlorophyll screening may modify ϕ . A problem with most surveys of green plants is that stem elongation has been related to ϕ measured in dark grown tissue, or estimated from the plot of ϕ /R:FR in dark grown tissue. This does not take into account either the effects of chlorophyll screening, or reflection and refraction within the tissue. The extent to which these are important depends on the localisation of phytochrome, but as yet there has been no systematic exploration of this. In etiolated pea epicotyls, phytochrome has been immunofluorescently visualised in cortical cells and stomatal guard cells, but not in other epidermal cells (Saunders et al, 1983). In green Phaseolus vulgaris, phytochrome is abundant in subepidermal

tissues, whilst the epidermis (except for the guard cells), meristems and vascular tissues contain very little phytochrome (Verbelen, unpublished). Attempts have been made to calculate how ϕ may vary in a model green leaf as a result of chlorophyll screening and they show that a gradient of ϕ will be established between the upper and lower epidermis (Holmes & Fukshansky, 1979). Morgan & Smith (1978) have pointed out that the linear relationship between ϕ and stem elongation can only be strictly true if phytochrome is in the epidermal, or subepidermal layers, as chlorophyll screening reduces ϕ ; but that this relationship will only significantly be affected if the phytochrome is located deep within a layer of chlorophyllous tissue.

It is not only chlorophyll screening that can reduce the R:FR within the tissue. Using a single glass fibre inserted at various depths into a succulent leaf of Crassula falcata, Vogelmann and Bjorn (1984) have monitored the changes in quality of light entering the leaf.

Internal reflection was found to increase the relative amount of FR by about 3 times that measured in the incident light within the initial 1mm of the leaf. Differences in spectral quality may, therefore, be amplified by plant tissue and could affect the relationship between ϕ and stem elongation rate.

Finally, an inverse relationship between ϕ and stem elongation rate may justifiably be interpreted as a simple correlation between Pfr concentration and response, provided P_{tot} remains constant. Smith (1981), however, has demonstrated that in light-grown maize, P_{tot} is

not constant. In norflurazon-treated plants, grown in WL for 7 days and then transferred for 72h to a range of cabinets giving equal PAR, but different R:FR, levels of phytochrome were very low; but differences in P_{tot} were measured. After 72h there was almost twice as much phytochrome in the leaves of plants grown at the lowest R:FR ($\phi=0.36$), compared with those in the highest R:FR ($\phi=0.65$). These observations can be explained in terms of differences in the amount of Pfr available for destruction. Moreover, Smith verified the prediction made by Schafer (1975), that Pfr concentration becomes independent of wavelength under continuous irradiation, and showed that the elongation rate of the leaves in both herbicide-treated and green plants was inversely and linearly related to ϕ . These results are not consistent with the theory of Pfr as active form, and suggest that the relative amounts of Pr and Pfr are important in the control of elongation in light-grown plants. Schmidt & Mohr (1982) have proposed that light-grown maize plants may measure ϕ , whereas mustard seedlings measure Pfr concentration. Such a submission may not be inconsistent with the increasing volume of evidence for several independent and, perhaps, physiologically distinct populations of phytochrome. Whether or not this is correct, the relationship between ϕ and R:FR shows that phytochrome is very sensitive to R:FR values in that range (i.e., 1.15 to 0.05) found in vegetational shade (Smith & Holmes, 1977b) and is, therefore, splendidly adapted for monitoring natural variations in light quality.

Experimental Rationale

The main aim of these experiments was to discriminate between the

contribution of cycling rate, the concentration of Pfr and photoequilibrium to phytochrome-modulated stem elongation. Phytochrome cycling rate is dependent on the fluence rate and the quality of incident radiation; so there are two ways of assessing the importance of cycling rate. Because stem elongation rate has been shown to be related to ϕ , then the effect of fluence rate or wavelength on the functional relationship between elongation rate and ϕ should reveal the extent of the influence of cycling rate.

The first approach adopted here has been to compare the functional relationship between elongation rate and ϕ at different fluence rates. This was achieved by giving supplementary FR to the first internode in different levels of background white fluorescent light. The theory is that if cycling rate is important, then the functional relationship (i.e., the slope of elongation rate v ϕ) should be different when ϕ is determined by light of different fluence rates. The rapid response of internode elongation to FR given to the internode itself was chosen as the probe for this examination because it can be measured within 45 min. This means that it occurs before any large changes in P_{tot} can take place. Furthermore, only the internode needs to be irradiated with FR and this reduces the possibility of affecting process in other organs, and it is technically much easier to obtain the high fluence rates of FR required to generate low ϕ values when irradiating a small area. The technical problems associated with obtaining low ϕ values also limits the range of background WL fluence rates which can be used

for these experiments. It was the intention to use plants grown under conditions that were as natural as possible within the bounds of the experimental procedures. These conditions involved growing plants in a suitable photoperiod at a sufficiently high level of PAR to allow normal development.

Schafer (1981) has quoted some unpublished work of Smith and Holmes, in which the light above a stand of the arable weed Tripleurospermum maritimum had a photon fluence rate (in the 400-800 nm waveband) of $1182 \mu\text{mol}/\text{m}^2\text{s}$, while the value within the canopy was $52 \mu\text{mol}/\text{m}^2\text{s}$. The high fluence rate of daylight is beyond the technical limits of these experiments, but fluence rates of the order of those found within such an arable weed stand are easily attained. Background WL fluence rates of 50, 100 and $150 \mu\text{mol}/\text{m}^2\text{s}$ were used for these experiments and these should provide sufficient differences in cycling rate while allowing low ϕ to be generated, and being of the order of fluence rates found under canopies of arable weeds. The use of different levels of background WL is, however, fraught with potential difficulties. In addition to modifying phytochrome cycling rate, the different WL fluence rates may induce responses involving photosynthesis or a B-photoreceptor. Before embarking on the main experiment it, therefore, was important to determine the effects of WL fluence rate on stem elongation. A good way of determining the involvement of a B-photoreceptor in non-directional stem elongation is to follow the technique of adding B to background R. Background R can be obtained from sodium discharge lamps, and it provides photosynthetically active radiation and maintains a high ϕ (see, Thomas, 1981).

B given to plants in background R would not affect ϕ significantly, yet if a B-photoreceptor were involved, then a rapid reduction in stem elongation rate would be anticipated, and this would be dependent on the continuity of the B.

The effects of WL levels on photosynthesis may lead to complex differences between plants; particularly if the plants were grown at different WL levels over several days (indeed experiments described later show this to be true). To reduce the possible long-term effects of different WL levels, plants were grown to a particular physiological stage at one light level, and transferred to the experimental light level and equilibrated before giving supplementary FR. This method required the short-term effects of changes in WL fluence rate to be monitored.

The second approach for assessing the importance of phytochrome cycling rate in the modulation of stem elongation rate, was to compare the functional relationship between elongation rate and ϕ set up by supplementary FR of different wavelengths added to low fluence rate background white fluorescent light. Action spectra for phytochrome cycling rate, measured *in vivo* (Kendrick & Spruit, 1973) and calculated (Hartmann, 1966; Johnson & Tasker, 1979), show a peak in the region of 680-700 nm, which declines gradually at shorter wavelengths, but steeply above 700 nm. If cycling rate is important in determining elongation rate, then the functional relationship between elongation rate and ϕ should vary at different wavelengths, in response to the extent to which a particular wavelength stimulates cycling rate.

Johnson & Tasker (1979) calculated that the action maximum for net cycling rate (i.e., cycling rate \times P_{tot}) increases with the duration of irradiation, and shifts from about 680 nm to about 715 nm after 24 h of continuous monochromatic irradiation. This is because P_{tot} is wavelength dependent: phytochrome accumulates under conditions which establish a low ϕ , because less Pfr is available for destruction. As described above, the rapid response of internode elongation to FR can be measured within 45 min; so problems associated with changes in P_{tot} levels are avoided.

Two wavelengths were chosen for the experiments: λ max 700 nm and λ max 719 nm. FR λ max 700 nm is, according to Johnson & Tasker (1979), about 2.5 times more effective at stimulating cycling rate than FR λ max 719 nm. The results obtained by Morgan et al (1980) for FR λ max 739 nm were included in the analysis, because FR λ max 739 nm is about 4.5 times less effective than FR λ max 700 nm at stimulating cycling rate.

To distinguish between the contributions of Pfr concentration and ϕ to phytochrome-modulated elongation, an attempt has been made to vary P_{tot} , following the work of Hunt & Pratt (1980). Hunt & Pratt demonstrated that in oats, soluble phytochrome levels increase in darkness and that this increase is greatest when plants are given a brief irradiation with FR at the onset of the dark period (a so-called end-of-day FR treatment).

It is possible, therefore, at least in theory that P_{tot} levels may similarly be modified in *S. alba*. If these plants subsequently

were given a photoperiod of light establishing a low ϕ and stem elongation was monitored continuously, then the elongation rate could be related to the level of phytochrome. Because ϕ should be the same in each plant, the absolute amount of Pfr will depend on P_{tot} . If Pfr inhibits stem elongation, as suggested by the theory of Pfr as active form, the lowest elongation rates would be expected in plants having the highest P_{tot} levels (i.e., those given end-of-day FR).

As with the previous experiments, the rapid response to FR offers a good opportunity to investigate this scenario. End-of-day FR can be given to the internode specifically, using fibre-optic probes, thus avoiding affects on other organs and the elongation rates in the subsequent photoperiod should be measurable before there is any large change in P_{tot} .

Because all these experiments rely on measuring the rapid response of internode elongation to supplementary FR, a detailed knowledge of the nature of the response is a pre-requisite for the investigation. Finally, in the General Introduction it was mentioned that stem elongation was to be analysed in terms of internode, rather than hypocotyl elongation. This was because differences between hypocotyl and internode elongation are already known to exist (even though many people extrapolate from one to the other without regard for these differences), but experiments have been carried out here to compare and contrast the effects of light quantity and light quality on hypocotyl and internode elongation in plants grown in normal conditions.

MATERIALS AND METHODS

Plant Material

Seeds of Sinapis alba L. (Carters seeds, Llangollen, U.K) were sown in 65 mm pots with John Innes No.1 potting compost and watered daily with tap water. For most experiments these plants were grown under white fluorescent light ($100 \mu\text{mol/m}^2\text{s}$; 1500 mm, 65/80 W Crompton "warm-white" tubes) with a 16h photoperiod at $20 \pm 2^\circ\text{C}$.

Stem Elongation Measurements

(i) Long-Term Measurements

Daily measurements were made at 14-00h with a steel rule (calibrated in divisions of 0.5 mm).

(ii) Short-Term High Resolution Continuous Recording

This was achieved using miniature linear displacement transducers, mounted in custom-built apparatus. The apparatus was designed to alleviate the difficulties encountered in making fine measurements, namely:

(a) Noise from vibration.

(b) Noise from temperature-related expansion/contraction of the apparatus, or plant, caused by changes in light treatment or air temperature.

(c) Disturbance of the test plant.

Apparatus 1

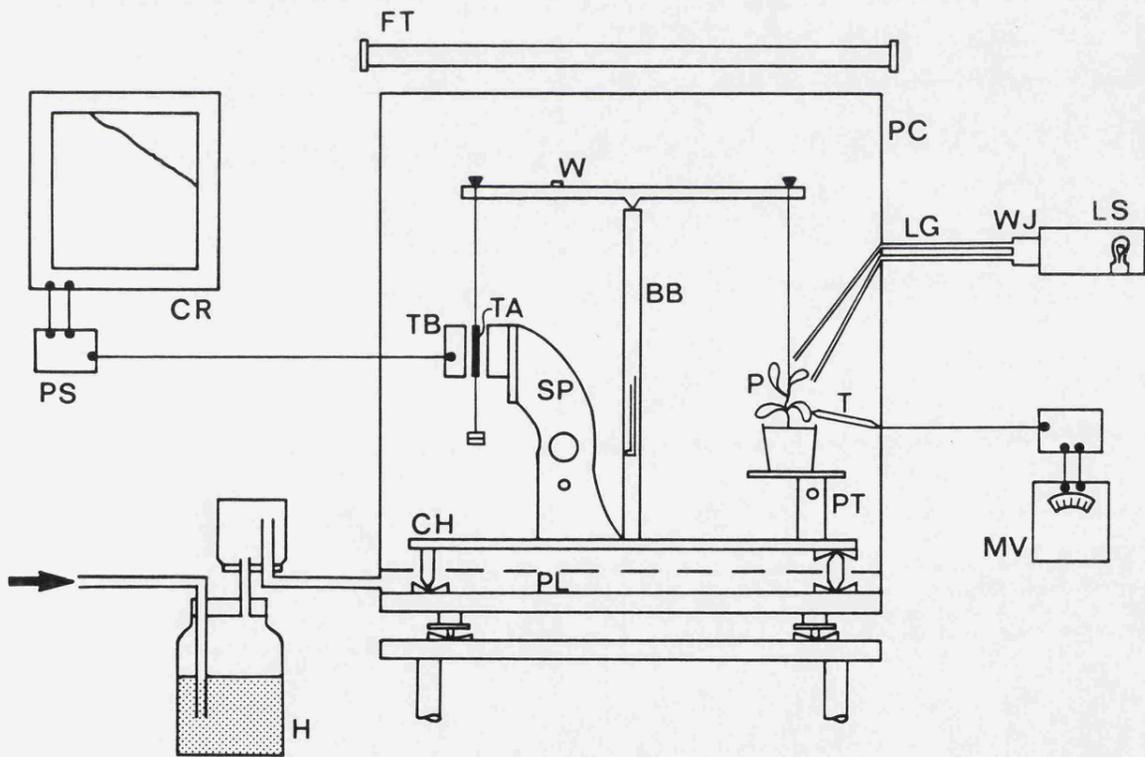
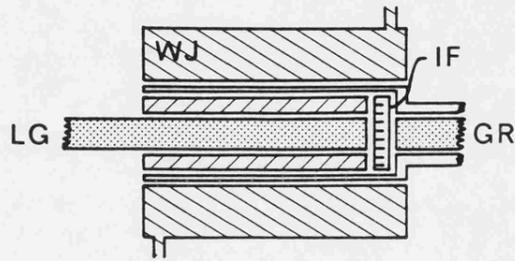
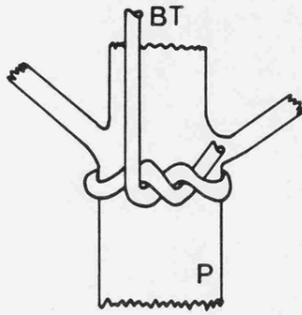
This is shown in Figure 2. The transducer (Dc/Dc, LVDT, Type DF/1.0 mm; Sangamo-Schlumberger, Bognor Regis, U.K.) was mounted in an adjustable supporting pillar with the armature running freely through the transducer body. This armature was attached to one end of a balance beam, via a glass rod. The plant was fastened to the other end of the balance beam using a length of button-thread tied at the node, and then looped over a glass hook. A counter-balance weight on the armature applied an upward force (equivalent to 2g) on the plant. The transducer used was linear in distance/output over a 2mm stroke, and the balance allowed the apparatus to be re-set without disturbing the plant (using a remote attachment to a rack and pinion in the supporting pillar). To accommodate plants of different height the plant table was also adjustable. The measuring apparatus was mounted on a length of channel-steel fitted with legs designed to eliminate lateral and vertical distortions of the enclosing plastic chamber. Humidified air was pumped through the chamber at about $0.06\text{m}^3/\text{min}$, to buffer the internal air temperature.

Background WL was from two 600mm, 40W Philips "colour-matching" fluorescent tubes, which could be raised or lowered to adjust the fluence rate. Supplementary irradiation to specific parts of the plant was from fibre-optic light sources (Schott KL 150B) fitted with a 3.55mm active diameter, three-way, fibre-optic bundle (Carl Zeiss, London, U.K.). Interference filters (25mm diameter; Ealing

Figure 2

Diagram of transducer apparatus 1.

Key: BB: beam-balance.	P: plant.
BT: button thread.	PC: plant chamber.
CH: channel steel.	PL: plenum chamber.
CR: chart-recorder.	PS: power supply.
FT: fluorescent tube.	PT: plant table.
GR: glass rod.	SP: supporting pillar.
H : humidifier.	T : thermistor.
IF: interference filter.	TA: transducer armature.
LG: light-guide.	TB: transducer body.
LS: light source.	W : weight.
MV: millivolt-recorder.	WJ: water-jacket.



Beck Ltd, London, U.K.) were placed between the light source and the light guides, and were shielded by a piece of heat-absorbing glass (25mm diameter x 2mm; Schott KGI). The filters and glass were mounted in copper water-jackets cooled by a continuous flow of water, so that infra-red was reduced as much as possible.

Output from the transducer was fed directly to a single-pen potentiometric chart recorder (CR 650; JJ Instruments, Southampton, U.K.) and the charts were analysed by main-frame computer (using the Fortran programmes of Dr D C Morgan), or micro computer (using the Pascal programmes of Dr P M Hayward). The micro computer was an Apple II with graphics tablet (Apple Computer Inc, Copertino, Ca, USA).

The treatment chamber, fibre-optic unit and chart recorder were on separate tables to reduce vibration. The treatment chamber was isolated from its table by a sheet of anti-vibration matting and the complete system was enclosed in a temperature controlled room at 23 ± 1 °C.

Plants were attached to the apparatus at 17-00h the previous evening to allow the plant to overcome any shock resulting from handling. No significant temperature or vibration effects were experienced. Additional FR light (λ max 719nm) caused a temperature rise of 0.25 °C at the maximum fluence rate used, measured by a copper-constantan thermocouple junction embedded in the internode. Furthermore, the kinetics of responses to radiant heat are different from those to the supplementary light sources used here (Morgan, unpublished).

Apparatus 2

This system was designed to improve on Apparatus 1 in three ways:

- (i) Three transducers were positioned side by side, allowing three plants to be studied each day.
- (ii) The transducer assembly was remote from the light sources; so the possibility of temperature effects was reduced.
- (iii) Higher fluence rates of both WL and supplementary light were possible.

The apparatus is shown in Figure 3. The transducers, supporting pillars and beam balances were fixed to a piece of laminated board and a cement slab, with a sheet of anti-vibration matting beneath the slab. The transducers were enclosed in a thermally-insulated chamber, fitted with extended arms for remotely re-setting the transducers.

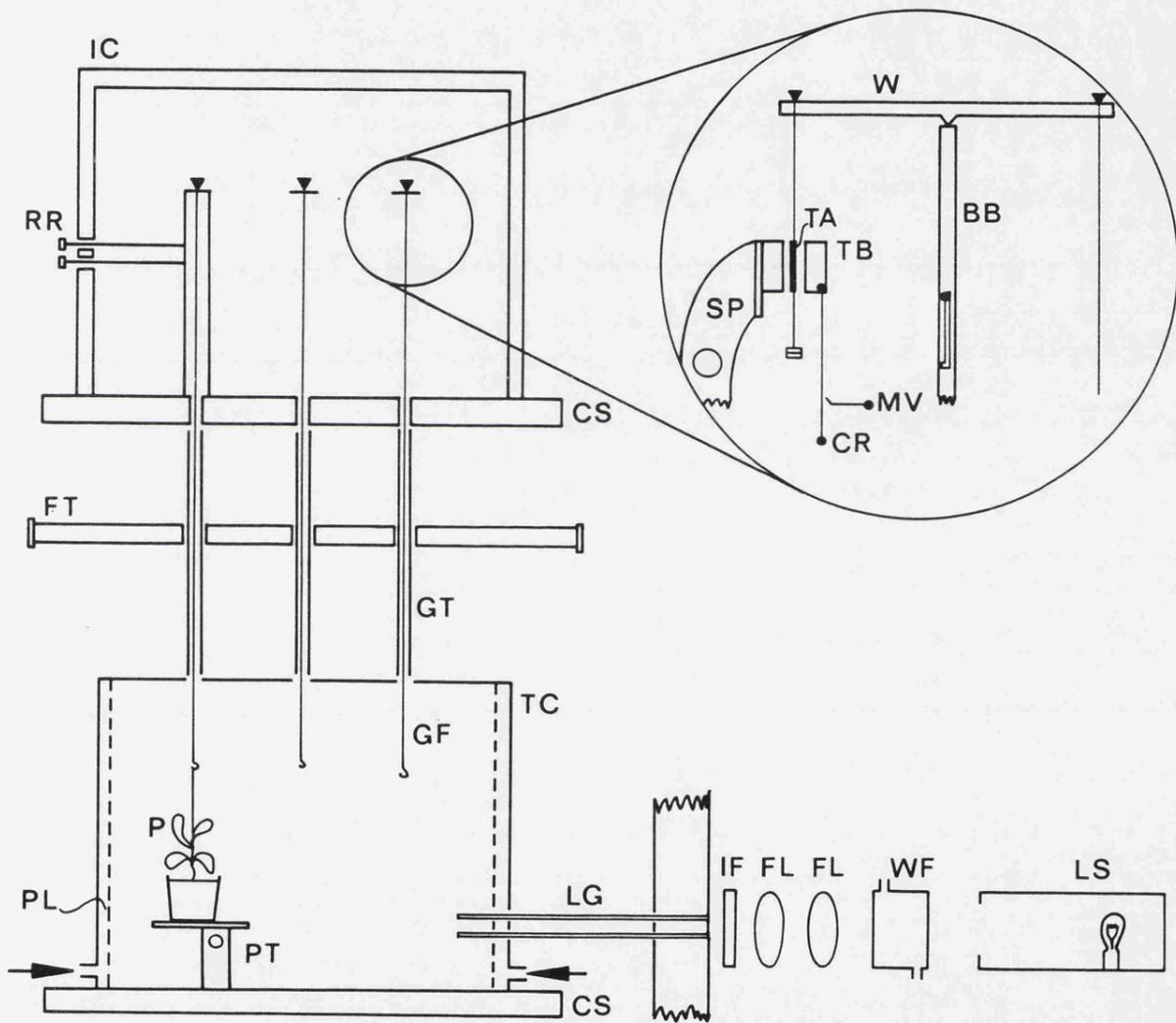
The connections between the beam balances and plants was by 900mm x 0.75mm glass fibres (shielded within glass tubes) and button-thread. Within the plant-chamber the plants stood on adjustable tables. The plant chamber and transducer chamber were mounted on adjustable shelves attached to the wall.

Background WL was from up to six 600mm, 40W Philips "colour-matching" fluorescent tubes, which could be lit in pairs and raised or lowered to alter the fluence rate. Background R was from a 775mm, 135W low-pressure sodium discharge lamp (Thorn Ltd, U.K.) mounted on an adjustable gantry.

Figure 3

Diagram of transducer apparatus 2.

Key: BB: beam-balance.	MV: lead to millivolt-recorder.
CR: lead to chart-recorder.	P: plant.
CS: cement slab.	PL: plenum-chamber.
FL: focusing lens.	PT: plant table.
FT: fluorescent tube.	RR: remote re-adjusting arms.
GF: glass fibre.	SP: supporting pillar.
GT: glass tube.	TA: transducer armature.
IC: insulated chamber.	TB: transducer body.
IF: interference filter.	TC: test chamber.
LG: light-guides.	W : weight.
LS: light source.	WF: water filter.



Supplementary irradiation was from fibre-optic light guides (1000 x 5mm; Volpi AG, Urdorf, Switzerland) held in position by adjustable clamps, with magnetic bases, standing on a sheet of galvanized steel fixed to the floor of the plant chamber. The light for the fibre-optics was from a theatre-spotlight fitted with a 1KW tungsten-halogen bulb (Type T84; Rank Strand Electric, Brentford, U.K.) and housed in an adjacent room to the plant chamber, to avoid overloading the temperature-control system. Radiant heat was first reduced by a 160mm thick running-water filter. The beam was then focused, by a lens system, onto an interference filter (50mm ; Balzers, Liechtenstein) flush with the ends of the fibre-optic light guides. The filter was protected by a piece of heat-absorbing glass (50mm x 2mm thick; Schott KGI), and both were cooled by a fan. The fluence rate of the additional light was varied by neutral density filters (50mm gelatin Wratten No 96; Eastman Kodak, U.K.) sandwiched between the filter and the glass.

Output from the three transducers was logged by a standard six-channel potentiometric chart-recorder (Clearspan P250L; Foster-Cambridge Ltd, Cambridge, U.K.). Each transducer could be run on one of five calibrated scales using a custom-made back-off system. The chart traces were analysed either by microcomputer, or by hand.

Light Measurement

All light measurements were made in $\mu\text{mol}/\text{m}^2\text{s}$, (the SI units for photon fluence rate) because photochemical reactions depend upon the absorption of specific photons, rather than the energy of incident

radiation.

The light sources were analysed with a spectroradiometer (Gamma Scientific, San Diego, Ca, USA) fitted with a miniature cosine-corrected receptor at the end of a flexible fibre-optic probe. The spectroradiometer scanned the 400-800nm waveband and was regularly calibrated against a tungsten-halogen standard source. The scans of light sources were analysed by microcomputer using the Pascal programmes of Dr P. M. Hayward.

Fluence rates of photosynthetically active radiation (PAR) from WL sources were measured with a quantum meter (Lambda L1-185; Lambda Instrument Corp. Lincoln, Nebraska, USA).

Light Sources

(i) Background Sources

(a) White Light: 1500mm, 65/80W Crompton "warm-white" fluorescent tubes (Figure 4). 600mm, 40W Philips "colour-matching" fluorescent tubes (Figure 4).

(b) Red Light: 775mm, 135W Thorn SoX low-pressure discharge lamp (Figure 4).

(c) Variable R:FR: for variable background R:FR a custom-built growth cabinet was used which was divided into four compartments, each giving equal values of PAR but, varying the R:FR (Figure 5). This cabinet is described in detail by Heathcote et al, (1979).

Figure 4

Relative spectral photon fluence rates (SPFR) for light sources.

A: background WL from Crompton "Warm-White" fluorescent tubes.

Full scale response = $1.04 \mu\text{mol}/\text{m}^2\text{s nm}$.

R:FR = 3.3, $\phi_e = 0.71$

B: background WL from Philips "Colour-Matching" fluorescent tubes.

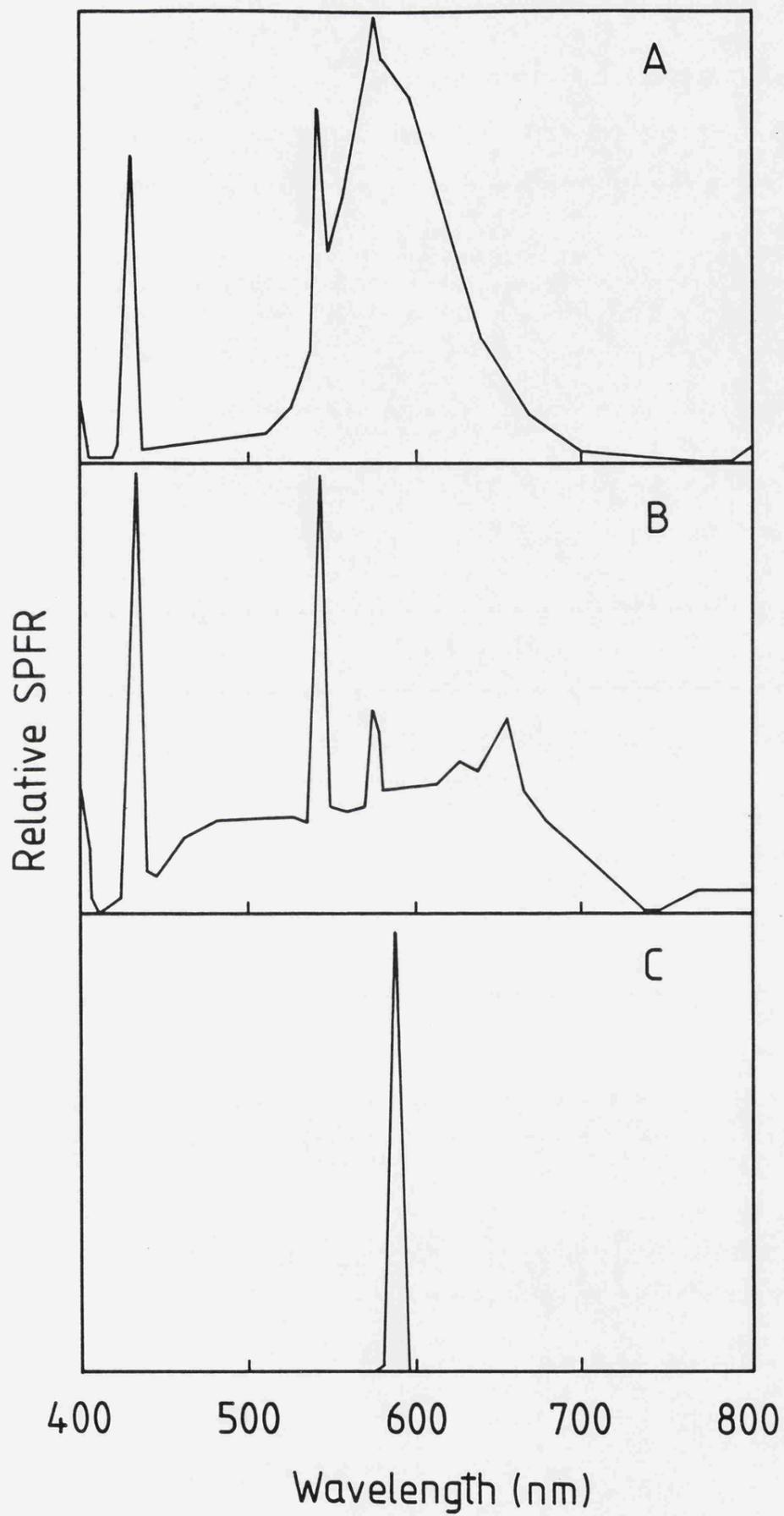
Full scale response = $2.23 \mu\text{mol}/\text{m}^2\text{s nm}$.

R:FR = 3.4, $\phi_e = 0.72$

C: background R from Thorn low-pressure sodium discharge lamp (SOX).

Full scale response = $1.41 \mu\text{mol}/\text{m}^2\text{s nm}$.

$\phi_m = 0.76$.



(ii) Supplementary Sources

(a) Far-red: from 25mm diameter filters λ_{\max} 700nm, 719nm or 739nm (Figure 5) or a 50 mm filter λ_{\max} 730nm (Figure 5).

(b) Blue: from 50mm filter λ_{\max} 452nm (Figure 5).

Phytochrome Measurement

Since the background WL sources were at 90° to the supplementary light from fibre-optic probes it was not possible to measure the R:FR using a spectroradiometer. By using preparations of dark-grown tissue it was possible to determine the effects of light treatments on phytochrome by measuring ϕ directly. Although as previously mentioned this method has limitations (see Introduction).

ϕ was measured by irradiating partially purified extracts (see Woolman, 1980), or coleoptile segments, from dark-grown Avena sativa var Mostyn tissue. The partially purified extracts were irradiated in cuvettes (fitted with nylon inserts to give internal dimensions of 5 x 10 x 10 mm) mounted on the plant table. ϕ was measured in a Perkin-Elmer 156 dual-wavelength spectrophotometer. When oat coleoptile tissue was used, 1.5mm segments were packed into cuvettes fitted with plastic inserts to give a volume of tissue 10mm high, 2.5mm thick and 10mm wide (2.5mm is roughly the diameter of the internode in 14d old S. alba). This enabled the fibre-optic probes to be placed in exactly the same geometrical position as in the irradiation of plant stems - that is, 10mm from the stem. This allowed a more accurate representation of the situation during the

Figure 5

Relative spectral photon fluence rates (SPFR) for light sources.

A: variable R:FR cabinet.

Full scale response = $1.50 \mu\text{mol}/\text{m}^2 \text{ s nm}$.

1. R:FR = 1.84, $\phi_e = 0.66$

2. R:FR = 0.35, $\phi_e = 0.38$

3. R:FR = 0.10, $\phi_e = 0.17$

4. R:FR = 0.07, $\phi_e = 0.12$

B: supplementary B (λ max 452 nm) and FR (λ max 730 nm) from Balzer
50 mm² interference filters.

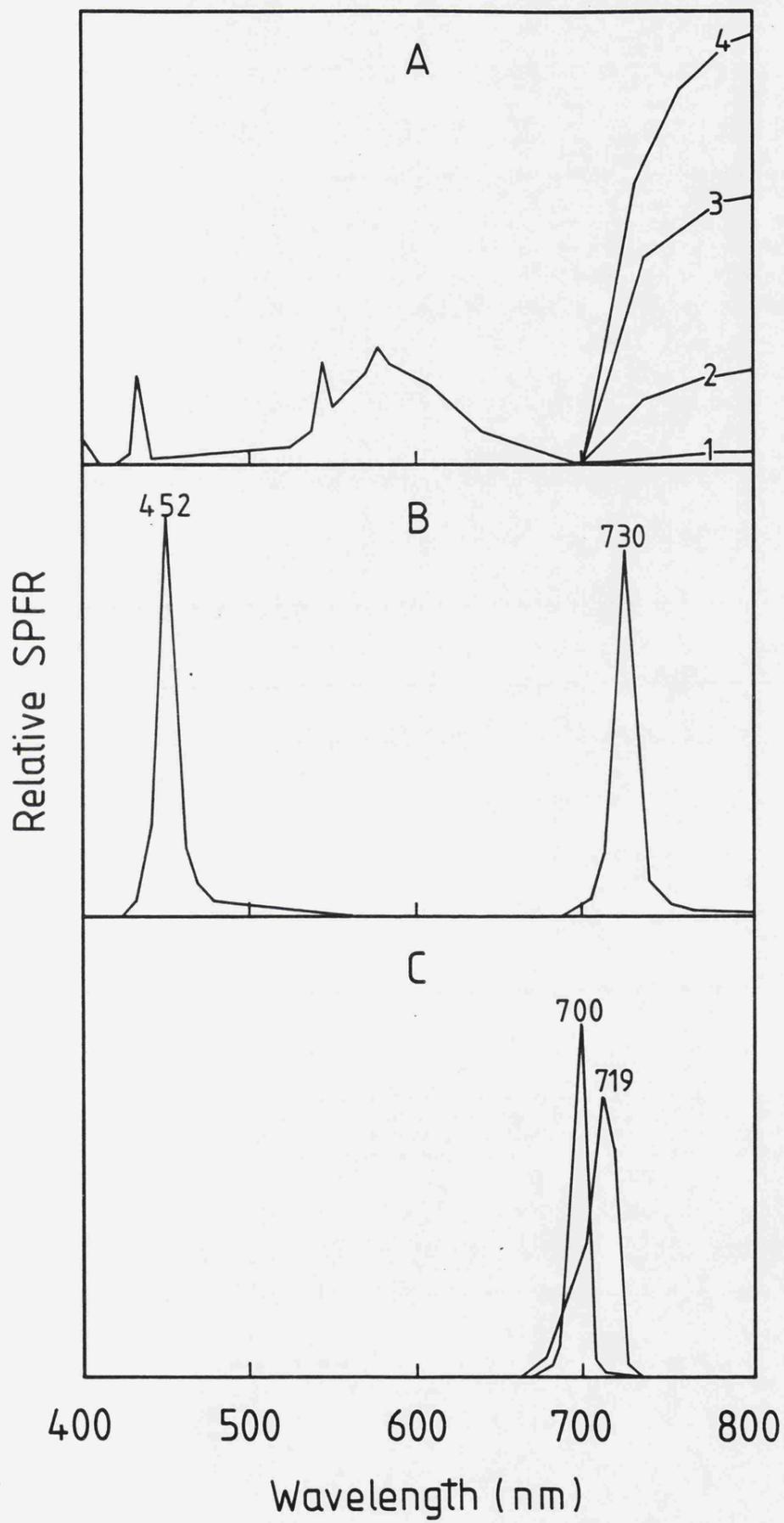
Full scale response B = $1.50 \mu\text{mol}/\text{m}^2 \text{ s nm}$.

Full scale response FR = $6.00 \mu\text{mol}/\text{m}^2 \text{ s nm}$.

C: supplementary FR (λ max 700 nm and λ max 719 nm) from Ealing
25 mm diameter interference filters.

Full scale response 700 nm = $3.00 \mu\text{mol}/\text{m}^2 \text{ s nm}$.

Full scale response 719 nm = $6.00 \mu\text{mol}/\text{m}^2 \text{ s nm}$.



experiment. The tissue was irradiated in the light source for 5 min and transferred to the spectrophotometer (Aminco DW-2aUV-Vis) in a light-tight box.

Experimental Procedures

The Effect of WL Fluence Rate on Stem Elongation

For long-term experiments, plants of S. alba were grown from seed in white fluorescent light (Crompton "warm-white" tubes) at fluence rates of 50, 100 or 150 $\mu\text{mol}/\text{m}^2\text{s}$ (R:FR=3.3; $\phi=0.76$), with a 16h photoperiod. Temperature was $20\pm 2^\circ\text{C}$ throughout the experiment.

Measurements of total height, hypocotyl length and first internode length, were made at 14-00h each day, after which each plant was given an equal volume of tap water. The plants were harvested on day 20 after sowing; the dry weight of the internodes was recorded, and the number of elongating internodes was noted.

In short-term experiments, 14d old S. alba plants, grown in WL (100 $\mu\text{mol}/\text{m}^2\text{s}$), were mounted in transducer apparatus 2 and equilibrated overnight in white fluorescent light (R:FR=3.4; $\phi=0.72$) at $21\pm 1^\circ\text{C}$.

In the following photoperiod stem elongation was continuously monitored for a period of about 6h, during which the WL fluence rate was changed once, by switching pairs of fluorescent tubes on or off.

The Effect of Added B on Stem Elongation

14d old S. alba plants, grown in WL (100 $\mu\text{mol}/\text{m}^2\text{s}$; R:FR=3.3;

$\phi=0.76$), were mounted in transducer apparatus 2 and equilibrated overnight in R ($50 \mu\text{mol}/\text{m}^2\text{s}$). During the following day, supplementary B ($\lambda_{\text{max}} 452\text{nm}$; $20 \mu\text{mol}/\text{m}^2\text{s}$) from fibre-optic probes, was directed at the primary leaves and first internode. A similar experiment was performed in which the plants had been grown in continuous R, but only the first internode was irradiated with additional B.

The Effect of R:FR on Stem Elongation

For long term experiments, plants of S. alba were grown from seed in the custom-built growth cabinets giving equal fluence rates of PAR (about $40 \mu\text{mol}/\text{m}^2\text{s}$) but different R:FR (Heathcote et al, 1979). The photoperiod was continuous, because a dark period would have put the plants under stress and may have caused premature flowering (Grime, 1979). The temperature was $20 \pm 1^\circ\text{C}$.

Measurements of total height, hypocotyl length and first internode length were made at 14-00h each day, after which each plant was given an equal volume of tap water. On day 15 the plants were harvested and the dry weights of the first internodes were measured. The number of elongating internodes was noted.

In short-term experiments, 14d old or 20d old S. alba plants, grown in WL ($100 \mu\text{mol}/\text{m}^2\text{s}$), were mounted in transducer apparatus 2 and equilibrated overnight in white fluorescent light (R:FR=3.4; $\phi=0.72$) at $21 \pm 1^\circ\text{C}$. The 20d old plants were used for one experiment in which they were given FR ($\lambda_{\text{max}} 730\text{nm}$; $100 \mu\text{mol}/\text{m}^2\text{s}$) added to WL ($100 \mu\text{mol}/\text{m}^2\text{s}$). The 14d old plants were used for two groups of experiments: the first to investigate the kinetics of the rapid response to added FR, the second to analyse the FR dose/response.

relationship at different background WL fluence rates.

In the first group of experiments, 14d old plants equilibrated in WL ($50 \mu\text{mol}/\text{m}^2\text{s}$) were given added FR ($\lambda_{\text{max}} 730\text{nm}$; $100 \mu\text{mol}/\text{m}^2\text{s}$) for periods of time ranging from 1 min to 180 min. The lag time, duration of the first phase, and the decay kinetics of the rapid response were measured, together with the ability of plants to respond to intermittent pulses. In the second group of experiments plants were equilibrated at 50, 100 or $150 \mu\text{mol}/\text{m}^2\text{s}$ WL and then given two 90 min pulses of FR at fluence rates between $1.4\text{--}102 \mu\text{mol}/\text{m}^2\text{s}$ ($\lambda_{\text{max}} 730\text{nm}$), with a 2h time interval between each pulse. The fibre-optic probes were positioned at exactly 10mm from the stem, because an error of 1mm in either direction between 2.5 and 15mm gives a change in fluence rate of about $8 \mu\text{mol}/\text{m}^2\text{s}$. Care was taken to choose plants at the same physiological stage of development: about 40mm tall with a 5mm first internode. The Wratten neutral density filters used to modify the FR fluence rate gave a measured FR transmission about 2-5% higher than that stated in the catalogue. Phytochrome measurements were made under the same irradiation conditions. Photoequilibrium was measured at time intervals in one of the lowest fluence rates used for the experiment.

The Effect of Different FR Wavelengths on Stem Elongation

14d old plants grown under WL ($100 \mu\text{mol}/\text{m}^2\text{s}$; R:FR=3.4; $\phi=0.72$) were mounted in transducer apparatus 1. Following an 8h dark period they were given background WL ($23 \mu\text{mol}/\text{m}^2\text{s}$; R:FR= 3.4; $\phi = 0.72$), to which was added FR at wavelengths of 700, 719 or 739nm (λ_{max}) and various fluence rates. Fluence rates were adjusted by using muslin filters adjacent to the interference filter and/or the dimmer switch on the

light source.

The Effects of End-of-Day Treatments on Stem Elongation

13d old S. alba plants grown in WL were mounted in transducer apparatus 2 and given light treatments according to the scheme shown in Figure 6. For each day's experiment, two plants were used, one was given an end-of-day FR pulse and the other only a WL-darkness transition. To avoid scattered light from the fibre-optics a sheet of opaque black paper was used to divide the plant chamber.

Elongation rates were monitored at the WL-D transition and then 2h before the onset of the subsequent photoperiod.

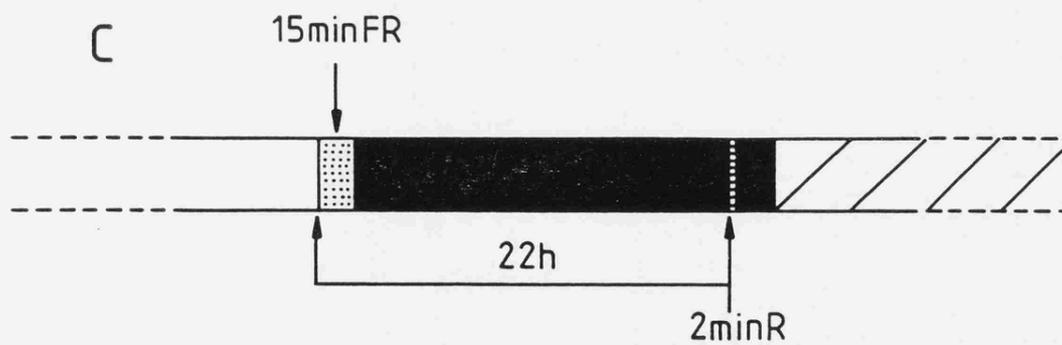
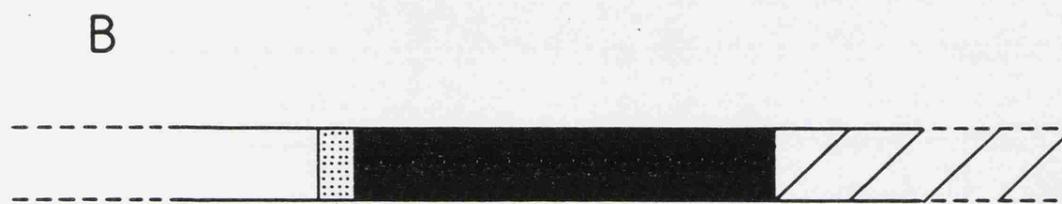
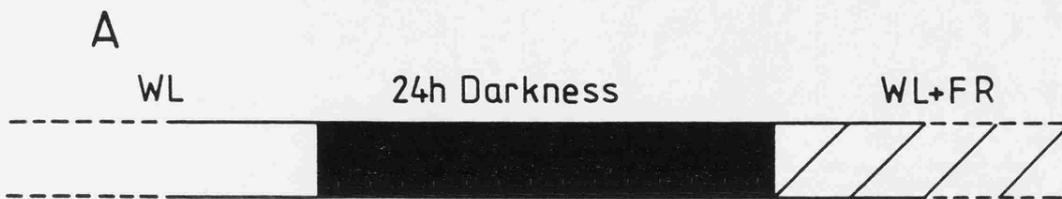
Figure 6

Scheme for the end-of-day treatment of Sinapis alba plants.

Plants grown for 13 d in WL ($100 \mu\text{mol}/\text{m}^2 \text{ s}$, R:FR = 3.3, $\phi_e = 0.71$) were mounted in transducer apparatus 2 in WL ($50 \mu\text{mol}/\text{m}^2 \text{ s}$, R:FR = 3.4, $\phi_e = 0.72$).

After equilibration the plants were given one of the following treatments.

- A: 24 h darkness.
- B: 24 h darkness with a 15 min FR treatment (λ max 730 nm, $100 \mu\text{mol}/\text{m}^2 \text{ s}$) given, at the onset of the darkness, to the internode by fibre-optic probes.
- C: 24 h darkness with a 15 min FR treatment (λ max 730 nm, $100 \mu\text{mol}/\text{m}^2 \text{ s}$) given at the onset of the darkness. After 22h a 2 min R pulse (λ max 660 nm, $100 \mu\text{mol}/\text{m}^2 \text{ s}$) was given to the internode by fibre-optic probes.



Statistical Treatment of Results

The mean values quoted are for at least five replicates. The examples of single experiments were chosen as representative of the results as a whole. The standard error is given to indicate the variability. Mean values were compared using "Student's" t-test for small samples:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{s^2 \times \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}}$$

where: t = the value of P in the t distribution

\bar{x}_1 , \bar{x}_2 = the respective means.

n_1 , n_2 = the respective number of replicates.

Regression analysis was done using the methods described by Sokal & Rohlf (1969), these are summarised in the appropriate figure legends.

Results and Discussion

The Effect of WL Fluence Rate on Stem Elongation

These experiments had two main purposes: to provide background information on first internode elongation for the FR dose/response experiments; and to compare the effect of fluence rate on hypocotyl and internode elongation. The experiments were not intended to be a detailed study of the effects of light

quantity on morphogenesis. Such effects have already been extensively analysed (see, introduction); although it is interesting to note that their physiological basis is not well understood.

Stem elongation in S. alba was biphasic at all light levels (Figure 7). For the first 15d after sowing height was inversely related to fluence rate, but by day 20 the reverse was true. This was because height initially was determined by hypocotyl elongation and subsequently became dependent on internode growth.

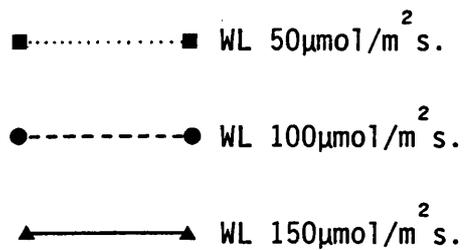
Hypocotyl elongation was most rapid during the 4d following emergence and was inversely related to fluence rate (Figures 8 and 9). After this period the elongation rate slowed to a steady-state independent of fluence rate (Figure 8), but the seedlings at the high light levels attained this steady-state earlier than those at low fluence rates (Figure 8A). The fluence rate dependency of hypocotyl elongation in S. alba has been reported by Holmes et al (1982). They believe that the dependency is manifest before the seedlings are fully photosynthetic and that the photoreceptor may be phytochrome. However, a B-photo-receptor must also be considered (Cosgrove, 1982).

Internode elongation started when hypocotyl growth was slowing (Figure 10). At 150 and 100 $\mu\text{mol}/\text{m}^2\text{s}$ internode elongation was detected 2 days earlier than at 50 $\mu\text{mol}/\text{m}^2\text{s}$ (Figure 10), but by day 18 the first internodes of plants in 150 $\mu\text{mol}/\text{m}^2\text{s}$ had entered the stationary phase and by day 20 those at 100 $\mu\text{mol}/\text{m}^2\text{s}$ also appeared to be entering the stationary phase.

Figure 7A

The effect of fluence rate on stem elongation in Sinapis alba.

Plants were grown from seed in WL (R:FR = 3.3, $\phi_e = 0.71$) with a 16 h photoperiod at $20 \pm 2^\circ\text{C}$. The bars are \pm S.E. for 10 plants.



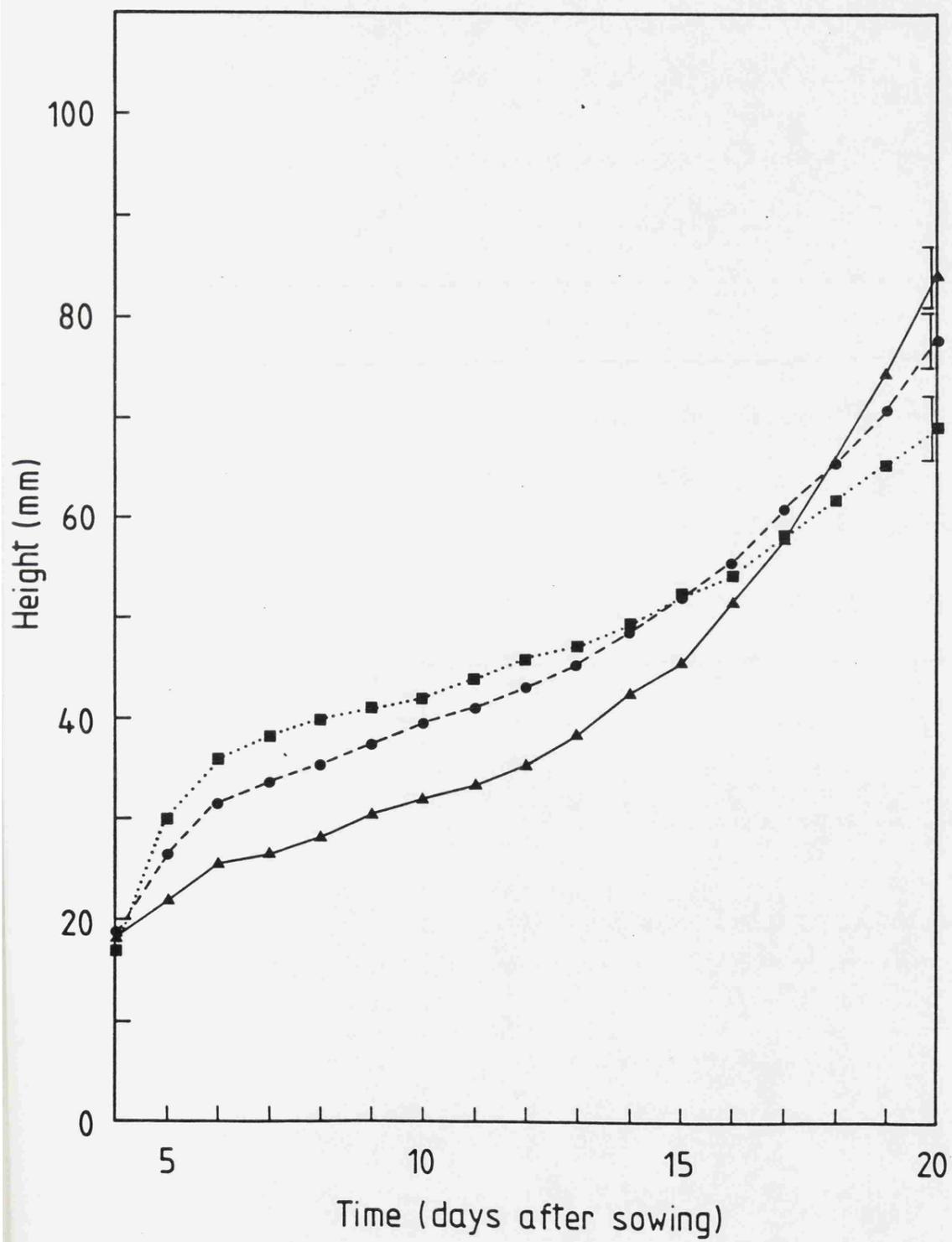


Figure 7B

The effect of fluence rate on stem elongation in Sinapis alba.

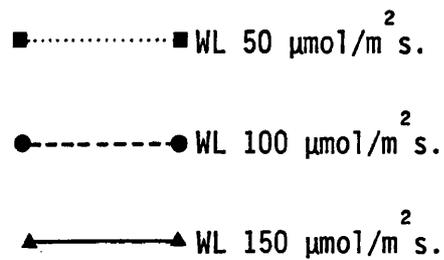
Plants were grown from seed in WL (R:FR = 3.3, $\phi_e = 0.71$) with a 16 h photoperiod at $20 \pm 2^\circ\text{C}$. The photographs were taken on day 20 after sowing and the plants are representative examples grown in WL at (from left to right) 150, 100 and $50 \mu\text{mol/m}^2 \text{s}$. The upper photograph shows the different number of elongating internodes. The lower photograph shows the difference in leaf development.



Figure 8

The effect of fluence rate on hypocotyl elongation in Sinapis alba.

Plants were grown from seed in WL (R:FR = 3.3, $\phi_e = 0.71$) with a 16 h photoperiod at $20 \pm 2^\circ\text{C}$.



A: the relationship between height and time. The bars are \pm S.E. for 10 plants.

B: the relationship between \log_{10} height (mm) and time.

Note: the first point is the height on the first day that the hypocotyl was measurable; i.e. when the hook had opened.

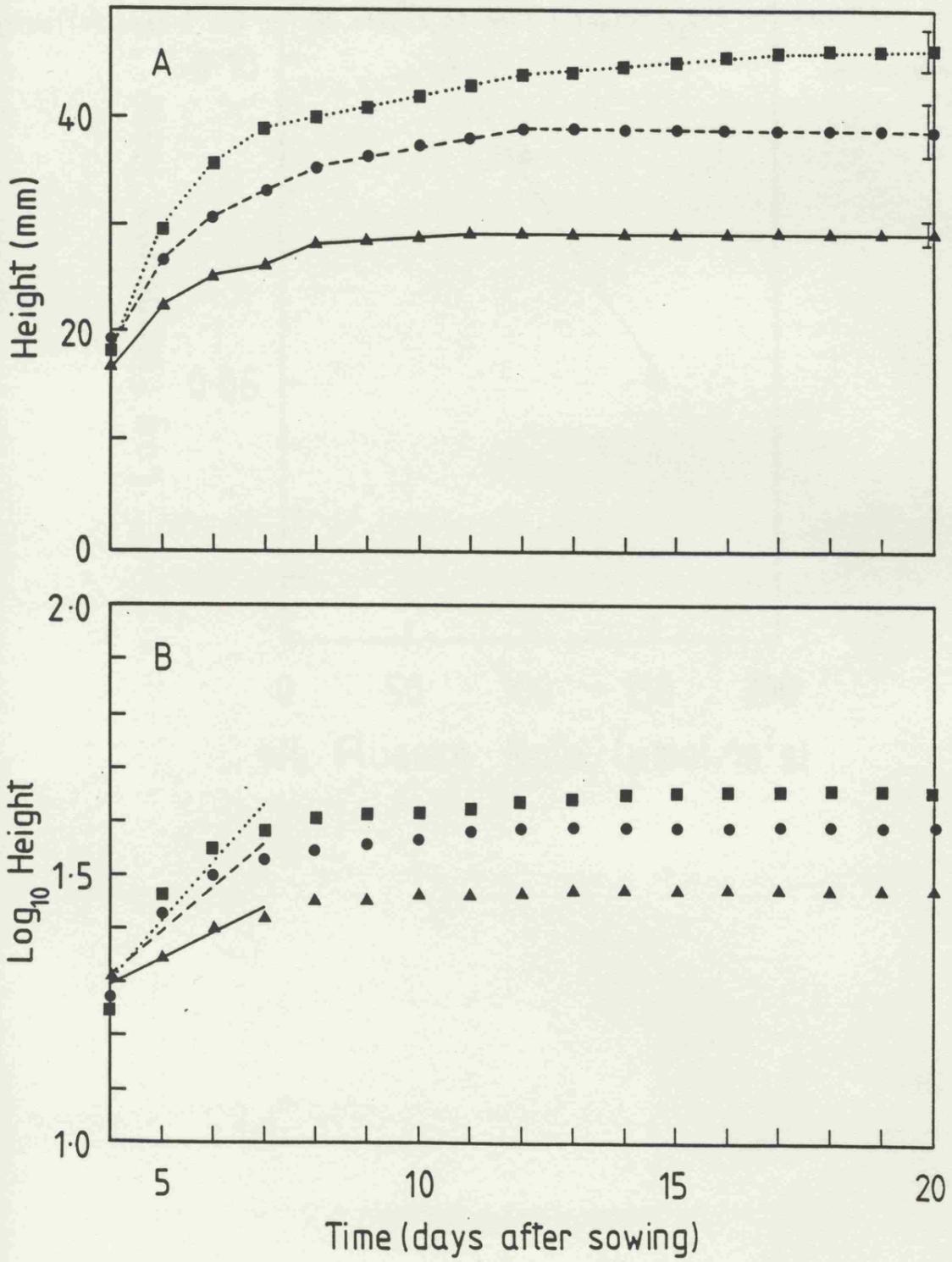


Figure 9

The relationship between hypocotyl elongation and WL fluence rate in Sinapis alba.

The values for logarithmic elongation rate (\log_{10} mm/d) are the slopes of the lines for \log_{10} height v time in Figure 8B for the 3 days following emergence.

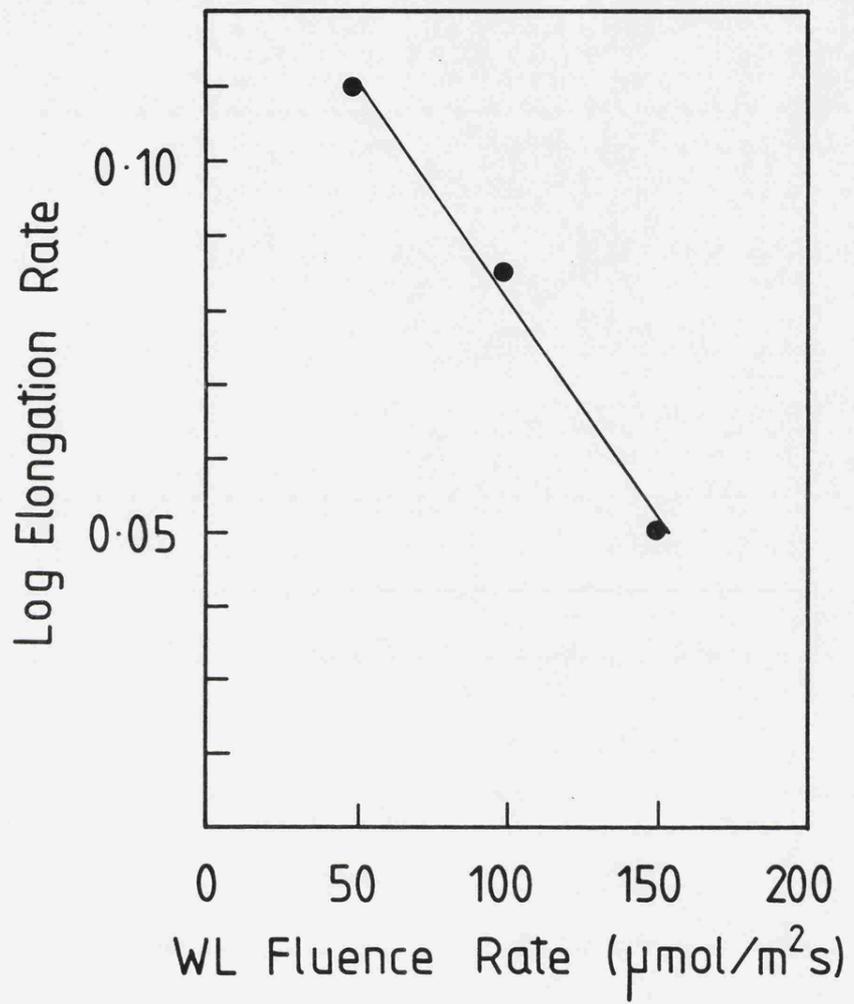


Figure 10

The effect of fluence rate on first internode elongation in Sinapis alba.

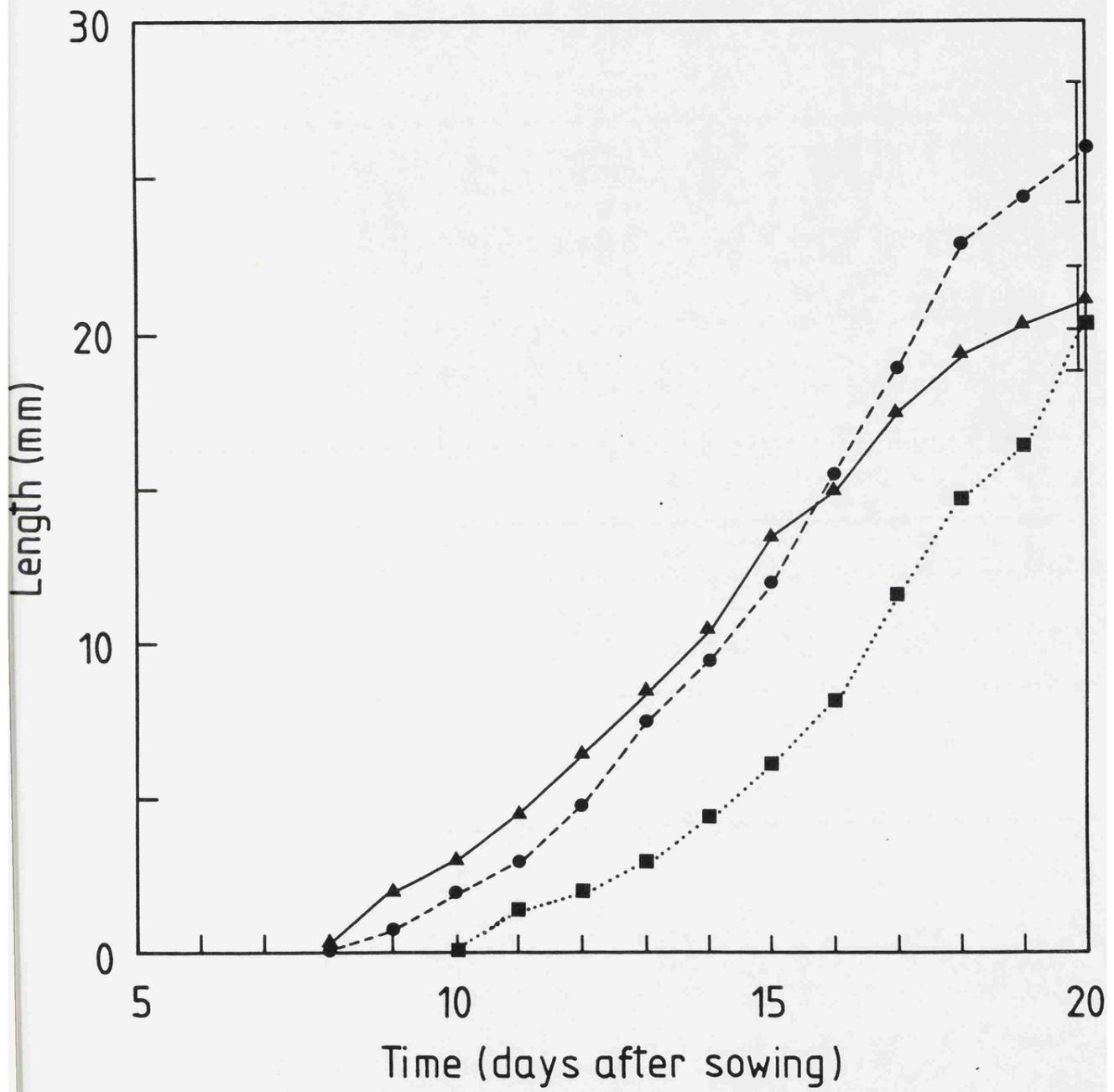
Plants were grown from seed in WL (R:FR = 3.3, $\phi_e = 0.71$) with a 16 h

photoperiod at $20 \pm 2^\circ\text{C}$. The bars are \pm S.E. for 10 plants.

■.....■ WL $50 \mu\text{mol}/\text{m}^2 \text{ s}$.

●-----● WL $100 \mu\text{mol}/\text{m}^2 \text{ s}$.

▲————▲ WL $150 \mu\text{mol}/\text{m}^2 \text{ s}$.



The first internodes of plants in the other light treatments showed no sign of having entered the stationary phase, even by day 20. These results indicate that light accelerates the phases of development, with the consequence that at high light levels the elongation of an internode is completed earlier than in low fluence rates. In addition, the specific length of the first internodes was inversely related to fluence rate and this suggests that at low light levels resources were directed into elongation at the expense of radial growth (Figure 11). As a result of this acceleration of internode development in high fluence rates, plants had different numbers of actively elongating internodes: an average of 3, 4 and 6 internodes longer than 1mm were measured on day 20 in 50, 100 and 150 $\mu\text{mol}/\text{m}^2\text{s}$ respectively.

These results indicate that the observations made by Grime & Jeffrey (1965) and Morgan & Smith (1981) on the effects of fluence rate on stem elongation (described in the introduction) can be explained in terms of different effects on hypocotyl and internode elongation. Grime & Jeffrey included hypocotyl elongation, which is depressed by high fluence rates, in their measurements. In contrast, Morgan & Smith transferred 9 day old plants, which had been grown at a high fluence rate, to a range of WL levels and, therefore, would have measured little, if any, significant effect on hypocotyl elongation.

The short-term effects of changes in WL fluence rate on internode elongation were progressive and statistically insignificant (Table 1). In plants transferred from 100 to 50 $\mu\text{mol}/\text{m}^2\text{s}$

Figure 11

The relationship between specific length (mm/mg) and WL fluence rate for the first internode of Sinapis alba. The bars are \pm S.E. for 10 internodes.

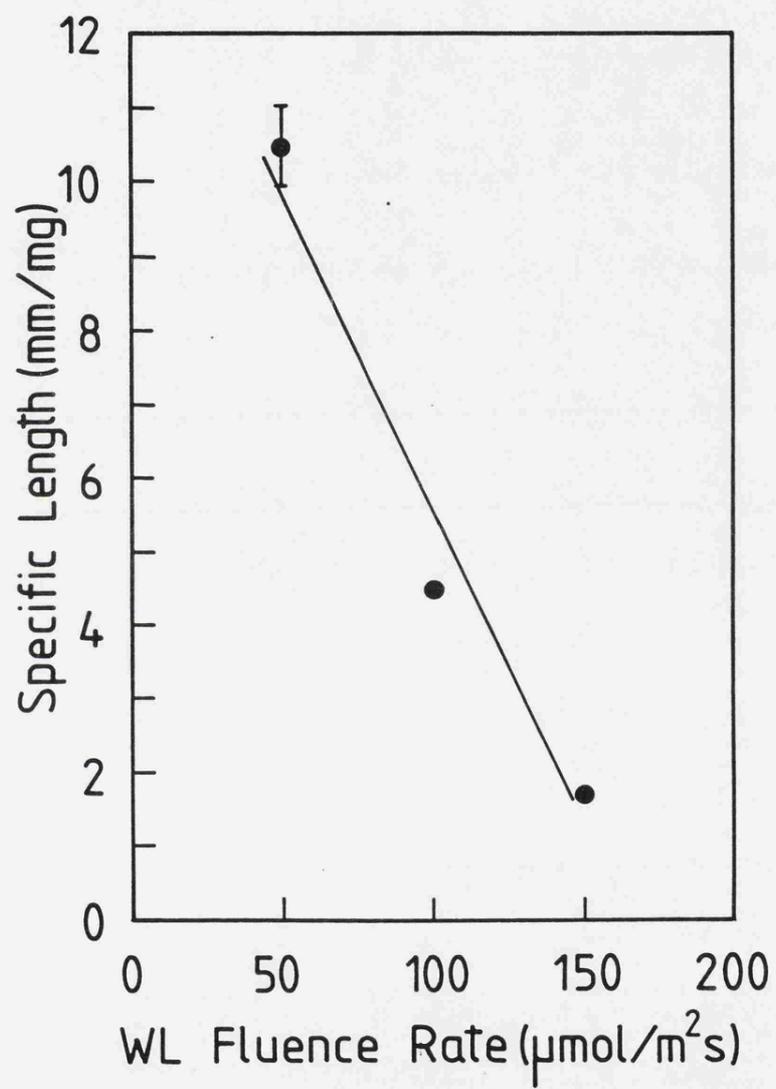


Table 1

The short-term effect of changes in fluence rate on stem elongation in Sinapis alba.

Plants were grown for 14 d in WL (R:FR = 3.3, $\phi_e = 0.71$, $100 \mu\text{mol}/\text{m}^2 \text{s}$), then mounted in transducer apparatus 2 and equilibrated overnight in WL (R:FR = 3.4, $\phi_e = 0.72$, $100 \mu\text{mol}/\text{m}^2 \text{s}$). The change in fluence rate, indicated in the table, was made during the following day. The elongation rate was measured over a 120 min period before and after the change in fluence rate.

ΔER = the change in elongation rate. The values are the means of 6 plants \pm S.E.

WL Fluence Rate ($\mu\text{mol}/\text{m}^2\text{s}$)	Elongation Rate ($\mu\text{m}/\text{min}$)	ΔER
100 \longrightarrow 50	$3.50 \pm 0.40 \longrightarrow 3.30 \pm 0.33$	-0.20 N.S.
100 \longrightarrow 150	$3.51 \pm 0.48 \longrightarrow 3.88 \pm 0.36$	+0.37 N.S.

there was a gradual decline in elongation rate over several hours (Figure 12), while an increase in fluence rate from 100 to 150 $\mu\text{mol}/\text{m}^2\text{s}$ caused a small increase (Table 1).

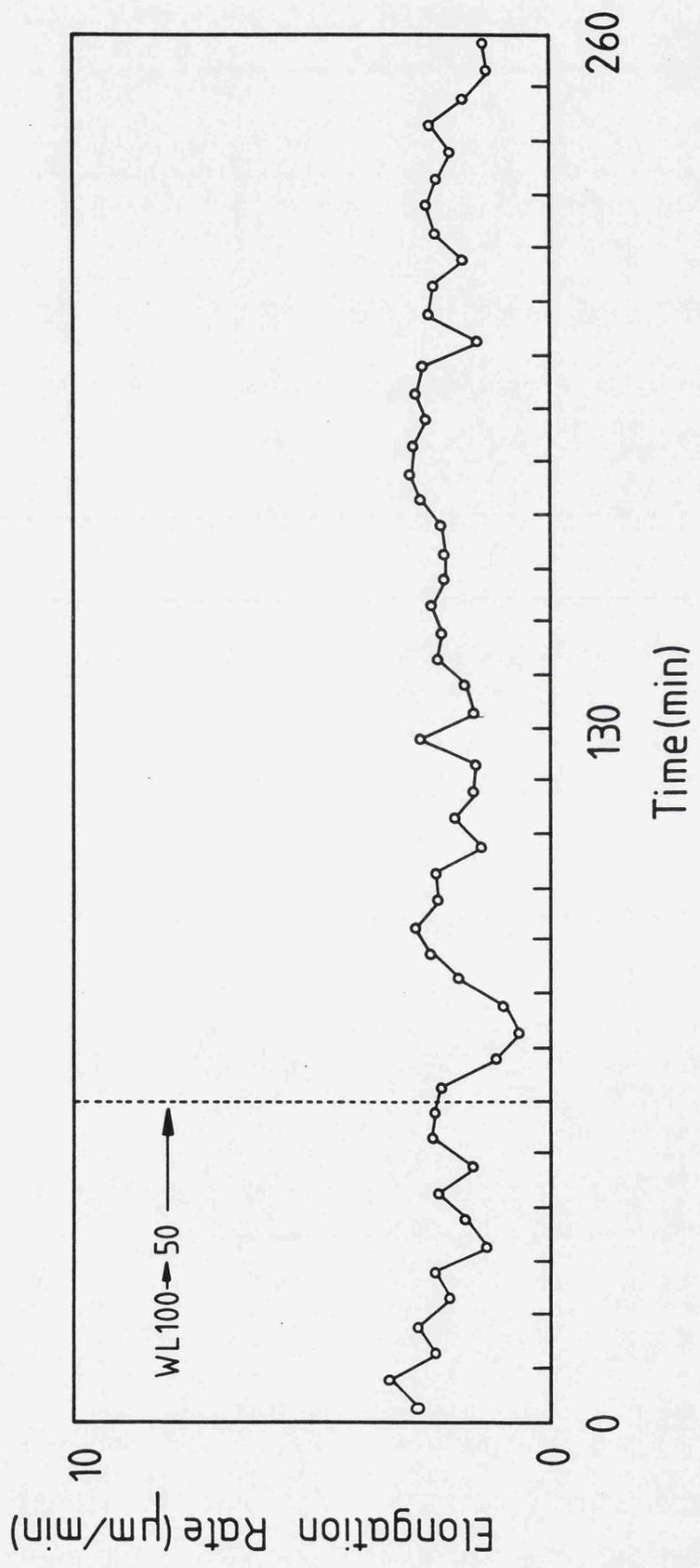
It is known that an increase in light quantity stimulates leaf expansion (see, introduction; and Van Volkenburgh & Cleland, 1979) and this occurred in S. alba (Figure 7B). At high light levels plants have a large area for light capture, combined with a high rate of photosynthesis and, therefore, should produce more photosynthate than plants growing in low fluence rates.

Went (1974) believes that the rate of entry of sugars into a region may alter the pattern of development profoundly, and should be regarded as being of equal importance as the movement of growth regulators - such as auxin or gibberellin. This idea is supported by observations that the levels of sugar and growth regulators interact to modify cell differentiation within callus tissue (e.g., Wetmore & Rier, 1963). Went (1941) found that the light treatment of pea seedlings inhibited the growth of an internode subjacent to a leaf, yet stimulated elongation of the internode directly above the leaf. He suggested that the inhibition of the lower internode reduced its consumption of growth substances produced in the roots and leaves, and made them available to the younger internode. An alternative explanation is that the availability of growth substances is determined by the rate and direction of their translocation from the leaves. In some species more sugar is exported from the leaves at high light levels than at low light levels and

Figure 12

The short-term effect of a reduction in fluence rate on stem elongation in Sinapis alba.

A representative plant grown in WL (R:FR = 3.3, $\phi_e = 0.71$, $100 \mu\text{mol}/\text{m}^2 \text{s}$) for 14 d, then mounted in transducer apparatus 2 and equilibrated overnight in WL (R:FR = 3.4, $\phi_e = 0.72$, $100 \mu\text{mol}/\text{m}^2 \text{s}$). The fluence rate was reduced the following day.



the bulk of this goes towards the stem apex (see, introduction). The idea that fluence rate can modify stem elongation by affecting the partitioning of substances between organs has been questioned by Gaba & Black (1983). They point out that isolated stem segments also show differential responses to light, which implies that photocontrol occurs in the organ itself.

Thomson (1950) noted that the response to light depended on the developmental stage of the organ - older internodes were inhibited by light, while young ones were promoted - but that all stages were accelerated by an increase in fluence rate. The results for the elongation of internodes in S. alba are consistent with this hypothesis. Elongation started earlier at 150 and 100 $\mu\text{mol}/\text{m}^2\text{s}$ than at 50 $\mu\text{mol}/\text{m}^2\text{s}$, but was completed earlier at the highest fluence rates; so the cells of internodes at 150 $\mu\text{mol}/\text{m}^2\text{s}$ may enter the maturation phase sooner than those at the lower fluence rates and have less time to enlarge. The results for hypocotyl elongation cannot be interpreted in quite the same way, because elongation started at the same time in all light treatments. Although the period of maximum elongation was shortest at high light levels, this was not as important as in the internodes, because in all the hypocotyls the majority of the elongation took place within 4 days of emergence when the rate was inversely related to fluence rate. In corresponding internodes at different light levels, the phases of maximum elongation did not coincide and were of different duration; this makes direct comparisons of elongation rates difficult.

The identity of the photoreceptors involved in controlling these patterns of development is unclear and there are problems in evoking any of the known photoreceptors in photoperception of light quantity by the internode itself (see, the following sections).

The Effect of B on Stem Elongation

Supplementary B to either the internode, or the internode and the leaves, had no significant effect on stem elongation in plants grown in a variety of conditions (Table 2, Figure 13). These results are consistent with the view that there is no specific B-photoreceptor involved in the control of non-directional axial elongation in S. alba (Wildermann et al, 1978). The photocontrol of stem elongation by B acting through a B-photoreceptor is characterised by a rapid inhibition of elongation (see, introduction).

Phytochrome could not detect B via photoequilibrium under these conditions, because R is over an order of magnitude more efficient at photoconverting phytochrome than B (Gardner & Graceffo, 1982); so the photoequilibrium in either WL or R will be high, and unaffected by the additional B.

The lack of involvement of a B-photoreceptor in this species could explain the differences in the responses to WL of S. alba, Chenopodium album and Vigna sinensis. A B-photoreceptor may be responsible for the rapid inhibition of elongation in C. album transferred from low to high fluence rate WL (Morgan & Smith, 1978a), because in the closely related C. rubrum, B inhibits hypocotyl

Table 2

The effect of supplementary B on stem elongation in Sinapis alba.

- Treatments: 1: plants grown for 13 d in WL (R:FR = 3.3, $\phi_e = 0.71$, $100 \mu\text{mol/m}^2 \text{s}$), then equilibrated in continuous R (SOX, $\lambda \text{ max } 585 \text{ nm}$, $\phi_m = 0.76$, $50 \mu\text{mol/m}^2 \text{s}$) for 24 h in transducer apparatus 2. B ($\lambda \text{ max } 452 \text{ nm}$, $20 \mu\text{mol/m}^2 \text{s}$) was given simultaneously to the first internode and primary leaves by fibre-optic probes.
- 2: plants grown for 14 d in continuous R (SOX, $\lambda \text{ max } 585 \text{ nm}$, $\phi_m = 0.76$, $50 \mu\text{mol/m}^2 \text{s}$). B ($\lambda \text{ max } 452 \text{ nm}$, $20 \mu\text{mol/m}^2 \text{s}$) was given to the first internode by fibre-optic probes.

The elongation rate was measured over a 120 min period before and after the addition of supplementary B. ΔER = the change in elongation rate. The values are the means of 6 plants \pm S.E.

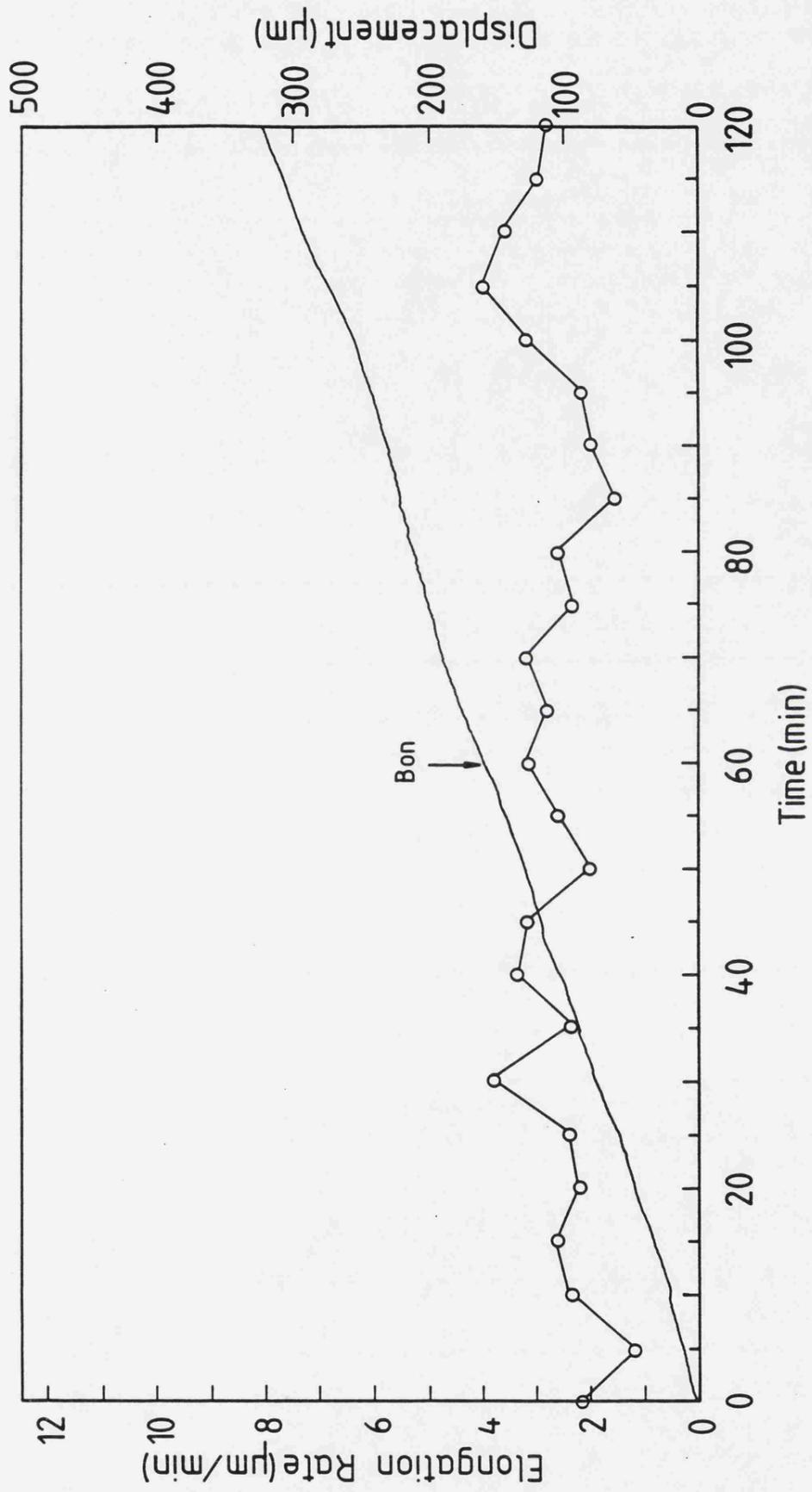
Treatment	Elongation Rate ($\mu\text{m}/\text{min}$)		ΔER
	-B	+B	
1	3.66 ± 0.55	3.85 ± 0.57	+0.19 N.S.
2	3.52 ± 0.69	4.21 ± 0.88	+0.69 N.S.

Figure 13

The effect of supplementary B on stem elongation in Sinapis alba.

A representative plant grown for 13 d in WL ($100 \mu\text{mol}/\text{m}^2 \text{ s}$, R:FR = 3.3, $\phi_e = 0.71$) with a 16 h photoperiod at $20 \pm 2^\circ\text{C}$, then mounted in transducer apparatus 2 in continuous R (SOX, $\lambda_{\text{max}} 585\text{nm}$, $50 \mu\text{mol}/\text{m}^2 \text{ s}$, $\phi_m = 0.76$).

B ($\lambda_{\text{max}} 452 \text{ nm}$, $20 \mu\text{mol}/\text{m}^2 \text{ s}$) was given simultaneously to the first internode and primary leaves via fibre-optic probes as indicated.



elongation (Ritter et al, 1981). The more progressive responses of V. sinensis to light quantity (Lecharny & Jacques, 1980) are not typical of B-inhibition, but this cannot be ruled out.

The Effect of R:FR on Stem Elongation

The inverse relationship between stem elongation and R:FR or ϕ is well-established for many species, including S. alba, in which seedlings have been transferred from WL to light regimes representing different degrees of vegetational shade (see, introduction; and O'Brien, 1980). Rarely, however, have plants been grown from seed in different R:FR, so that both hypocotyl elongation and the inception of internode elongation can be followed.

At different R:FR, as with different WL fluence rates, the most extensive hypocotyl elongation occurred in the 4 days after emergence, and was inversely related to ϕ (Figures 14 and 15). Following this phase of rapid growth, hypocotyl elongation slowed (at the same time in all treatments) to a steady-state, independent of light quality (Figure 14). These results show that in the rapid phase of hypocotyl development following emergence, elongation was inversely related to both WL fluence rate and R:FR, and they agree with those of Holmes et al (1982). A difference between the responses to light quantity and light quality is that the duration of the log phase of elongation, which was lower at high WL fluence rates than at low light levels, was unaffected by R:FR.

Figure 14

The effect of R:FR on hypocotyl elongation in Sinapis alba.

Plants were grown from seed in custom-built growth cabinets giving variable R:FR at equal PAR ($40 \mu\text{mol/m}^2 \text{s}$). The photoperiod was continuous at $20 \pm 1^\circ\text{C}$.



A: the relationship between height and time. The bars are \pm S.E. for 10 plants.

B: the relationship between \log_{10} height (mm) and time.

Note: the first point is the height on the first day that the hypocotyl was measurable; i.e. when the hook had opened.

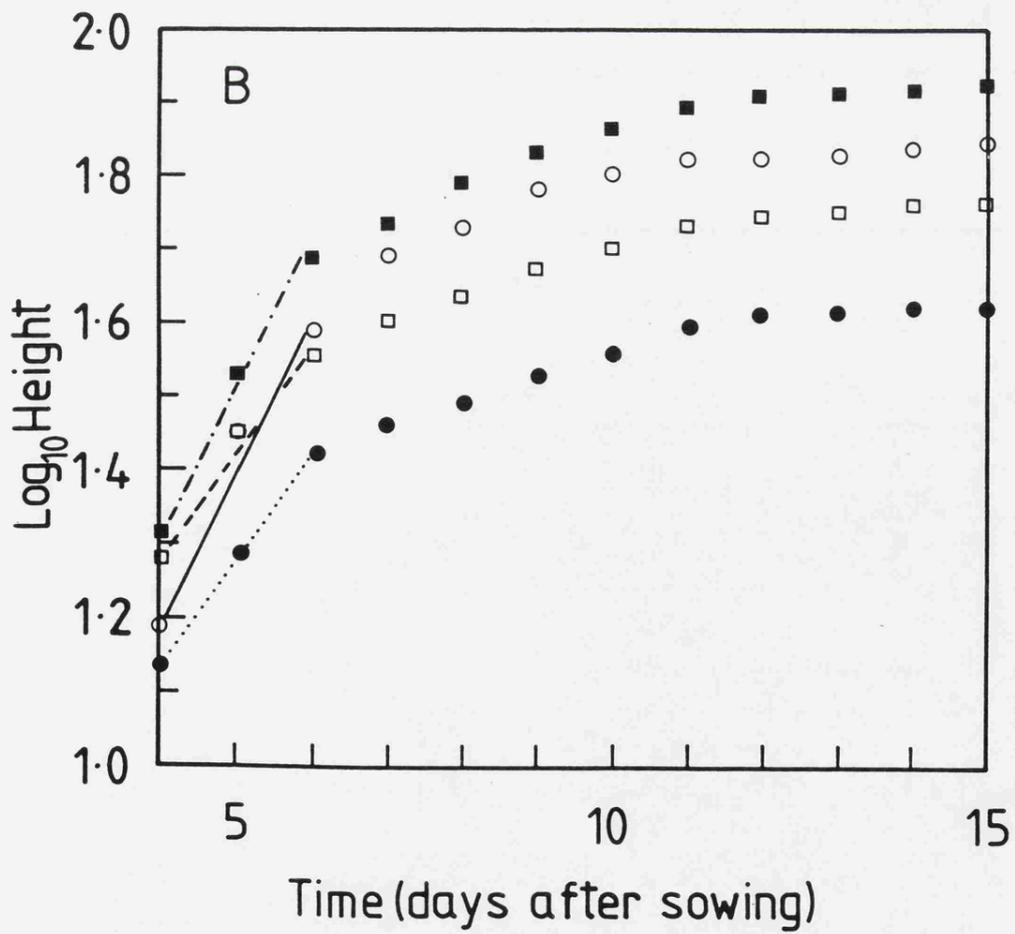
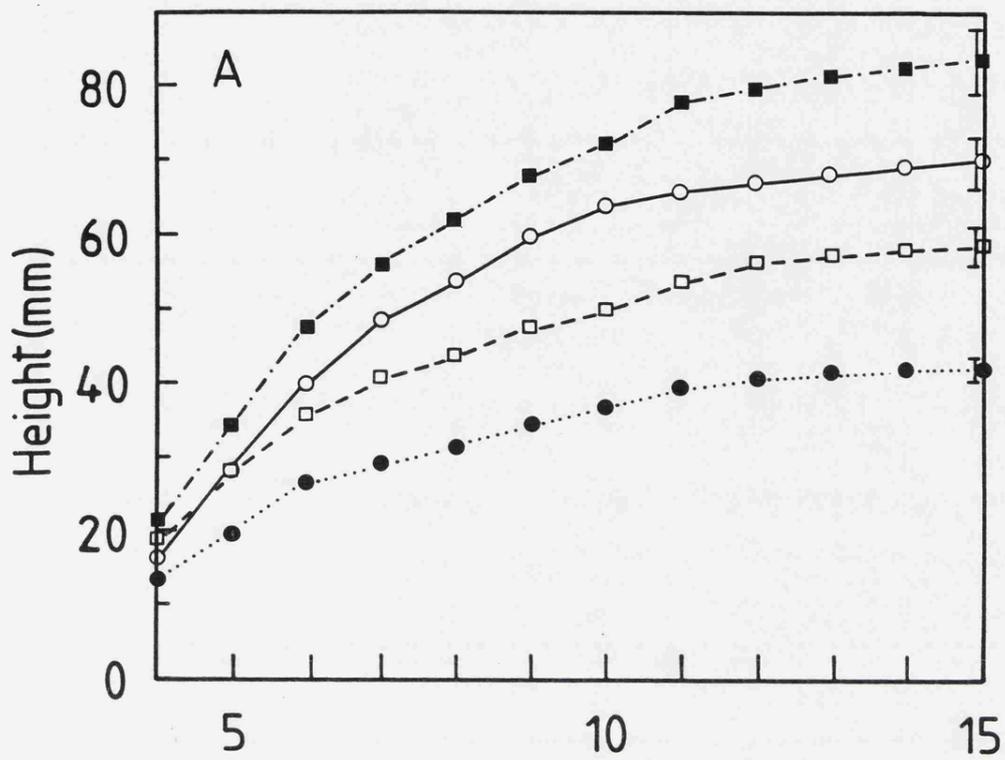
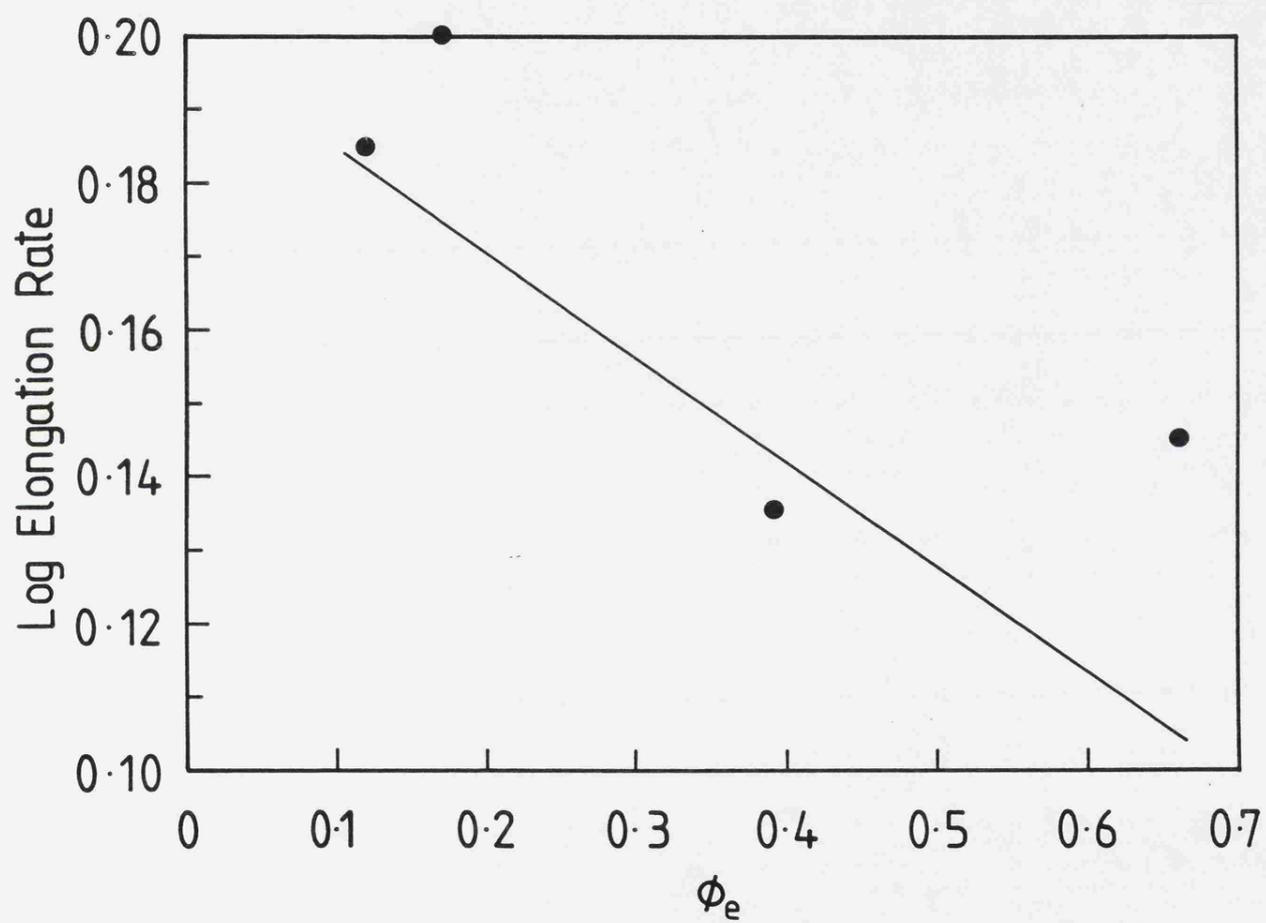


Figure 15

The relationship between hypocotyl elongation and ϕ_e in Sinapis alba.

The values for logarithmic elongation rate (\log_{10} mm/d) are the slopes of the lines for \log_{10} height v. time in Figure 14B for the 3 days following emergence.



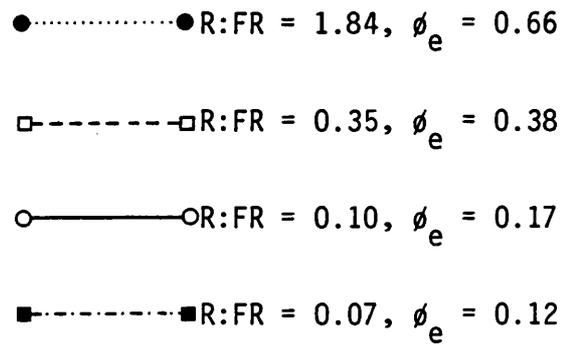
The onset of internode elongation in the four R:FR was spread over 2 days and was earliest in the lowest R:FR (Figure 16), but there was no detectable slowing of elongation rate at day 15. Despite this apparent acceleration of development at low R:FR, the plants in each treatment had on average 3 internodes longer than 1mm on day 15. These results are consistent with those reported for C. album, in which the elongation phase of internodes is accelerated at low R:FR, but light quality does not affect the rate of production of new internodes (Child et al, 1981a). In contrast to light quantity, R:FR did not appreciably affect the specific length of the first internode (Figure 17), nor was there any change in the relationship between R:FR and plant height during the course of the experiment - that is, height was always inversely related to ϕ (Figure 18) (at different WL fluence rates height initially was inversely related to fluence rate, but by day 20 was positively related to fluence rate).

The short-term experiments demonstrate that both 14 and 20d old plants responded to supplementary FR given to the first internode in background WL, with a rapid increase in elongation rate (Figure 19). In 14d old plants stem elongation occurred mainly in the first internode, which is in the log phase of its growth (Figures 7 and 10) and is ideal for examining the effect of R:FR on internode elongation. It was important to determine whether 20d old internodes responded in the same way as their younger counterparts, because they were used in the experiments on isolated segments (see, section 2).

Figure 16

The effect of R:FR on first internode elongation in Sinapis alba.

Plants were grown from seed in custom-built growth cabinets giving variable R:FR at equal PAR ($40 \mu\text{mol/m}^2 \text{s}$). The photoperiod was continuous at $20 \pm 1^\circ\text{C}$. The bars are \pm S.E. for 10 plants.



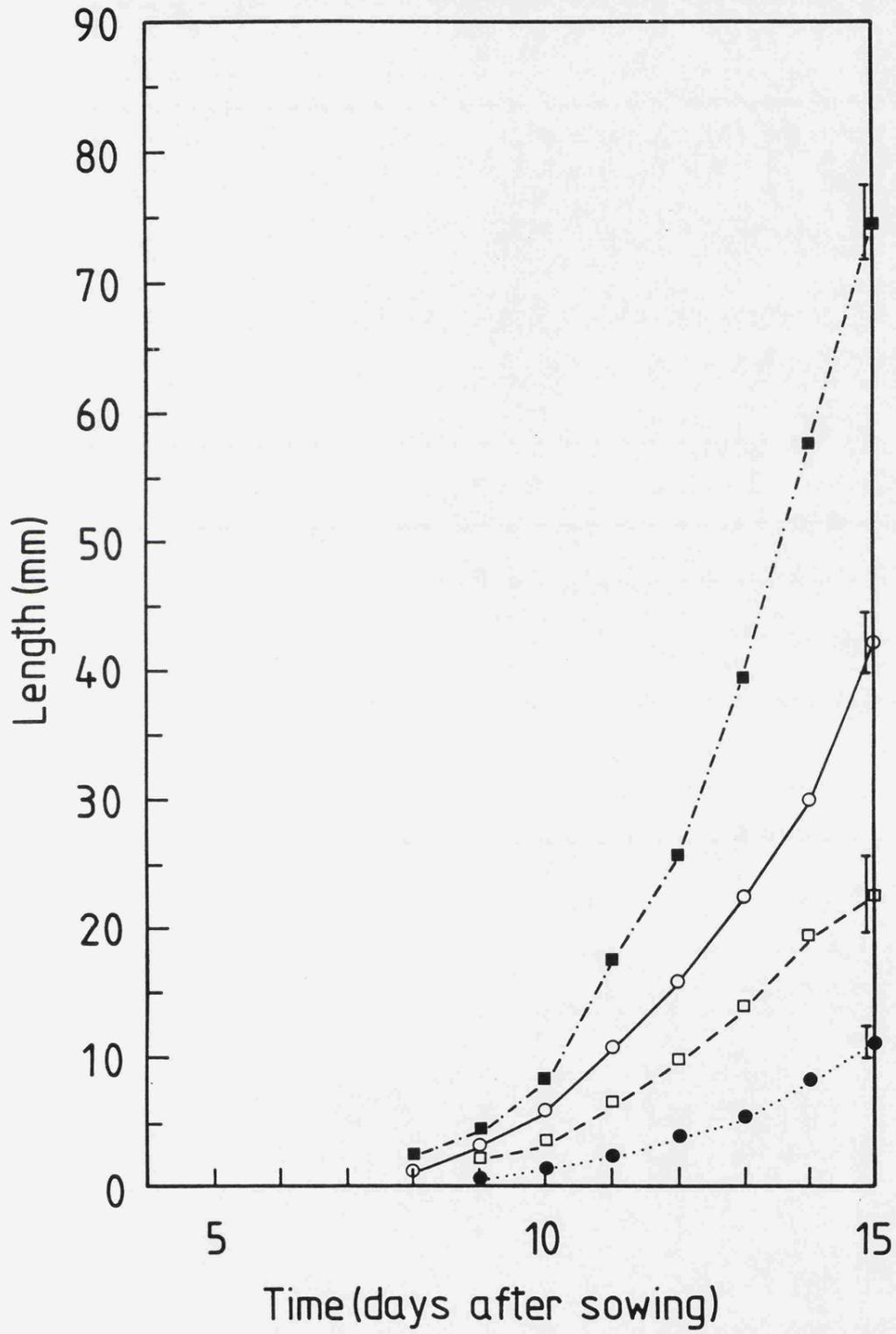


Figure 17

The relationship between specific length (mm/mg) and ϕ_e for the first internode of Sinapis alba. The bars are \pm S.E. for 10 internodes.

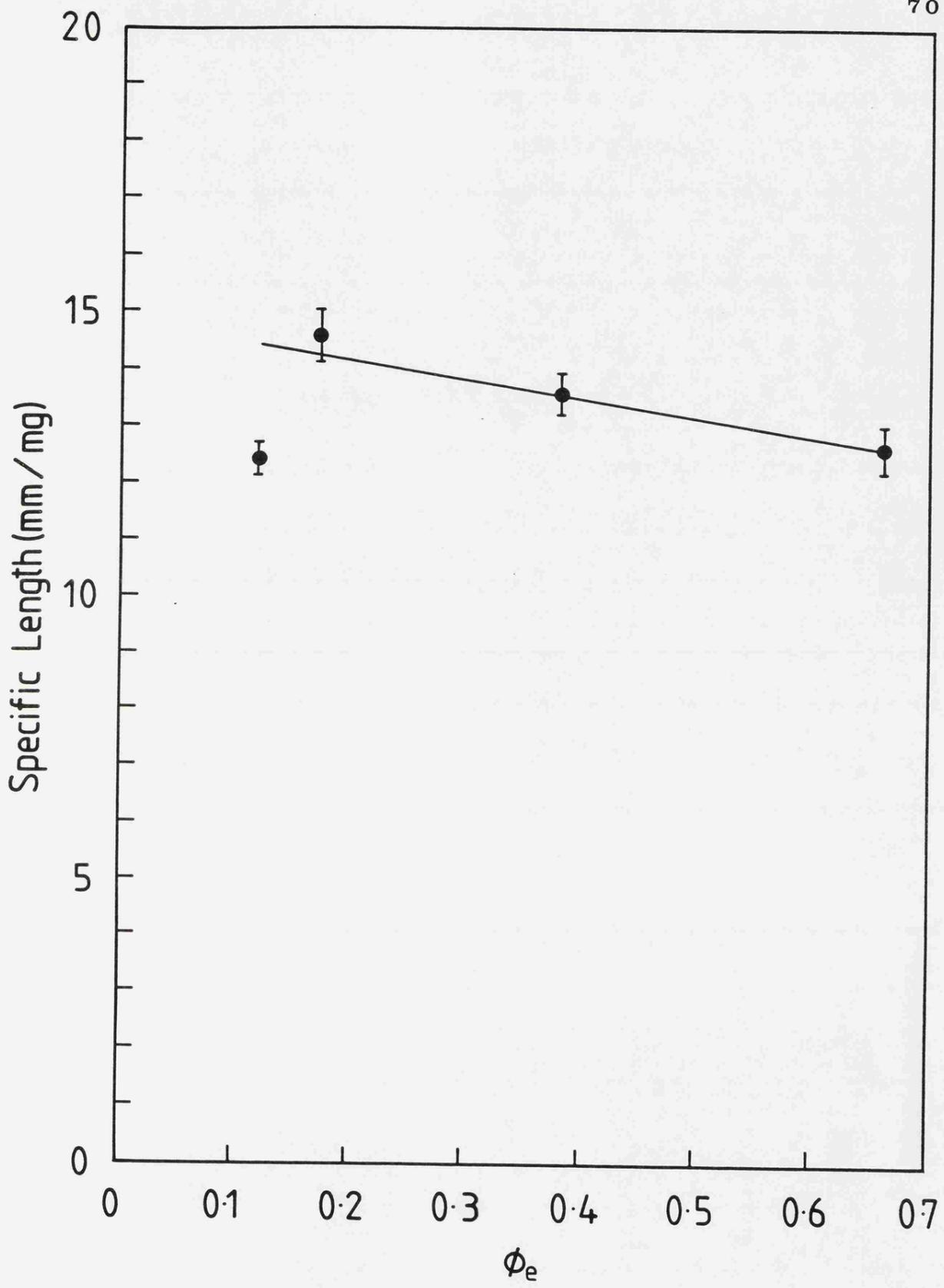


Figure 18A

The effect of R:FR on stem elongation in Sinapis alba.

Plants were grown from seed in custom-built growth cabinets giving variable R:FR at equal PAR ($40 \mu\text{mol}/\text{m}^2 \text{s}$). The photoperiod was continuous at $20 \pm 1^\circ\text{C}$.



The bars are \pm S.E. for 10 plants.

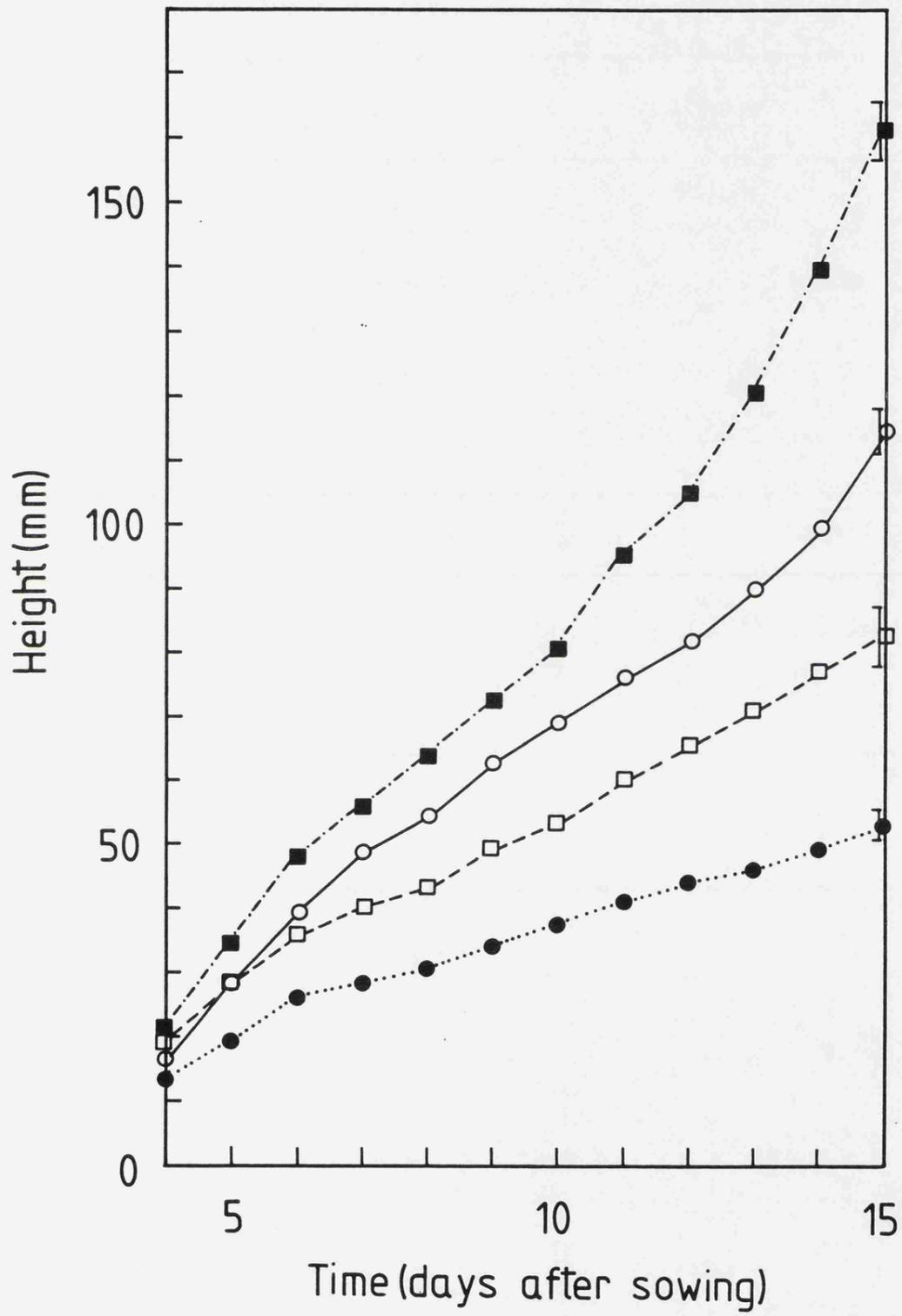


Figure 18B

The effect of R:FR on stem elongation in Sinapis alba.

Plants were grown from seed in custom-built growth cabinets giving variable R:FR at equal PAR ($40 \mu\text{mol}/\text{m}^2 \text{ s}$). The photoperiod was continuous at $20 \pm 1^\circ\text{C}$. The photograph was taken on day 15 after sowing and the plants are representative examples grown in R:FR of (from left to right) 0.07, 0.10, 0.35, 1.84. After 15 d the plants at the lowest R:FR had developed flower primordia.

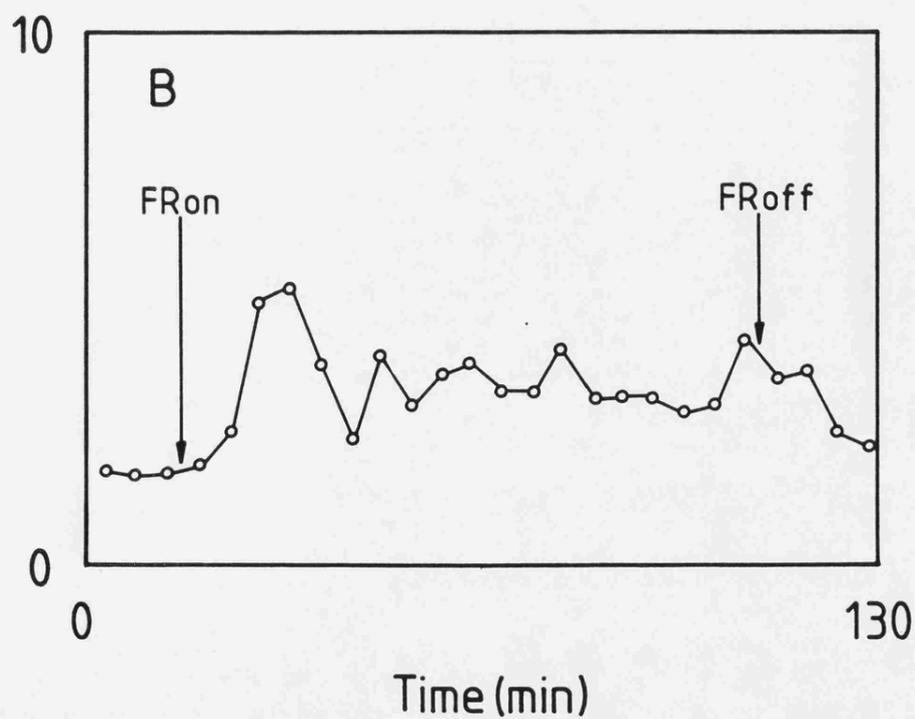
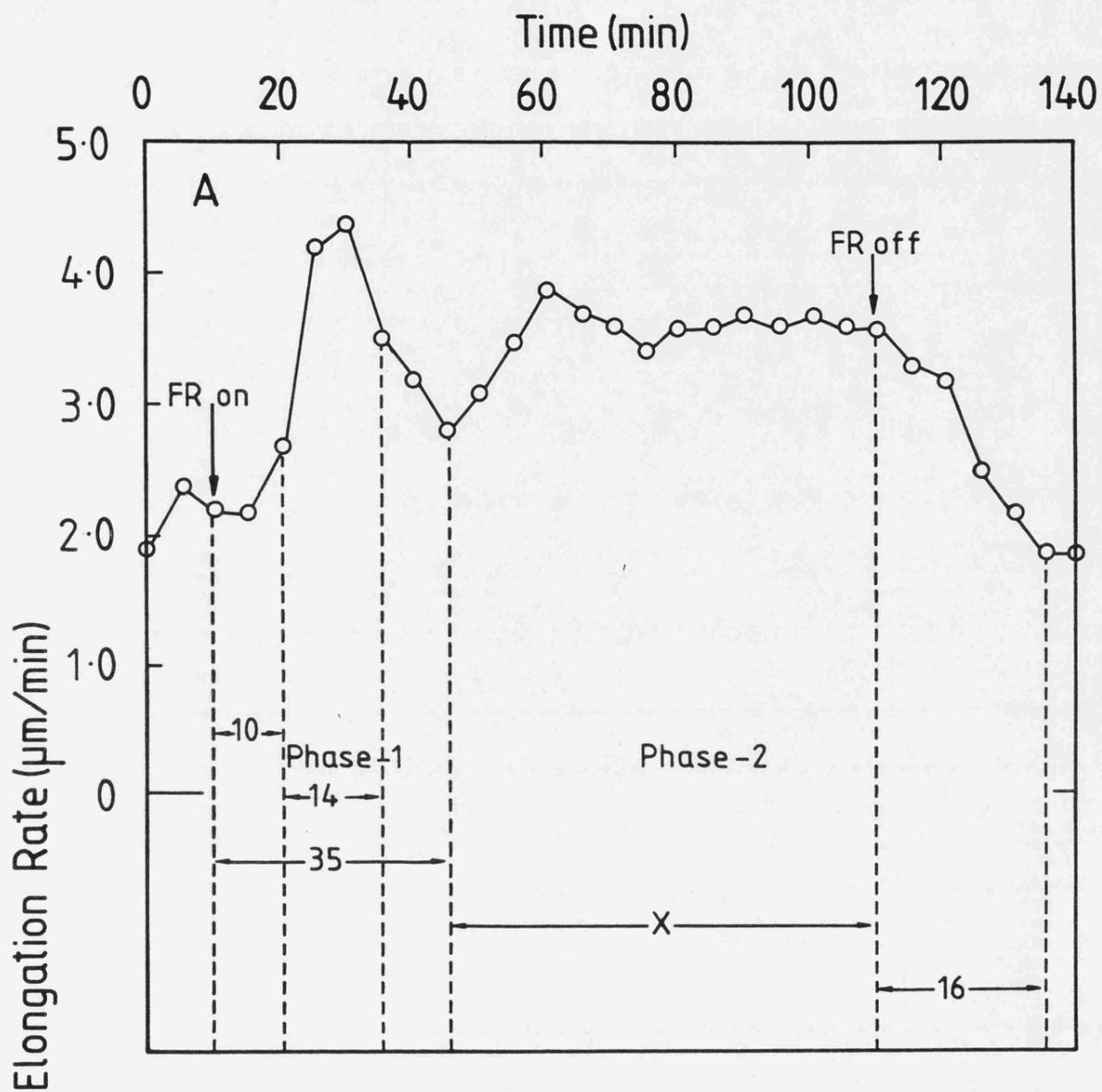


Time (min)

Figure 19

The rapid response of first internode elongation to supplementary FR in Sinapis alba.

- A: 14 d old plants grown in WL ($100 \mu\text{mol}/\text{m}^2 \text{ s}$, R:FR = 3.3, $\phi_e = 0.71$) were mounted in transducer apparatus 2 and equilibrated in WL ($50 \mu\text{mol}/\text{m}^2 \text{ s}$, R:FR = 3.4, $\phi_e = 0.72$). Supplementary FR ($\lambda_{\text{max}} 730 \text{ nm}$, $100 \mu\text{mol}/\text{m}^2 \text{ s}$) was given to the first internode via fibre-optic probes as indicated. The values for each phase of the response are the mean values, for 10 plants, to the nearest minute. The duration of the second phase, represented by X, depends upon the duration of the supplementary FR. Each data point is the mean of 10 plants.
- B: A representative 20 d old plant given an identical treatment to that described above except that the background WL was $100 \mu\text{mol}/\text{m}^2 \text{ s}$.



A detailed analysis of the response of 14d old plants is shown in Figure 19A. The lag period was about 10 min and was followed by a large increase in growth rate - phase-1 - which lasted for about 14 min. The elongation rate then fell to a level that was still above the WL rate, before rising again to a steady-state rate. This steady-state - phase-2 - was attained about 35 min after the FR was given and remained constant while the FR was on. When the FR was switched off the elongation rate started to decline within 2-3 min and reached the original WL rate after about 16 min. The decay time for the return to the WL level was independent of the duration of the FR treatment (Table 3).

Much of this agrees with the kinetics for the response of the first internode to added FR described by Morgan et al (1980), but there are two important differences: first, they do not report the dip between phases 1 and 2; second - and these are considered together because they are probably related phenomena - they found that when the FR was switched off the elongation rate declined, but not to the original rate, and that with prolonged FR there was a third increase in rate. The reason why they did not report the dip is probably trivial: that they simply were not looking for it, and it is not as clear as the two early phases. The second difference is more serious; it may be the result of the responses of internode elongation to FR given to the leaves and to the stem, being superimposed on each other. The two fibre-optic probes, which were positioned 20 mm from the first internode, could have irradiated the leaves;

Table 3

The decay kinetics for the rapid response of first internode elongation to supplementary FR in Sinapis alba.

The time taken for the elongation rate to return to the original WL level following FR treatments of different duration. Plants were grown for 14 d in WL (R:FR = 3.3, $\phi_e = 0.71$, $100 \mu\text{mol/m}^2 \text{s}$) then equilibrated in WL (R:FR = 3.4, $\phi_e = 0.72$, $50 \mu\text{mol/m}^2 \text{s}$) in transducer apparatus 2. Supplementary FR (λ max 730 nm, $100 \mu\text{mol/m}^2 \text{s}$) was given to the first internode by fibre-optic probes for the period of time indicated. The values are the means of 10 plants \pm S.E.

Duration of FR (min)	Decay Time (min)
35	15.7 ± 0.65
60	16.1 ± 1.30
90	14.5 ± 1.89
180	16.4 ± 3.40

an examination of the results of Morgan et al indicates that this explanation is tenable. The rapid modulation of stem elongation using pulses of R added to WL + FR show that the elongation rate did return to the original rate (or even less than the original rate) when FR treatments were for less than 100 min. If, however, the FR was given for more than about 100 min the rate did not fall to the WL level when the FR was switched off. The third phase occurred 80 to 300 min after the onset of FR to the internode, while the response to FR given to the primary leaves had a latent period of about 120 min. It is possible, therefore, that irradiation of the primary leaves with FR caused the release of a substance which was translocated to the internode where it stimulated elongation; and that this stimulation persisted for some time after the FR was removed.

The internode of 14d old S. alba was sensitive to brief pulses of FR (Figures 20 and 21). With pulses of FR less than about 40 min (i.e., the time taken for a full display of the response), that portion of the response was exhibited which could occur within the duration of the FR treatment, plus the normal time taken for the response to decay (i.e., 16 min). A FR pulse of 1 min gave a response equivalent to part of the first phase (Figure 20), and this rapid escape from reversibility shows that phytochrome action is swift in this response. The internode was also able to respond to repeated short pulses of FR (Figure 20) and this will be a great advantage for plants living in a canopy in which light quality is continually fluctuating (e.g., because

Figure 20

The rapid response of first internode elongation to supplementary FR in Sinapis alba: the effect of FR treatments of different duration.

14 d old plants were treated as described in Figure 19A. Each trace is of a representative plant and the duration of the FR is indicated.

●————● = elongation rate ($\mu\text{m}/\text{min}$).
———— = the original chart trace.

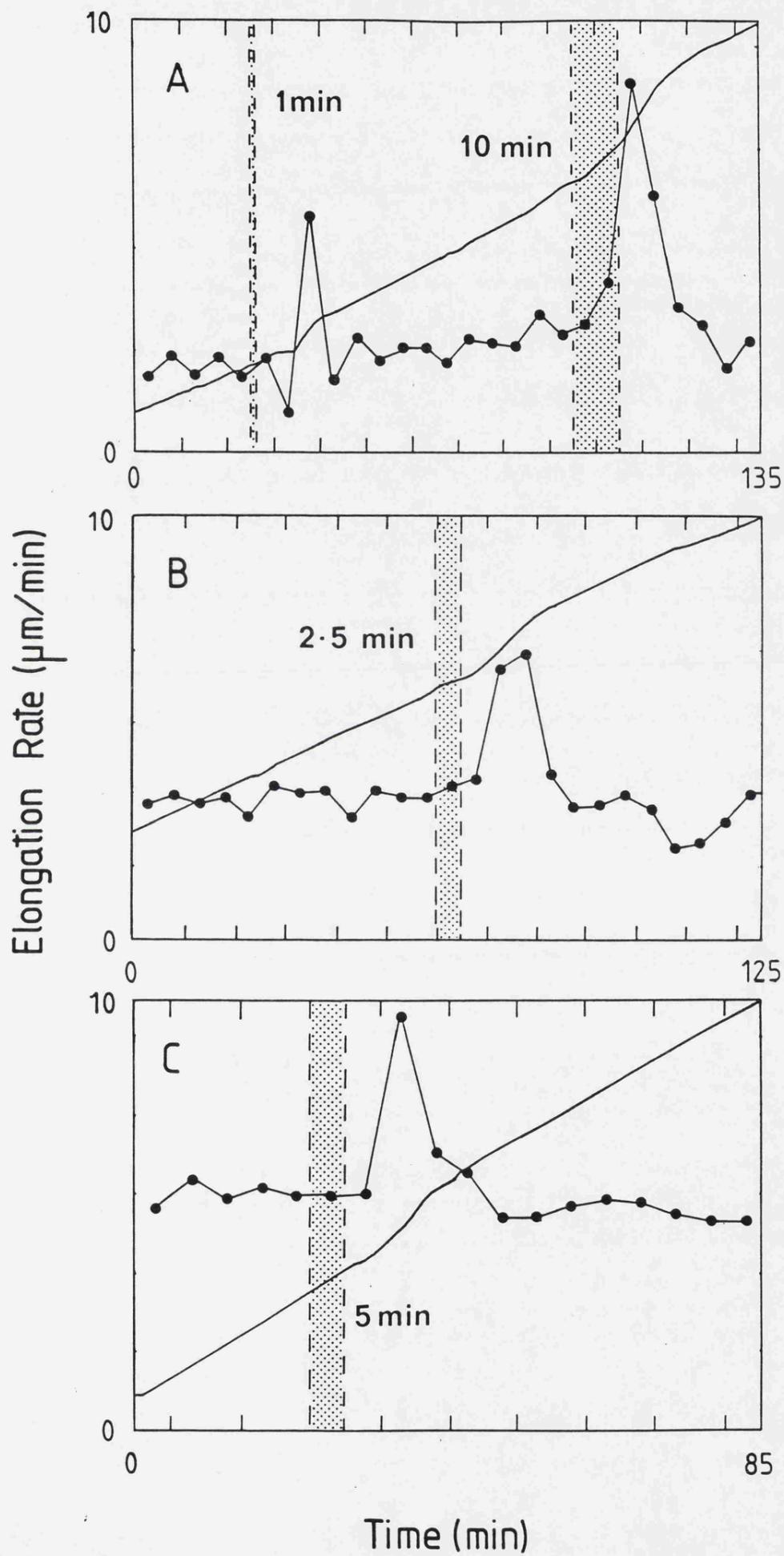
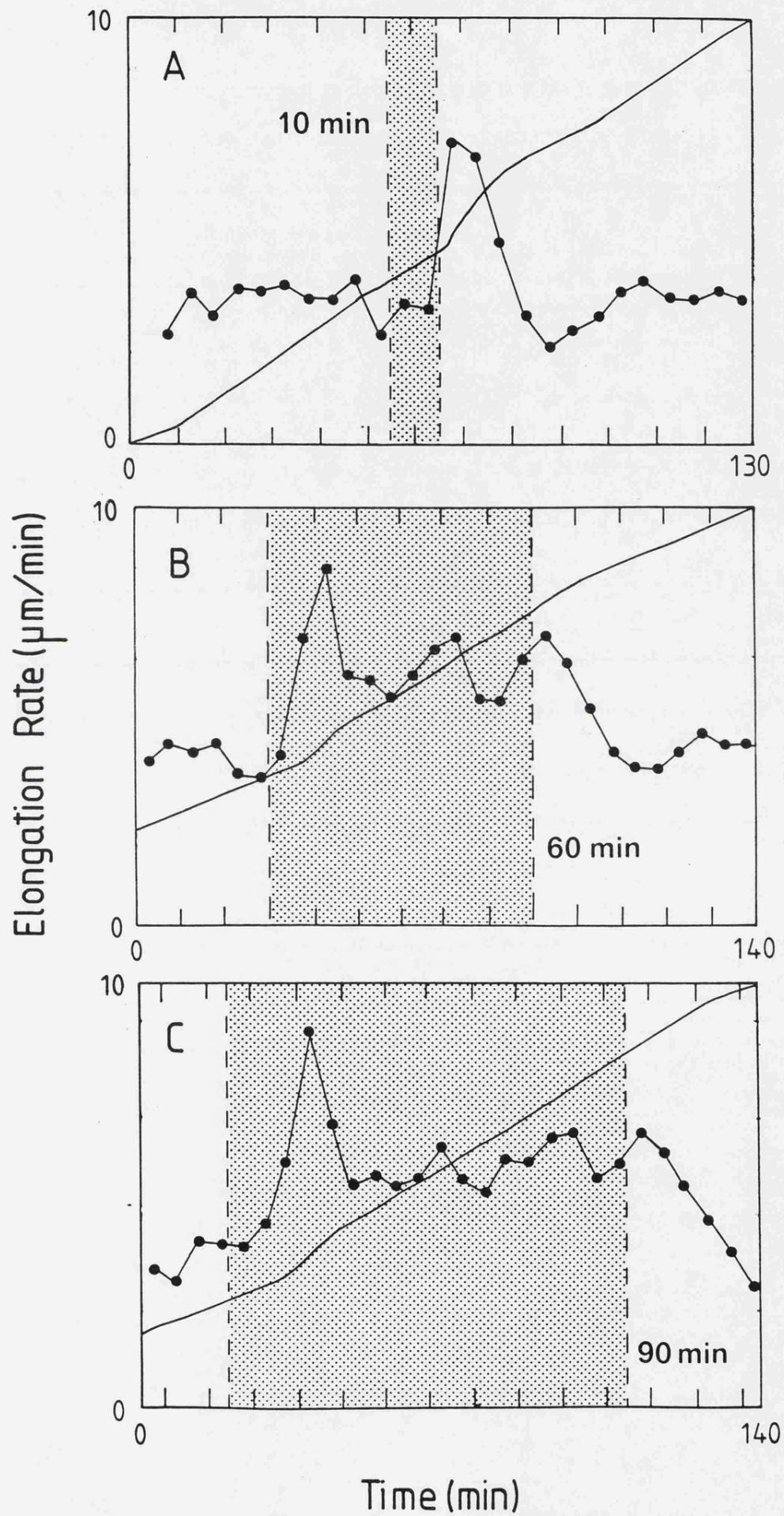


Figure 21

The rapid response of first internode elongation to supplementary FR in Sinapis alba: the effect of FR treatments of different duration.

14 d old plants were treated as described in Figure 19A. Each trace is of a representative plant and the duration of the FR is indicated.

●————● = elongation rate ($\mu\text{m}/\text{min}$).
———— = the original chart trace.



of leaf movement). The plants will be capable of rapidly modulating their stem elongation in accordance with the duration of reductions in R:FR and of integrating a number of short periods of shade. The rapid response to supplementary FR was inversely related to ϕ (ϕ measured in etiolated oat coleoptile tissue) over the range $\phi = 0.17$ to 0.63 (Figures 22 & 23). This is a wider range than previously has been reported for this response (Morgan et al, 1980; 1981) and elongation rate $v \phi$ was linear over the whole range.

The fluence rates used in this experiment were considerably higher than those of Wall & Johnson (1982) and a photoequilibrium was reached in under 2 min (Figure 24); so there were no complications resulting from the effects of thermal phytochrome reactions on photoequilibrium.

The first phase of the FR response has been explained in terms of the release of a potential for elongation which has accumulated in WL, and the second phase may represent the state when elongation is limited by the rate at which this potential is replenished (Morgan et al, 1980). In this hypothesis the two phases are regarded as different expressions of the same underlying phytochrome response and it is supported by the results shown in Figures 22 and 23, where both phases were related to ϕ in a similar way.

In Figures 22 and 23 the elongation rate was determined predominantly by ϕ in each of the three background WL fluence rates, namely: 50, 100 and $150 \mu\text{mol/m}^2\text{s}$. The experiments of the two preceding

Figure 22

The relationship between elongation rate and ϕ_m^* for the rapid response to supplementary FR in Sinapis alba: the relationship for the phase-1 response in different background WL fluence rates.

14 d old plants grown in WL ($100 \mu\text{mol/m}^2 \text{s}$, R:FR = 3.3, $\phi_e = 0.71$) were mounted in transducer apparatus 2 in WL (R:FR = 3.4, $\phi_e = 0.72$) and given two 90 min FR (λ max 730 nm) treatments at fluence rates between 1.4-102 $\mu\text{mol/m}^2 \text{s}$ to the first internode. Each data point is the mean of at least 10 measurements, but the statistics were based on all the measurements and were done according to Sokal & Rohlf (1969).

Linear Regression

Background ₂ WL fluence rate ($\mu\text{mol/m}^2 \text{s}$)	Y intercept	Regression Coefficient	S.E.	d.f.	t
■.....■ 50	9.11	- 9.05	1.841	7	4.915 **
●-----● 100	11.43	-11.43	1.181	7	9.682 ***
▲-----▲ 150	11.73	-12.87	1.396	7	9.216 ***
———— Average	10.70	-10.70			

Analysis of Variance of Regression

Source	d.f.	s.s.	m.s.	F
among slopes	2	31.8	15.9	1.562 N.S.
weighted average	21	213.8	10.18	

* ϕ_m was measured 3 times at each treatment and the mean value was used for the graph.

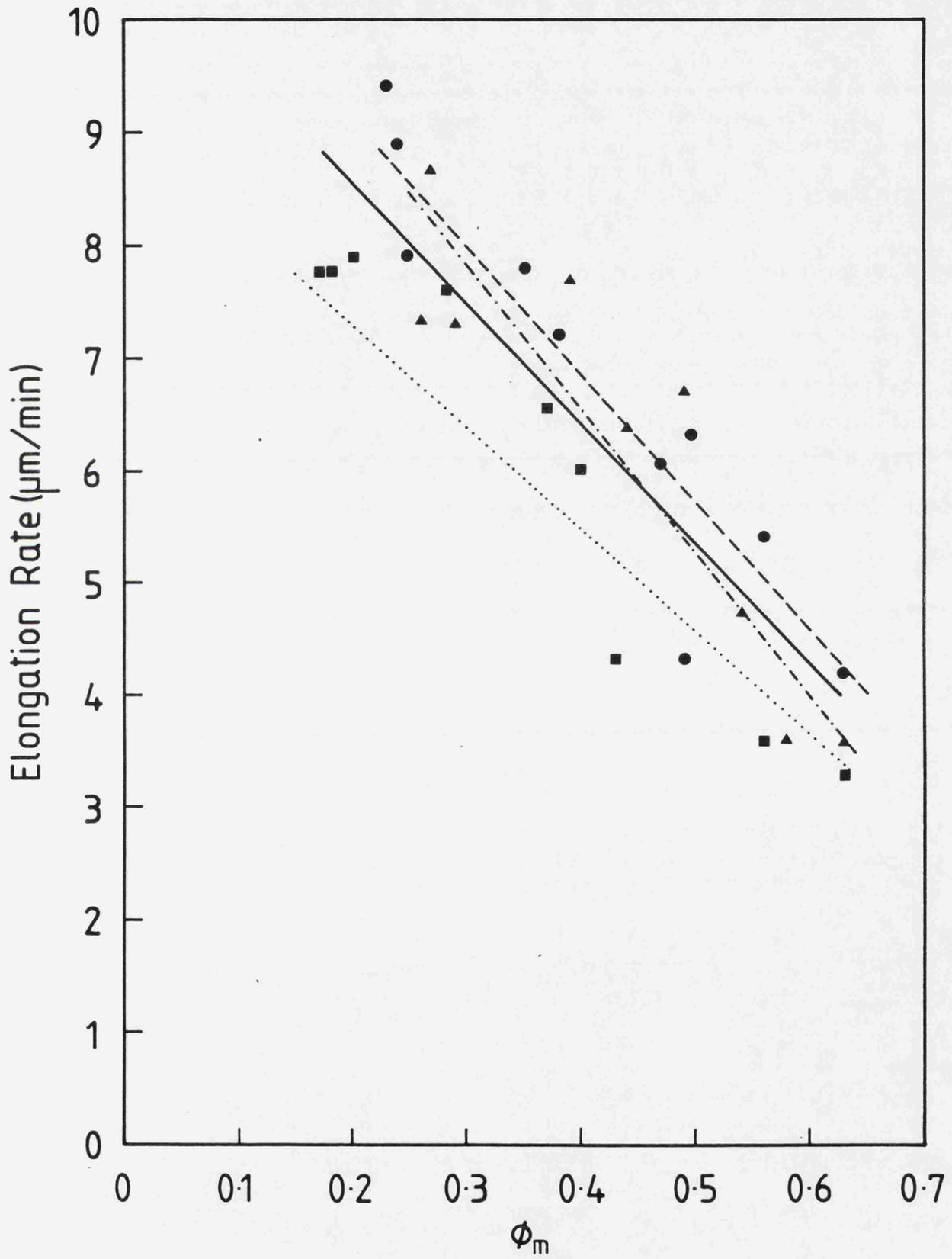


Figure 23

The relationship between elongation rate and ϕ_m for the rapid response to supplementary FR in Sinapis alba: the relationship for the phase-2 response in different background WL fluence rates.

14 d old plants grown in WL ($100 \mu\text{mol}/\text{m}^2 \text{ s}$, R:FR = 3.3, $\phi_e = 0.71$) were mounted in transducer apparatus 2 in WL (R:FR = 3.4, $\phi_e = 0.72$) and given two 90 min FR ($\lambda_{\text{max}} 730 \text{ nm}$) treatments at fluence rates between $1.4 - 102 \mu\text{mol}/\text{m}^2 \text{ s}$ to the first internode. Each data point is the mean of at least 10 measurements, but the statistics were based on all the measurements.

Linear Regression

Background ₂ WL fluence rate ($\mu\text{mol}/\text{m}^2 \text{ s}$)	Y intercept	Regression Coefficient	S.E.	d.f.	t
■.....■ 50	5.82	- 4.00	1.128	7	3.546 **
●-----● 100	8.29	- 6.29	0.838	7	7.506 ***
▲-----▲ 150	7.98	- 6.93	1.065	7	6.507 ***
———— Average	7.20	- 5.40			

Analysis of Variance of Regression

Source	d.f.	s.s.	m.s.	F
among slopes	2	21.2	10.60	2.387 N.S.
weighted average	21	93.3	4.44	

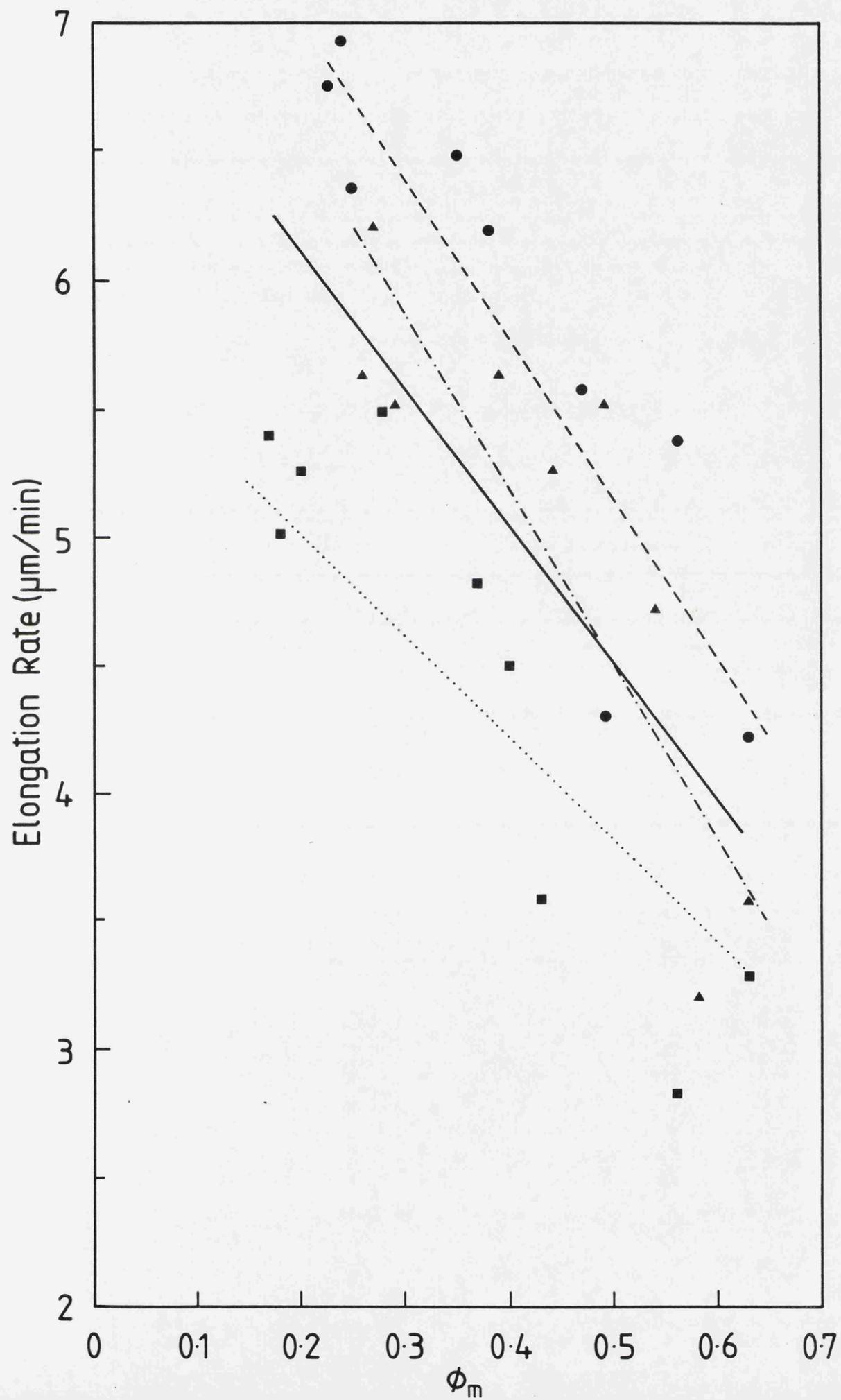
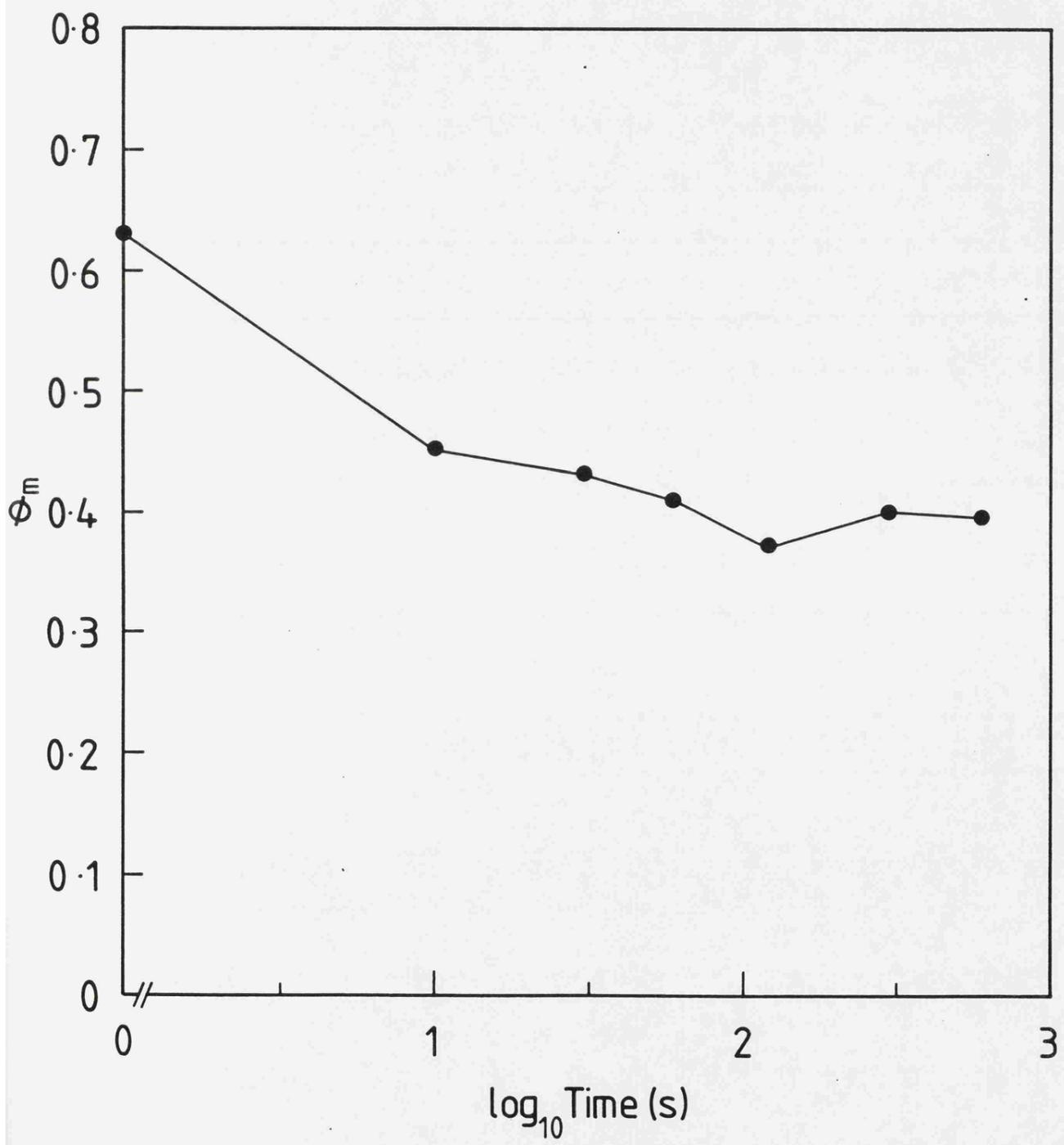


Figure 24

The time course for the achievement of photoequilibrium.

Dark-grown coleoptile tissue of Avena sativa var. Mostyn was irradiated in WL ($50 \mu\text{mol}/\text{m}^2 \text{s}$, R:FR = 3.4) for 5 min at 21°C in transducer apparatus 2. The tissue was then irradiated with FR (λ max 730 nm, $13.6 \mu\text{mol}/\text{m}^2 \text{s}$), from fibre-optic probes, in the background WL, for different times as indicated.



sections established that neither an increase in WL nor B fluence rate caused any significant change in elongation rate. The small changes found in the short-term WL fluence rate experiments can also be discerned in the scatter of points in Figures 22 and 23, but the regression analysis shows that the functional relationship between ϕ and elongation rate was similar in each treatment. The small differences at different WL levels could be due to variations in the rate of photosynthesis, but they are overridden by the effect of ϕ . This means that within the conditions of these experiments, the rapid response of internode elongation to changes in R:FR was dependent upon ϕ , and that any contribution from cycling rate was subordinate to changes in ϕ . Furthermore, the kinetics of the response were similar at all FR fluence rates, and FR λ max 730 would not have affected photosynthesis.

The Effect of Different FR Wavelengths on Stem Elongation

All the different FR wavelengths were added to background WL at $23 \mu\text{mol}/\text{m}^2\text{s}$, so the increase in elongation rate above the WL level is shown for the second phase of the response. The kinetics of the response were the same at each wavelength.

The FR fluence rate/response relationship shows that the increases in elongation rate are least at the wavelength giving the highest theoretical cycling rate - λ max 700 nm (Figure 25A). When, however, the results were plotted as increase in elongation rate against the ϕ set-up by each wavelength (Figure 25B), then the elongation rate correlated with ϕ in a similar way to the last experiment; so the response of internode elongation was dependent on wavelength, only in respect of the effectiveness

Figure 25

The effect of supplementary FR of different wavelengths on elongation rate in Sinapis alba.

A: the relationship between \log_{10} FR fluence rate and elongation rate.

14 d old plants grown in WL ($100 \mu\text{mol/m}^2 \text{ s}$, R:FR = 3.4, $\phi_e = 0.72$) were mounted in transducer apparatus 1 in WL ($23 \mu\text{mol/m}^2 \text{ s}$, R:FR = 3.4, $\phi_e = 0.72$) and supplementary FR was given to the first internode for 90 min at the following wavelengths:

λ max 700 nm ■-----■

λ max 719 nm ●————●

λ max 739 nm ▲-----▲ Data for 739 nm from Morgan et al (1980).

The elongation rates are for phase-2 of the rapid response and each point is the mean of 8 replicates.

B: the relationship between ϕ_m and elongation rate.

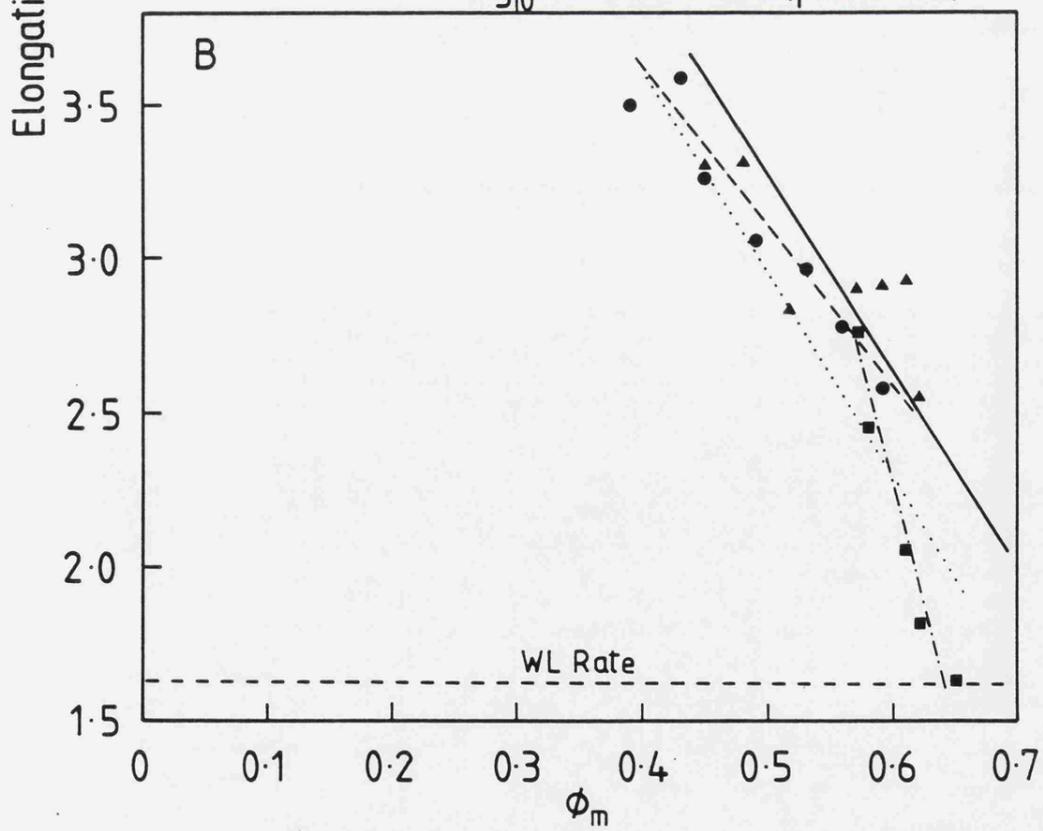
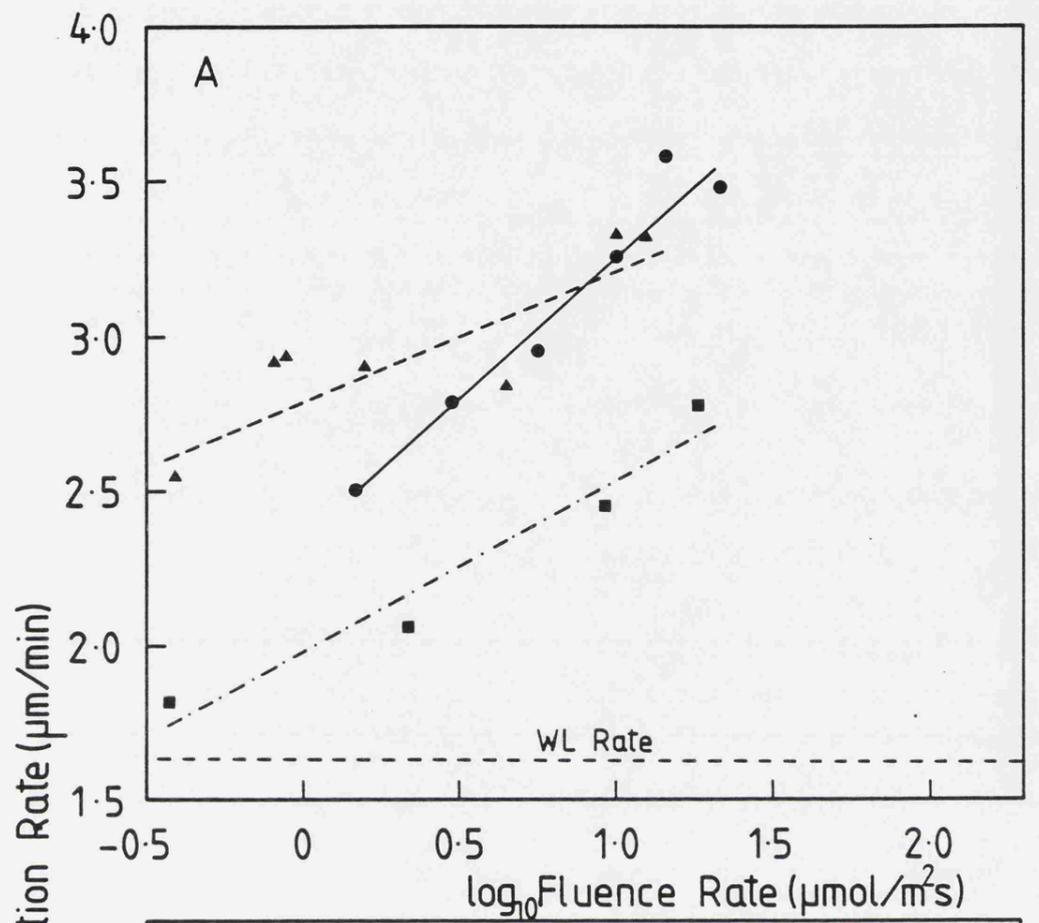
The data for elongation rates as in A plotted against the ϕ_m set up by each wavelength.

Linear Regression

Wavelength (λ max, nm)	Y intercept	Regression Coefficient	S.E.	d.f.	t
■-----■ 700	9.84	- 15.29	1.325	3	11.539 **
●.....● 719	4.67	- 6.65	0.998	6	6.660 ***
▲-----▲ 739	4.10	- 5.24	1.938	6	2.704 *
———— Average	4.86	- 6.41			

Analysis of Variance of Regression

Source	d.f.	s.s.	m.s.	F
among slopes	2	0.357	0.178	2.223 N.S.
weighted average	15	1.205	0.080	



with which each wavelength reduced ϕ , and was not related to fluence rate or wavelength per se and, thus, was independent of cycling rate. These results confirm the conclusions of the last section - that in this response elongation rate is determined by ϕ and not cycling rate.

These experiments were done in October 1980 and have been published: Morgan, D.C., Child, R., Smith, H. (1981), Absence of fluence rate dependency of phytochrome modulation of stem extension in light-grown Sinapis alba L., *Planta*, 151, 497-498.

The Effects of End-of-Day Treatments on Stem Elongation

When 13d old plants were transferred from WL to darkness there was a rapid decline in elongation rate, detectable within about 15 min (Figure 26). This decline was progressive and after 22h of continuous darkness the elongation rate was constant and very low (Table 4). In plants given a 15 min FR (λ max 730 nm, $100 \mu\text{mol}/\text{m}^2\text{s}$) pulse at the onset of the dark period (Figure 26) there was an increase in elongation rate which reached a peak after 25 min, but then gradually subsided, until after 22h the rate was constant and significantly different from the rate of plants given no FR treatment (Table 4). One of the axioms of plant physiology is that stem elongation is more rapid in the dark than in the light. For example: "It is a matter of common observation that internode growth is inhibited by light", (Smith, 1975); and, "Transfer of light-grown plants to darkness permits an increase in the growth rate of the stems", (Gaba & Black, 1983). This belief probably stems from the experiments of Sachs and Baranetzky, amongst others,

Figure 26

The effect of darkness on stem elongation in Sinapis alba.

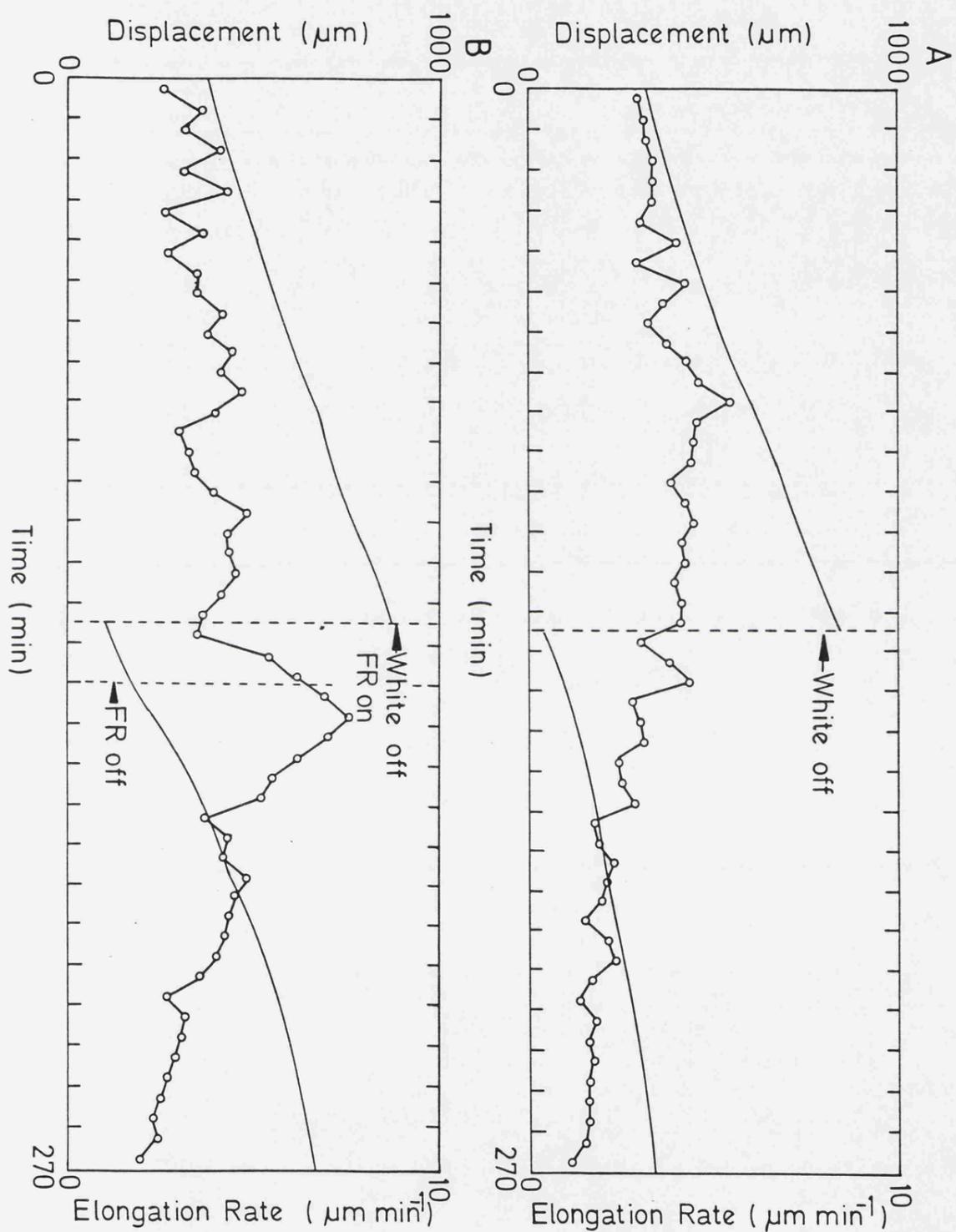
Plants were grown for 13 d in WL ($100 \mu\text{mol/m}^2 \text{s}$, R:FR = 3.3, $\phi_e = 0.71$) then mounted in transducer apparatus 2 in WL ($50 \mu\text{mol/m}^2 \text{s}$, R:FR = 3.4, $\phi_e = 0.72$). After equilibration in WL the plants were given a 24 h dark period.

A: a representative plant given a WL to darkness transition with no end-of-day FR.

B: a representative plant given a WL to darkness transition with a 15 min end-of-day FR (λ max 730 nm, $100 \mu\text{mol/m}^2 \text{s}$) treatment to the first internode as indicated.

○ — ○ elongation rate ($\mu\text{m}/\text{min}$)

— elongation shown on original chart trace (μm).



in the latter part of the last century and the early decades of this century; and it has received support from later studies, particularly those on hypocotyl elongation (see introduction). In plants possessing a B-photoreceptor, a transfer to darkness will release the plant from any inhibition by B. Also, during a dark period phytochrome in the Pfr form will be degraded and ϕ should decrease. The corollary is, therefore, that stem elongation should indeed increase in darkness, but this does not take account of other possible effects of darkness. In darkness, stem elongation could be limited by the availability of certain substances. The decline in elongation rate in darkness was rapid, but even after 22h it had not ceased. It is unlikely that the plants would become depleted in photosynthate so quickly as to cause the rapid reductions in elongation rate; but a reduction in the rate of sugar translocation from the leaves to the stem would limit the availability of photosynthate, and the kinetics of the decline in elongation rate are somewhat similar to those described for sugar movement from soyabean leaves transferred to darkness (Moorby et al, 1963). These results indicate that we must revise the supposition that stem elongation rates are always greater in darkness than in the light. We should consider a wider range of physiological processes which may be affected by a light to dark transfer. When the plants were transferred from the dark to WL + FR the elongation rate increased gradually and a steady-state was reached after 90-120 min (Figure 27). This time could represent the time required for the synthesis and/or mobilisation of substances necessary for the maintenance of a high elongation rate. There

Figure 27

The effect of end-of-day treatments on stem elongation in a subsequent photoperiod in Sinapis alba.

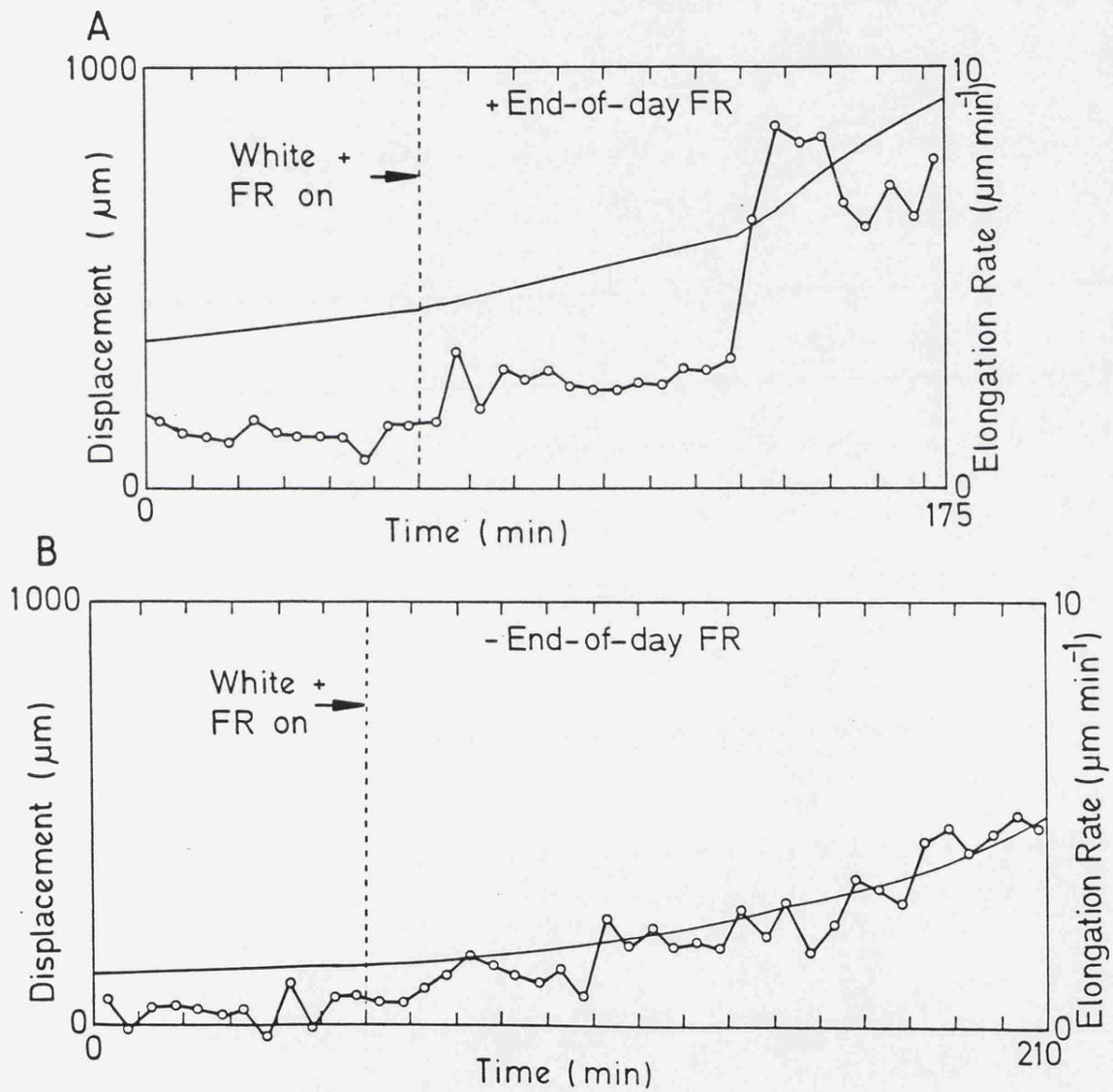
Plants were grown for 13 d in WL ($100 \mu\text{mol}/\text{m}^2 \text{s}$, R:FR = 3.3, $\phi_e = 0.71$) then mounted in transducer apparatus 2 in WL ($50 \mu\text{mol}/\text{m}^2 \text{s}$, R:FR = 3.4, $\phi_e = 0.72$). After equilibration in WL the plants were given a 24 h dark period followed by a subsequent photoperiod of WL + FR (background WL was $50 \mu\text{mol}/\text{m}^2 \text{s}$ and the FR was $\lambda \text{ max } 730 \text{ nm}$, $100 \mu\text{mol}/\text{m}^2 \text{s}$ to the first internode) with a ϕ_m of 0.17.

A: a representative plant given a 15 min end-of-day FR ($\lambda \text{ max } 730 \text{ nm}$, $100 \mu\text{mol}/\text{m}^2 \text{s}$) treatment to the first internode.

B: a representative plant given no end-of-day FR treatment

○——○ elongation rate ($\mu\text{m}/\text{min}$).

———— elongation shown on original chart trace (μm).



was no increase in elongation rate characteristic of phase-1 of the rapid response to FR in plants in WL. This suggests that the potential for a rapid increase in elongation rate was exhausted in the dark period and needed to be renewed. The analysis of variance (Table 4) shows that the plants given end-of-day FR and no subsequent R pulse (treatment B, Table 4), had significantly higher elongation rates, in darkness and WL + FR, than plants given the other two treatments. In the plants given a R pulse (treatment C), the elongation rates were not significantly different from those of plants given no end-of-day FR (Treatment A). This suggests that whatever process is stimulated by the end-of-day FR, it can rapidly be depressed by the R. The process is, therefore, probably under close and continuous regulation by phytochrome.

Ideally, the monitoring of stem elongation in these experiments should have been accompanied by parallel measurements of phytochrome levels. No routine method of making these measurements was available at the time, but this may now be possible using enzyme-linked-immunosorbant-assays (e.g., Saunders et al, 1983; Thomas et al, 1984).

To have some idea of what could be happening to phytochrome in the three treatments, the behaviour of phytochrome in other tissues must be considered. A danger of this is that the phytochrome system may be dissimilar in different species, in light or dark-grown plants, or even in organs of the same plant. For example, the thermal reversion of Pfr to Pr occurs in most classes of Dicotyledons, but not in Monocotyledons. With these

Table 4

The effect of end-of-day treatments on stem elongation in Sinapis alba.

Plants were given the end-of-day treatments described in Figure 6.

In the subsequent photoperiod of WL + FR, supplementary FR (λ max 730 nm, $100 \mu\text{mol/m}^2 \text{s}$) was given to the internode by fibre-optic probes and the background WL was $50 \mu\text{mol/m}^2 \text{s}$.

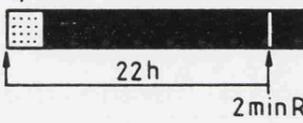
The elongation rates in darkness were measured between 22-24 h and the elongation rates in WL + FR were measured as the steady-state level over 120 min. The mean values are shown \pm S.E.

Analysis of Variance: elongation rates in darkness

Source	s.s.	d.f.	m.s.	F
among groups	5.150	2	2.575	4.940*
within groups	23.460	45	0.521	
Detailed Comparisons				
A v B	3.672	1	3.672	7.048*
A v C	0.014	1	0.014	0.027 N.S.

Analysis of Variance: elongation rates in WL + FR

Source	s.s.	d.f.	m.s.	F
among groups	56.360	2	28.180	5.420**
within groups	233.920	45	5.198	
Detailed Comparisons				
A v B	49.320	1	49.320	9.490**
A v C	3.350	1	3.350	0.640 N.S.

Treatment	Elongation Rate ($\mu\text{m}/\text{min}$)		
	Darkness	WL+FR	Reps.
<p>A</p>  <p>24h Darkness</p>	0.75 ± 0.17	6.21 ± 0.73	12
<p>B</p>  <p>15 min FR</p>	$1.43 \pm 0.16^*$	$8.69 \pm 0.47^{**}$	24
<p>C</p>  <p>22h</p> <p>2 min R</p>	0.80 ± 0.19	6.96 ± 0.73	12

potential problems in mind, the plants given treatment B should possess the highest levels of phytochrome. In plants transferred from WL ($\phi_m = 0.63$) to darkness, the bulk of the phytochrome should be in the Pfr form. This Pfr will be subject to destruction and thermal reversion. In dark-grown cotyledons of S. alba the half-life for Pfr destruction is about 45 min at 25°C, while the half-life for thermal reversion is about 8 min (Marme et al, 1971); so the amount of phytochrome that was in the Pfr form which survives the dark period, depends on the interplay between destruction and reversion. The plants which were given end-of-day FR will have had most of the Pfr converted to Pr, and in this state it will essentially be conserved during the darkness. In the plants given a brief R pulse after 22h of darkness this Pr, along with Pr synthesised in the dark, will mostly be converted to Pfr and liable to destruction in the following 2h. In addition to the phytochrome present at the onset of darkness, more phytochrome should be synthesised as Pr during the dark period. In oats, phytochrome synthesis is inversely related to ϕ (see introduction); so, if this is the case in S. alba, the rate of phytochrome synthesis will be greatest in plants given an end-of-day FR treatment.

According to this scheme, the three different treatments should result in plants with different levels of phytochrome after the 24h dark period. In the following photoperiod the WL + FR set up a ϕ of 0.17. At this low ϕ , most of the phytochrome will exist as Pr and will not be available for destruction; so the differences in phytochrome levels in the three treatments

should be maintained until the steady-state elongation rates were recorded (i.e., after 100 min in WL + FR). The plants which should have the most phytochrome (treatment B) had the highest elongation rate (Table 4). If the supposed differences in phytochrome levels actually occur, these results cannot fit the simple model of Pfr-as-active-form. This model states that Pfr inhibits stem elongation and that large physiological responses correlate with small changes in the absolute level of Pfr. Even if the differences in P_{tot} between the three treatments are very small, there should be measurable differences in the elongation rates.

An alternative explanation for these results is that plants possess more than one population of phytochrome which control different processes. Each population may have different properties associated with its function. For example, one of these populations may represent the majority of the phytochrome, and could govern elongation in the pre-emergent and newly emergent seedling. This population may become redundant in the light-grown plant, but changes in the size of the population may continue to take place. A second, and smaller, population may be relatively stable - that is, Pfr degradation is slow and is balanced by an equally slow rate of Pr synthesis. This population could control stem elongation in the emergent plant and, because the rates of synthesis and destruction are low, no major fluctuation in the size of the pool would occur in any light regime. Evidence from kinetic and immunological analyses of the phytochrome system, supports the existence of such populations of phytochrome, but at present no physiological functions can be assigned to them (see introduction). If the population of phytochrome

which modulates stem elongation is stable, these results can be interpreted in terms of either the concentration of Pfr or the ratio of Pr:Pfr, as the active component of the phytochrome system.

Finally, the significantly higher WL + FR elongation rate of plants given treatment B requires an explanation. If these plants possess the highest level of phytochrome, the implication is that there could be a co-action between ϕ and P_{tot} . A co-action of this kind would result in a progressive increase in elongation rate in conditions generating a constant, low ϕ , because phytochrome accumulates at low ϕ values. Morgan et al (1980) reported a gradual increase in elongation rate following 4-5h FR to the first internode, but this response had a definite lag time and can also be explained in terms of a response involving the leaves (see, earlier sections). A possible explanation is that the high elongation rate may result from the stimulation of a process, which increases stem elongation by the end-of-day FR. For example, the end-of-day FR may cause an increase in a parameter involved in bringing about cell enlargement. This may not be expressed fully in the dark period, perhaps because of the limited availability of substances required for growth. In the photoperiod, however, the potential may be retained, and could be expressed as the growth substances become available.

SECTION 2

THE PHOTOCONTROL OF ELONGATION IN ISOLATED INTERNODE SEGMENTS

Introduction

The rapid increase in stem elongation rate in response to supplementary FR given to the internode can only be brought about by cell enlargement; and because the first internode of S. alba perceives and responds to supplementary FR, excised internode segments offer an opportunity to probe the effects of light quality on cell enlargement.

Cell Enlargement

Cell enlargement is determined by several parameters, which Lockhart (1965) has combined in the following equation:

$$\frac{dv}{dt} = \frac{LM (\Delta\pi - Y)}{L + M}$$

where dv/dt = the relative rate of increase in volume.

L = the hydraulic conductivity. It is a function of membrane conductivity x area ÷ cell volume (1/bar . s).

M = wall extensibility (1/bar.s).

$\Delta\pi$ = the difference in osmotic potential between the internal cell sap and the external solution (bar).

Y = the wall yield stress threshold; i.e., the minimum turgor required to drive wall expansion (bar). The biochemical basis of Y may be the minimum elastic extension which must be attained before wall loosening can occur, rather than a minimum stress for physical extension as such.

Briefly, cell enlargement occurs when the wall yields to turgor stress. This yielding is a function of the initial stress threshold and the subsequent extensibility of the cell wall. Turgor pressure, the driving force for enlargement, relies on water movement into the cell and, hence, upon the maintenance of a low osmotic potential. Wall extensibility decreases with age and its ultimate loss results in the termination of cell enlargement, even though turgor pressure may be sustained (Wortmann, 1889).

The Effect of Light on Cell Enlargement

Most examinations indicate that light influences cell enlargement by altering wall extensibility. The B inhibition of hypocotyl elongation in cucumber and sunflower seedlings (Cosgrove & Green, 1981) and the R depression of stem elongation in peas (Lockhart, 1960) were accompanied by a reduction in extensibility. In dark-grown rice both R and FR caused a decrease in coleoptile extensibility (Masuda et al, 1970). This decrease was greatest with R, whilst FR reduced the R inhibition to the level brought about by FR alone, thus indicating that the response is phytochrome-controlled. Light can also promote extension (Warner &

Ross, 1981) and cell expansion (Van Volkenburgh & Cleland, 1979). The WL-promoted enlargement of leaf cells in Phaseolus vulgaris results from an increase in cell wall extensibility (Van Volkenburgh & Cleland, 1981) and requires sucrose and KCl to be present in the bathing solution (Van Volkenburgh & Cleland, 1979). Others have reported requirements for, or interactions with, chemicals in light-controlled responses. For example, the inhibition of elongation in etiolated maize coleoptile segments by R is reversed by FR or auxin (Vanderhoef & Briggs, 1978; Vanderhoef et al, 1979). The B and R inhibition of growth in green lettuce hypocotyl segments is reversed by gibberellin, and the effectiveness of the gibberellin is improved by sucrose and KCl (Silk & Jones, 1975). In addition to the reports of light affecting extensibility, it has been demonstrated that neither R nor FR alter the hydraulic conductivity of segments bathed in tritiated water (Pike, 1976).

The Control of Cell Enlargement

Much of our knowledge of the processes involved in cell enlargement has come from studies on the promotion of elongation and extensibility, by acidic solutions and auxins, in isolated stem segments.

The stimulation of elongation by acidic solutions was first demonstrated in the 1930s (e.g., Bonner, 1934). It subsequently has been confirmed in etiolated and green stem segments of many species (e.g., Rayle & Cleland, 1970; Hager et al, 1971; Cleland & Rayle, 1975; Mentze et al, 1977). The protons must continually be present for elongation to be sustained (Rayle & Cleland, 1970), and the optimum pH for promotion is about 5.2-5.7, where methods to allow protons easy entry to the tissues are used (e.g., by abrading or removing the cuticle) (see, Rayle, 1973). The response to a

decrease in pH has a latent period of about 1 min (Evans & Ray, 1969; Rayle & Cleland, 1970; Evans et al, 1971) and reaches a maximum within the first 2h (Rayle & Cleland, 1970; Bonner, 1934). After about 6h the response is exhausted in free-floating segments (Bonner, 1934), but in coleoptiles held under tension, extension may continue at a high level for up to 9h (Rayle et al, 1970).

Auxin has also been shown to induce elongation in excised stem segments (e.g., Ray, 1962; Cleland, 1967a, b) and acid-growth has been implicated in the response to auxin (see, Evans, 1974b).

Basically, the auxin-induced elongation response is characterised by two biochemically distinct growth phases following a latent period of 10-15 min (Penny, 1969; Evans & Ray, 1969; Barkley & Leopold, 1973): these are summarised in Figure 1.

Phase one is believed to result from an auxin-stimulated proton efflux which causes wall loosening. This is supported by observations that auxin increases proton efflux in isolated segments (Rayle, 1973; Marre et al, 1973a, b; Cleland, 1982). The lag time for this effect is 8-10 min, and within about 60 min the pH of the tissues equilibrates at pH 5.2-5.7 (Rayle, 1973; Evans & Vesper, 1980); thus, the rate and kinetics of proton efflux are parallel with the time course of auxin-induced elongation.

This first phase lasts for about 17 min and once initiated it cannot be reversed by respiratory inhibitors - such as KCN (Cleland, 1968). Moreover, it is unaffected by inhibitors of RNA synthesis (Evans & Ray, 1969), protein synthesis (Evans & Ray, 1969; Penny, 1971; Cleland, 1971a; Pope & Black, 1972) and certain polysaccharidases (Evans, 1974a; Perley & Penny, 1974).

In contrast, the second phase, which starts after about 35 min and can be maintained for at least 24h (Pope, 1978), is sensitive to metabolic inhibitors (Rayle et al, 1970; Vanderhoef & Stahl, 1975; Vanderhoef & Dute, 1981) and requires sustained protein synthesis (Cleland, 1971). The display of two distinct phases does not mean that there are necessarily two separate actions of auxin. It must be remembered that the organs used in these experiments were depleted in auxin, and the addition of auxin may initiate processes which are continuous in the intact plant, but manifest as two phases in excised segments. In whole plants, processes such as wall loosening, protein synthesis and the manufacture of new cell wall materials will take place concurrently. If, however, wall loosening is greatly reduced in excised segments, there may be a build up of products formed by the other processes. The sudden restoration of wall loosening could cause the large increase in growth rate seen in phase one, but as the pool of metabolites is consumed then continued elongation would become dependent upon the rate at which these substances are re-synthesised.

As well as modifying proton efflux, which would restore wall loosening, auxin has also been shown to alter the pattern of protein synthesis (Zurfluh & Guilfoyle, 1980) and cell wall metabolism (Ray, 1962; Ray & Baker, 1965; Labavitch & Ray, 1974a, b). It is not clear whether protein or RNA synthesis are involved in the initial action of auxin as the lag times for such changes in metabolism would need to be short. The determination of accurate lag times requires a high resolution assay; and although modulations in RNA synthesis have been detected within 1 min in Drosophila (see, Vanderhoef, 1981), auxin-stimulated modifications usually take over 60 min to become apparent (see, Evans, 1974b).

In summary, the response to auxin requires continuous acidification

of the cell wall - auxin-induced elongation is completely counteracted by neutral buffers (Durand & Rayle, 1973; Rayle & Cleland, 1980) - and it is dependent upon protein synthesis after about 90 min. Auxin does not appear to regulate hydraulic conductivity (Cosgrove & Cleland, 1983), and although turgor pressure does decline during auxin-induced elongation this is probably a consequence of wall loosening (Boyer & Wu, 1978). The uptake of permeant solutes, which maintain cellular osmotic potential, is proportional to the amount of elongation and does not take place when auxin-induced growth is inhibited (Stevenson & Cleland, 1981).

Proton Efflux

Cell wall acidification by auxin is believed to be brought about by an active secretion of protons into the apoplast (Cleland, 1982), rather than by changes in the uptake of apoplastically-generated bicarbonate ions (Lucas & Smith, 1973), or reductions in the efflux of hydroxyl ions (Johnson & Rayle, 1976). Despite suggestions that proton efflux may occur by pinocytosis (Ray, 1977), the most cogent hypothesis remains that propounded originally by Hager et al (1971), in which protons are pumped out of the protoplasm by an electrogenic ATPase situated in the plasma-membrane. The notion of such a pump is supported by evidence that plasma-membranes contain ATPases which play an important role in cation transport (see, Higinbotham & Anderson, 1974). Two other pumps have also been advanced: an electroneutral ATP driven H^+/K^+ exchange (Haschke & Luttge, 1975) and a $2H^+/Ca^{2+}$ exchange (Cohen & Nadler, 1976). Although there is evidence for the H^+/K^+ exchange, the second of these is improbable, because the requirement for Ca^{2+} is not absolute and the stoichiometry of the $2H^+/Ca^{2+}$ exchange is not fulfilled (Cleland, 1982).

The nature of these putative proton pumps has been investigated using auxin, and the fungal toxin fusicoccin which is more effective than auxin at increasing both elongation rate and proton efflux in stem segments (Lado et al, 1972; Cleland, 1976a). The results indicate that several proton pumps may exist. There is evidence for a fusicoccin-stimulated H^+/K^+ ATPase, and auxin-activated electroneutral and electrogenic proton pumps. Auxin and fusicoccin have been shown to actuate K^+ uptake by pea internode segments (Marre et al, 1974) and, correspondingly, K^+ enhanced proton efflux in the presence of auxin or fusicoccin (Lado et al, 1976). With fusicoccin the order of activity, and the stoichiometry of the K^+ uptake and proton efflux matched a capacity to activate an H^+/K^+ ATPase. Auxin, however, was less effective at stimulating K^+ uptake than was fusicoccin. In oat coleoptiles auxin-stimulated proton efflux was followed by a 1-2h lag period for K^+ uptake (Cleland, 1976b), and K^+ did not enhance proton efflux with auxin as it did in fusicoccin-stimulated proton efflux.

On the basis of the above results, together with the observations that fusicoccin and auxin cause membrane hyperpolarisation (Cleland & Lomax, 1977; Cleland et al, 1977), Cleland (1977) has proposed two proton pumps. One of these is stimulated by fusicoccin and normally exchanges H^+ and K^+ whenever K^+ is available, but can operate electrogenically in the absence of sufficient K^+ . The second, the auxin-activated pump, probably is foremost an electrogenic pump which induces passive K^+ uptake. In addition to these pumps, there is evidence to support the existence of electroneutral auxin-stimulated proton pumps. For example, in sunflower hypocotyl segments auxin-induced proton efflux could only be detected with concomitant K^+ influx (Ilan, 1973).

The method by which auxin modulates the activity of plasma-membrane

proton pumps is unknown. Marre (1977) has inferred from the 8-10 min latent period for auxin-induced proton efflux, that auxin does not have a direct effect on these proton pumps. Auxin may enter the cytoplasm via membrane-associated uptake sites (Lomax-Reichert et al, 1982) and influence the proton pumps by a second messenger.

Cell Wall Loosening

The rapid kinetics of acid and auxin-induced growth in excised segments suggest that wall loosening is a consequence of modifications to the existing wall structure resulting from wall acidification.

At present the model of plant cell walls ventured by Preston (1974) is considered valid. Preston envisages a system of cellulose microfibrils coated with a sheath of hemicellulose polymers and embedded in a matrix of pectic polysaccharides with some protein. The controversy rests on whether the extensibility of the wall is limited by particular covalent bonds requiring enzymic cleavage (Lampert & Catt, 1981), or if wall loosening can be achieved by a less specific breakage of some of the many weak interactions among the wall polymers and metal cations (Preston, 1979). There are few reports of direct effects of either low pH or auxin on cell wall structure. One of the most promising, however, is the observation that auxin increases the conversion of xyloglucan from an insoluble, to a water soluble form in oat coleoptiles (Labavitch & Ray, 1974a). This starts within 15 min of adding auxin and is proportional to the concentration of auxin in the same way as auxin-stimulated elongation (Labavitch & Ray, 1974b). In addition, the conversion is inhibited by Ca^{2+} and metabolic inhibitors. This effect is important, because in the model of cell wall structure described for sycamore cells by Keegstra et al (1973) the xyloglucan component is

a strategic intermediate between the cellulose microfibrils, to which it is bound by hydrogen bonds (the only non-covalent bonds in the wall), and the pectic matrix.

Keegstra et al (1973) considered that cleavage of the bonds between xyloglucan and cellulose would allow the cellulose fibres to move relative to each other. Chaotropic agents such as urea (which break hydrogen bonds), however, allow only a small amount of extension (Tepfer & Cleland, 1979), and it may be that the xyloglucan-pectin association is more important. The identity of cell wall enzymes which may achieve wall loosening is unclear, but indirect evidence suggests they may exist in oat coleoptiles. Cline (1979) has proposed a wall loosening enzyme with a half-life of 7-8h, following his observations that cycloheximide takes 6h to inhibit acid-induced elongation in frozen-thawed oat coleoptiles. The rapid kinetics of auxin and acid-induced growth preclude the *de novo* synthesis of wall enzymes from being involved in the early stages of the response, but activation of existing enzymes could occur. Johnson et al (1974) tested the pH dependency of enzymes in the cell wall and found some glycosidases to have an optimum of pH 5.0 or thereabouts, but the initiation of activity was too slow for them to be involved in the first stages of wall loosening. More encouraging are the reports that auxin stimulates the activity of a β -1,3-glucanase within 10 min (Masuda & Yamamoto, 1970), and that auxin-induced elongation in oat coleoptiles is accompanied by a massive release of glucose from the mixed-linked glucans of the cell walls (Masuda, 1980).

A further direct observation is that acid solutions release Ca^{2+} from the cell walls of oat coleoptiles (0.1% of the dry weight of these cell walls is Ca^{2+}) (Carr & Ng, 1959). There are many reports of the ability of very low concentrations of Ca^{2+} to inhibit elongation (e.g., Thimann & Schneider, 1938; Cooil & Bonner, 1957;

Cleland & Rayle, 1977; Metraux & Taiz, 1977). This inhibition occurs at concentrations which are too low to affect the osmotic balance of cells (Bennet-Clark, 1956), and at concentrations below 1 mM Ca^{2+} actually stimulates proton efflux (Cohen & Nadler, 1976). The Ca^{2+} must, therefore, affect the cell wall, and it appears to do this in several ways, which are represented to different extents in various species. In oat coleoptiles, for example, Ca^{2+} does not decrease extensibility when added to methanol-boiled wall preparations, but inhibits acid-growth in live segments when provided simultaneously with the protons (Cleland & Rayle, 1977). Moreover, the chelating agent EDTA is less effective than pH 4.0 at stimulating elongation, and elongation can be inhibited by 8M urea or boiling (Tepfer & Cleland, 1979). These results suggest that biochemical wall loosening predominates in oat coleoptiles. In contrast, a pretreatment with formaldehyde has no effect on acid-growth in light-grown sunflower hypocotyl segments and EDTA can substitute for acid (Soll & Bottger, 1982). Similarly, in the giant alga Valonia, acid-growth occurs in both live and urea-treated cell wall preparations and can be inhibited by Ca^{2+} and Mn^{2+} , and completely substituted for by 10mM EDTA (Tepfer & Cleland, 1979). Extension in wall preparations from Nitella is also inhibited by certain tri and divalent cations (La^{3+} , Al^{3+} , Ba^{2+} , Ca^{2+} , Sr^{2+}) and stimulated by EDTA (Metraux & Taiz, 1977). Indeed the promotion of elongation by buffered EDTA, and solutions of other chelating agents, has been demonstrated in whole plants and stem segments (Weinstein et al, 1956; Moll & Jones, 1981b).

These investigations affirm that wall loosening can involve biochemical and non-biochemical processes. Enzymic wall loosening may be stimulated directly by protons affecting enzyme activity, or liberating Ca^{2+} from the substrate. Ca^{2+} may compete for specific domains on certain molecules which are hydrolysed by cell wall

enzymes (Bates & Ray, 1981). Alternatively, metal cations may form structural bonds within the cell wall, such as with pectins (Tagawa & Bonner, 1957; Bennet-Clark, 1956), or glycoproteins (Kauss & Glaser, 1974), and may be displaced by protons. Consequently the H^+ : Ca^{2+} ratio may be very important, and Ca^{2+} movement into and out of cells may be as significant as proton efflux, under certain conditions (Taiz, 1984).

Experimental Rationale

The response of intact internodes to added FR bears a striking similarity to that of excised segments to auxin, in terms of both the shape and kinetics of the curve (Figure 28; Table 5).

Furthermore, the continuous presence of either FR or auxin is required for the respective responses. If auxin is given for only 1 to 15 min the first phase occurs, but then declines to the endogenous level (see, Penny & Penny, 1978) in a way resembling the response of internodes to brief pulses of FR (see, Section 1).

It is tempting, therefore, to hypothesise that auxin and light act in similar ways. Not necessarily that light affects auxin levels, but that they both may act at a particular locus in the process of cell enlargement.

Hanson and Trewavas (1982) have argued that the response to auxin may be an artifact resulting from the use of excised organs. They point out that when auxin is added to whole plants there is no appreciable increase in growth, and that correlations between auxin levels and growth rates are poor. Although it may be true that in whole plants auxin is not limiting, these criticisms have weaknesses. First, auxin levels are notoriously difficult to assay, because of the low concentrations and labile nature of the molecule. Second, the auxin level only may stimulate cell elongation in specific regions of the plant. For example, in hypocotyls of Vigna radiata the biphasic kinetics of auxin-stimulated elongation only are shown in certain segments, and the hook region never reacts to auxin, whatever the concentration (Bouchet et al, 1983).

The value of these investigations into auxin-stimulated growth in excised segments is, in part, that auxin has been used as a probe to

Figure 28

A comparison between the response of the intact Sinapis alba plant to supplementary FR and the response of excised segments to auxin.

A: the rapid response of intact 14 d old S. alba plants to supplementary FR (λ max 730 nm, $100 \mu\text{mol}/\text{m}^2 \text{s}$) given to the first internode in background WL ($50 \mu\text{mol}/\text{m}^2 \text{s}$). The data is as shown in Figure 19A.

B: the rapid response of excised stem segments to auxin. The figure is based on the data of Penny & Penny (1978) for a 23 mm lupin hypocotyl segment, but the response is typical of those reported in the literature.

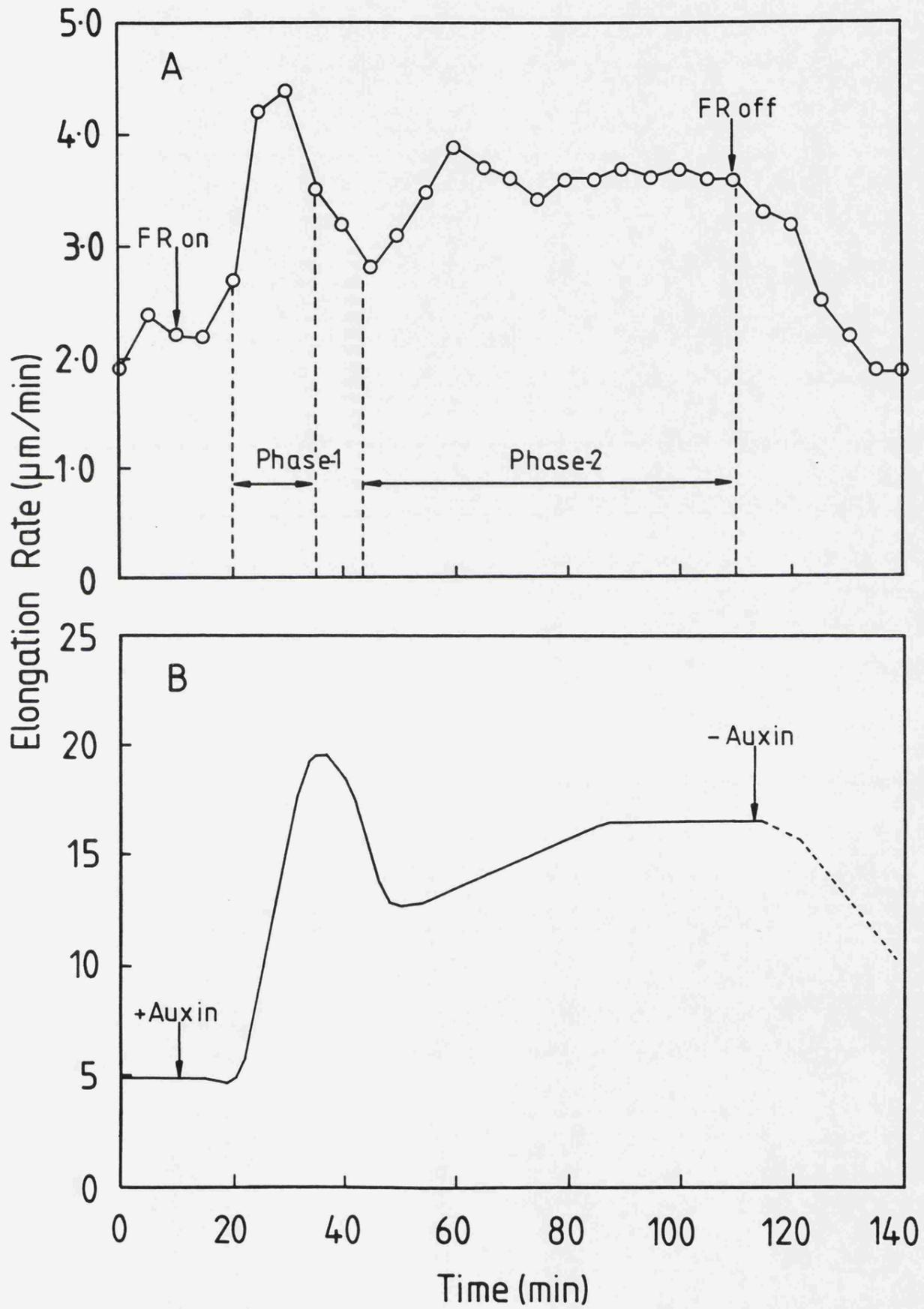


Table 5

A comparison between the kinetics for the response of the intact Sinapis alba plant to supplementary FR and the response of excised segments to auxin.

The data for the rapid response of intact 14 d old S. alba plants to supplementary FR (λ max 730 nm, $100 \mu\text{mol/m}^2\text{s}$) given to the first internode in background WL ($50 \mu\text{mol/m}^2\text{s}$) are mean values for 10 plants. The data for the kinetics of the auxin response are from Penny & Penny (1978) and Vanderhoef (1981).

* The decay kinetics following the removal of auxin depend on the concentration of auxin given originally. With an auxin concentration of $1 \mu\text{M}$ the rate started to decline 3 min after the removal of auxin (Penny, 1972) and the elongation rate reached the original level after 50 min (Evans & Hokanson, 1969).

	Treatment	
	WL→WL+FR	Addition of Auxin
Lag Time Phase-1	10.3 min	12 min
Duration of Phase-1	13.6 "	17 "
Lag Time Phase-2	35.0 "	35 "
Decay Time	15.6 "	*

examine the mechanisms involved in cell enlargement, even though its action may not be unique. In addition to auxin, gibberellin (Adams et al, 1973; Hebard et al, 1976; Moll & Jones, 1981a), ethene (in the presence of auxin) (Malone & Ridge, 1983) and abscisic acid (Khan & Tao, 1978) have been shown to modify elongation growth and, in some cases, proton efflux. In addition, WL stimulates cell enlargement and proton efflux in bean leaves (Van Volkenburgh & Cleland, 1980), and water stress may stimulate or depress proton efflux depending on the conditions (Cleland, 1975). These observations indicate that under certain circumstances a number of chemical and physical factors may modulate cell enlargement, probably by altering the activity of the plasma-membrane-bound proton pumps.

The purpose of these experiments was to establish whether, and under what conditions, the response to lowered R:FR occurs in isolated segments, and to determine if elongation is brought about by the photocontrol of cell wall acidification. The latter may be tested by examining the effect of added FR on segments elongating at the optimum pH. Under such conditions if light affects L , $\Delta\pi$ or Y , then, assuming M is high at the optimum pH, there should be a synergism between low R:FR and low pH. If, on the other hand, low R:FR has no effect at low pH, this would support the theory that light affects M , probably via wall acidification.

Materials and Methods

Plant Material

S. alba plants were grown under WL (R:FR=3.3, $\phi=0.71$, $100 \mu\text{mol}/\text{m}^2\text{s}$; 16h photoperiod; $20\pm 2^\circ\text{C}$) for 16-18d, by which time the first internode was about 20 mm long, but still able to respond to added FR (see section 1). This internode was swabbed with ethyl-acetate, to render the cuticle more permeable to ions, and excised. The use of organic solvents to increase cuticle permeability has been described by Penny & Penny (1978) and it is less destructive than either peeling or abrasion (see, Jones & Vanderhoef, 1981). The 20 mm excised segments usually were mounted immediately in the transducer apparatus, but in some cases they were incubated in aerated medium for up to 2h before mounting.

The Transducer Apparatus

The transducer apparatus (Figure 29) consisted of a linear displacement transducer fixed in an adjustable supporting pillar. This pillar also supported a clear plastic flow-chamber, and both this and the transducer were mounted in line on rack and pinion devices. The apical end of the internode segment was attached directly to the transducer armature via a plastic cone, and the basal end rested in a second plastic cone fixed to the floor of the flow-chamber. The whole assembly was mounted on a table fitted with anti-vibration legs, and isolated from the building by several

Figure 29.

The transducer apparatus for internode segments.

A: photograph.

B: diagram.

Key: AS: adjustable support for flow-chamber.

AW: adjusting wheel.

C: cup for stem segment.

FC: flow-chamber.

FO: fibre-optic probe.

IN: in flow.

OUT: out flow.

P: plug.

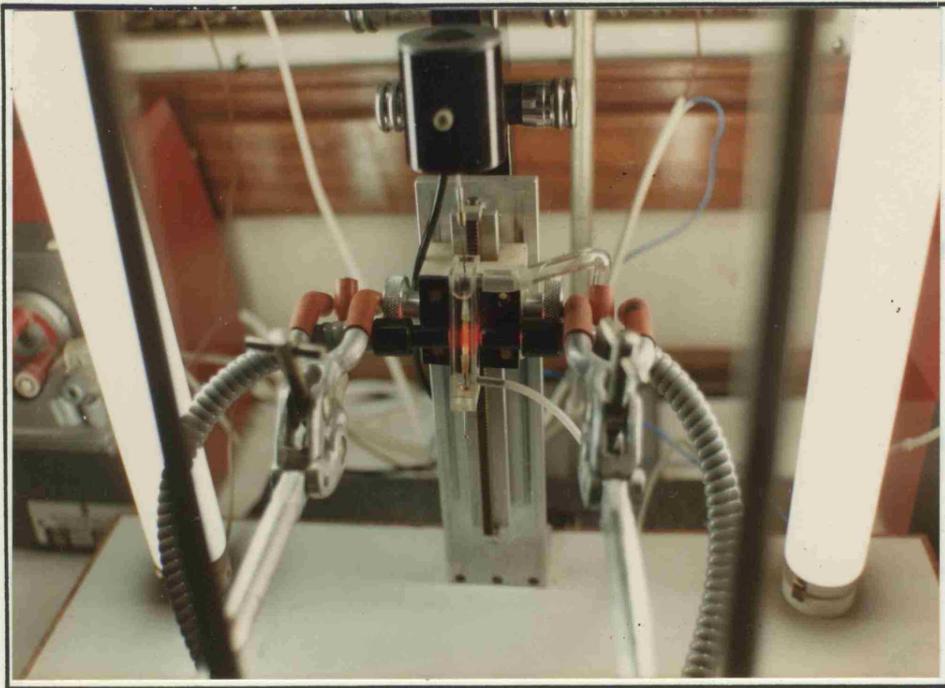
R: rack.

S: internode segment.

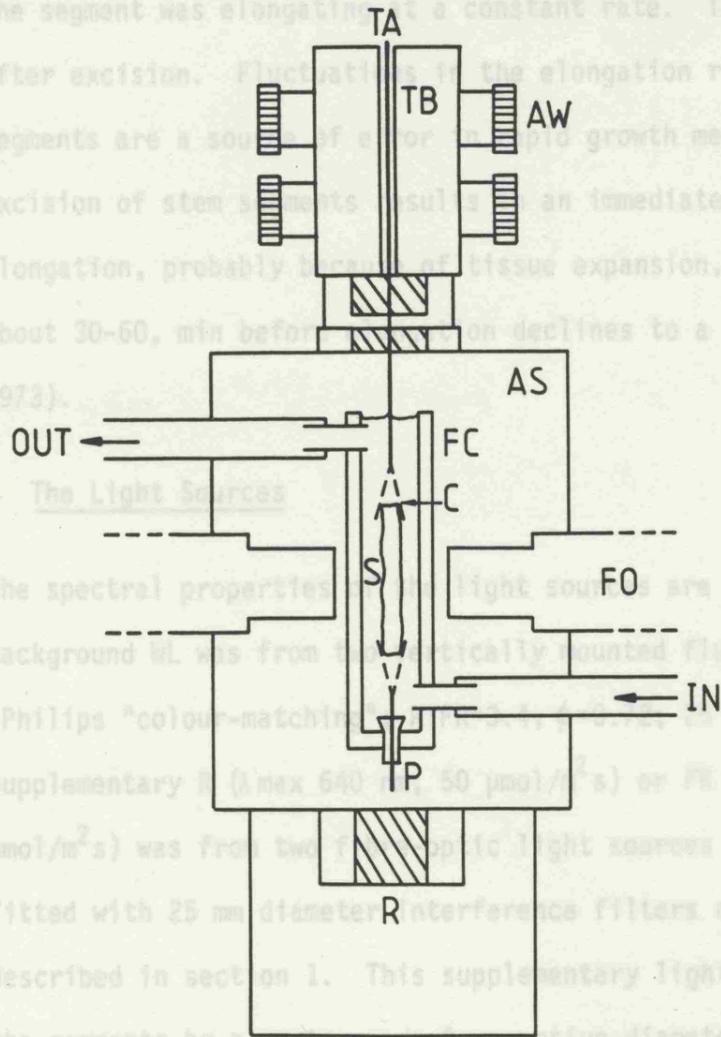
TA: transducer armature.

TB: transducer body.

Note: the adjusting wheel for the flow-chamber support has been omitted and the in flow tube has been re-positioned for the sake of clarity in the diagram.



B



Any changes in medium composition, or light regime, were made when the segment was elongating at a constant rate. This was usually 4h after excision. Fluctuations in the elongation rates of isolated segments are a source of error in growth measurements. Excision of stem segments results in an immediate stimulation of elongation, probably because of tissue expansion, and this lasts about 30-60 min before elongation declines to a steady rate (Evans, 1973).

The Light Sources

The spectral properties of the light sources FO are shown in Figure 2b. Background ML was from two vertically mounted fluorescent tubes (Philips "colour-matching" type, 40W, 50 $\mu\text{mol}/\text{m}^2/\text{s}$). Supplementary R ($\lambda_{\text{max}} 640 \text{ nm}$, 50 $\mu\text{mol}/\text{m}^2/\text{s}$) or PR ($\lambda_{\text{max}} 740 \text{ nm}$, 50 $\mu\text{mol}/\text{m}^2/\text{s}$) was from two diode laser light sources (Schott KL 150 B) fitted with 25 mm diameter interference filters and cooled as described in section 1. This supplementary light was directed onto the segments by a custom-made 5 mm active-diameter light guide

layers of anti-vibration matting. The internode segment was bathed in aerated medium pumped through the chamber at 1ml/min by peristaltic pumps. Medium was made-up each day using double-distilled water and analytical grade reagents, and allowed to equilibrate at the temperature of the treatment room ($23\pm 1^\circ\text{C}$). After each experiment distilled water was flushed through the apparatus. The transducer output was fed directly to a single pen chart-recorder and the charts were analysed by hand, or by microcomputer. The complete system was housed in a temperature controlled room at $23\pm 1^\circ\text{C}$.

Any changes in medium composition, or light regime, were made when the segment was elongating at a constant rate. This was usually 4h after excision. Fluctuations in the elongation rates of isolated segments are a source of error in rapid growth measurements. Excision of stem segments results in an immediate stimulation of elongation, probably because of tissue expansion, and this lasts about 30-60, min before elongation declines to a steady rate (Evans, 1973).

The Light Sources

The spectral properties of the light sources are shown in Figure 30. Background WL was from two vertically mounted fluorescent tubes (Philips "colour-matching"; R:FR=3.4, $\phi=0.72$; $25 \mu\text{mol}/\text{m}^2\text{s}$). Supplementary R ($\lambda_{\text{max}} 640 \text{ nm}$; $50 \mu\text{mol}/\text{m}^2\text{s}$) or FR ($\lambda_{\text{max}} 719 \text{ nm}$; $50 \mu\text{mol}/\text{m}^2\text{s}$) was from two fibre-optic light sources (Schott KL 150 B) fitted with 25 mm diameter interference filters and cooled as described in section 1. This supplementary light was directed onto the segments by a custom-made 9 mm active-diameter light guide

Figure 30

Relative spectral photon fluence rates (SPFR) for light sources.

A: background WL from Phillips "Colour-Matching" fluorescent tubes.

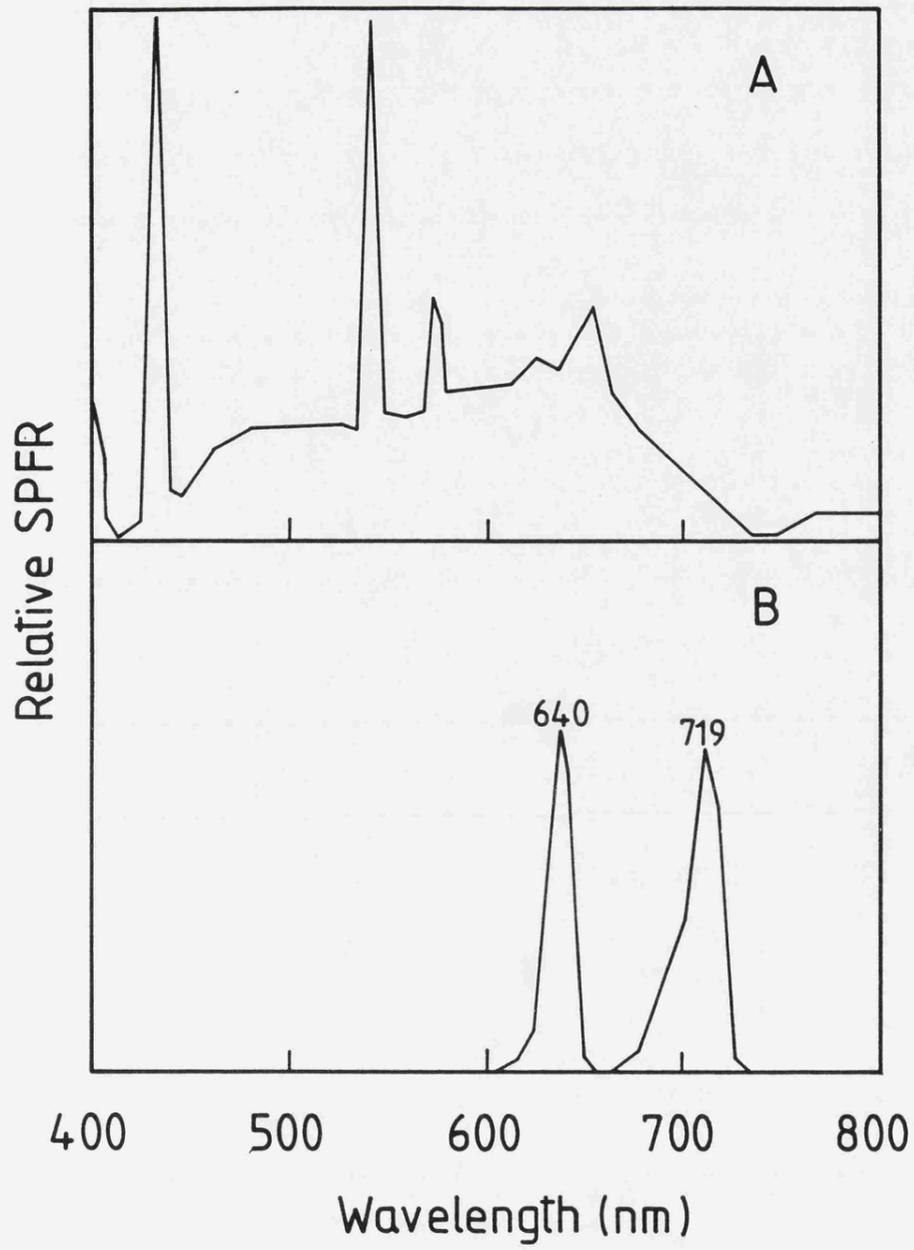
Full scale response = $2.23 \mu\text{mol}/\text{m}^2 \text{ s nm}$.

R:FR = 3.4, $\phi_e = 0.72$.

B: supplementary R (λ max 640 nm) and FR (λ max 719 nm) from Ealing
25 mm diameter interference filters.

Full scale response 640 nm = $1.5 \mu\text{mol}/\text{m}^2 \text{ s nm}$.

Full scale response 719 nm = $6.00 \mu\text{mol}/\text{m}^2 \text{ s nm}$.



(Volpi AG, Switzerland). The light-guide randomly mixed light from the two sources, and then redivided into two probes, allowing segments to be irradiated with completely mixed light of two wavelengths.

A control experiment was done to examine the effect of IR on segment elongation rate. A dry-mounting iron placed 40 mm from the front of the flow-chamber was the IR source, and temperature changes were monitored with a miniature bead thermistor touching the epidermis. The thermistor output was fed into a millivoltmeter.

Light Measurement

This was as described in section 1.

Phytochrome Measurement

The effect of the light treatments on ϕ was assessed by measuring ϕ in tissue of dark-grown Avena sativa var Mostyn as described in section 1. The cuvette was irradiated for 5 min in the same position as the flow-chamber of the transducer apparatus.

Statistical Treatment of Results

The mean values quoted are for at least five replicates. Where examples of single experiments are shown these were chosen as representative of at least three experiments. In most cases ten replicates were obtained for light treatments. The standard error (SE) is, strictly speaking, not applicable to small samples of less than thirty replicates (Bailey, 1959), but it is given to indicate the variability in the experiments.

Differences in elongation rate (ΔER) were analysed using the method of "paired comparisons":

$$t = \frac{\bar{x} - \mu}{\sqrt{s^2} / \sqrt{n}}$$

where: t = the value of P in the t-distribution

\bar{x} = the difference in elongation rate (ΔER)

$\mu = 0$ as the null hypothesis suggests that the hypothetical mean will be the same before and after the treatment.

Results and Discussion

Spontaneous Changes in Elongation Rate

Figure 31 shows that a number of spontaneous changes in elongation rate occurred in the internode segments in the first 3h after excision. In water or 10 mM KCl the rate initially was about 1.1 $\mu\text{m}/\text{min}$, but this declined to a low steady-state rate of between 0.2-0.3 $\mu\text{m}/\text{min}$ after about 35 min. When the bathing medium contained sucrose the initial elongation rate was very high (circa 18 $\mu\text{m}/\text{min}$), but again this declined to a lower rate after 40 min (about 1 $\mu\text{m}/\text{min}$), and then to approximately 0.4 $\mu\text{m}/\text{min}$ at 100 min. The rate remained constant for 50 min and then gradually increased to reach a plateau 230 min after excision. This level could be maintained for up to 24h (Figure 32) and was highest in medium containing 10 mM KCl in addition to 100 mM Sucrose (Figure 31 and Figure 33). All treatments were carried out at least 4h after excision to avoid the complications of these spontaneous changes. Similar fluctuations have been described in excised coleoptile segments from oats and maize (Cline et al, 1974; Evans, 1973; Evans & Schmitt, 1975). Typically, in these the initial elongation rate is high (about 12 $\mu\text{m}/\text{min}$ in 10mm oat segments), but this rate gradually declines, to reach a low steady value (circa 0.7 $\mu\text{m}/\text{min}$) after 60 min. The rate remains constant for a further 90-120 min before increasing to a new high level. In maize this level is sustained for up to 6h, but in oats a second decline occurs before a constant rate of about 0.7 $\mu\text{m}/\text{min}$ is achieved (Evans & Schmitt, 1975).

Vesper & Evans (1978) found that during the first few hours following excision the sensitivity of oat coleoptiles to auxin

Figure 31

Spontaneous changes in elongation rate in internode segments from Sinapis alba.

The elongation rates are for representative segments.

- · · · · · ■ distilled H₂O
- · · · · · □ 10 mM KCl (follows distilled H₂O for the first 40 min).
- ————— ● 100 mM Sucrose
- ▲ · · · · · ▲ 100 mM Sucrose + 10 mM KCl

Time 0 is when the segments were excised and fixed in the apparatus immediately.

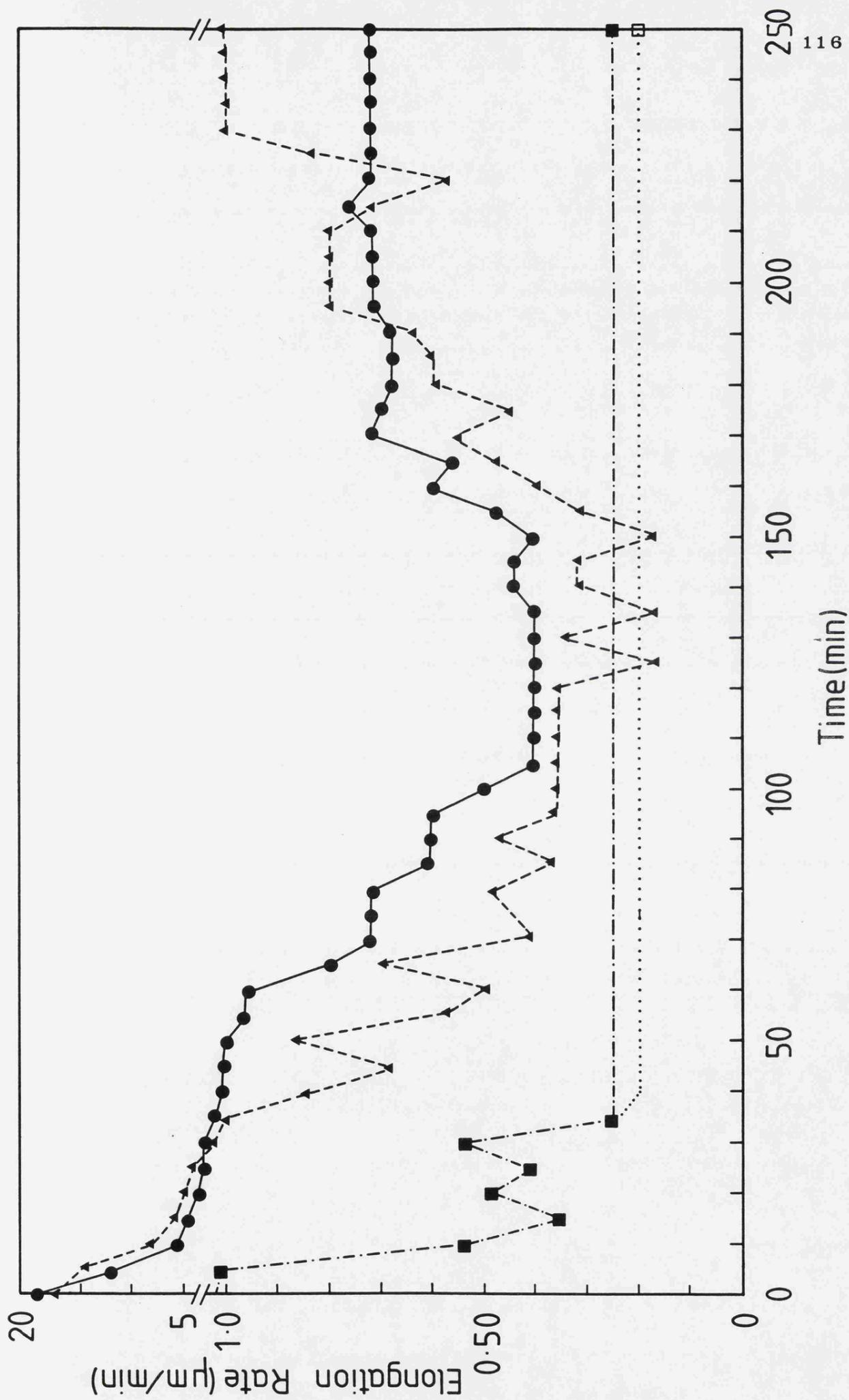


Figure 32

The effect of supplementary FR on elongation in excised internode segments from Sinapis alba.

These are copies of traces for representative segments incubated in 100 mM sucrose + 10 mM KCl in background WL ($25 \mu\text{mol/m}^2 \text{ s}$, R:FR = 3.4).

Supplementary FR ($\lambda \text{ max } 719 \text{ nm}$, $50 \mu\text{mol/m}^2 \text{ s}$) was from fibre-optic probes.

A: FR given 24 h after the segment was excised.

B: FR given 4 h after the segment was excised.

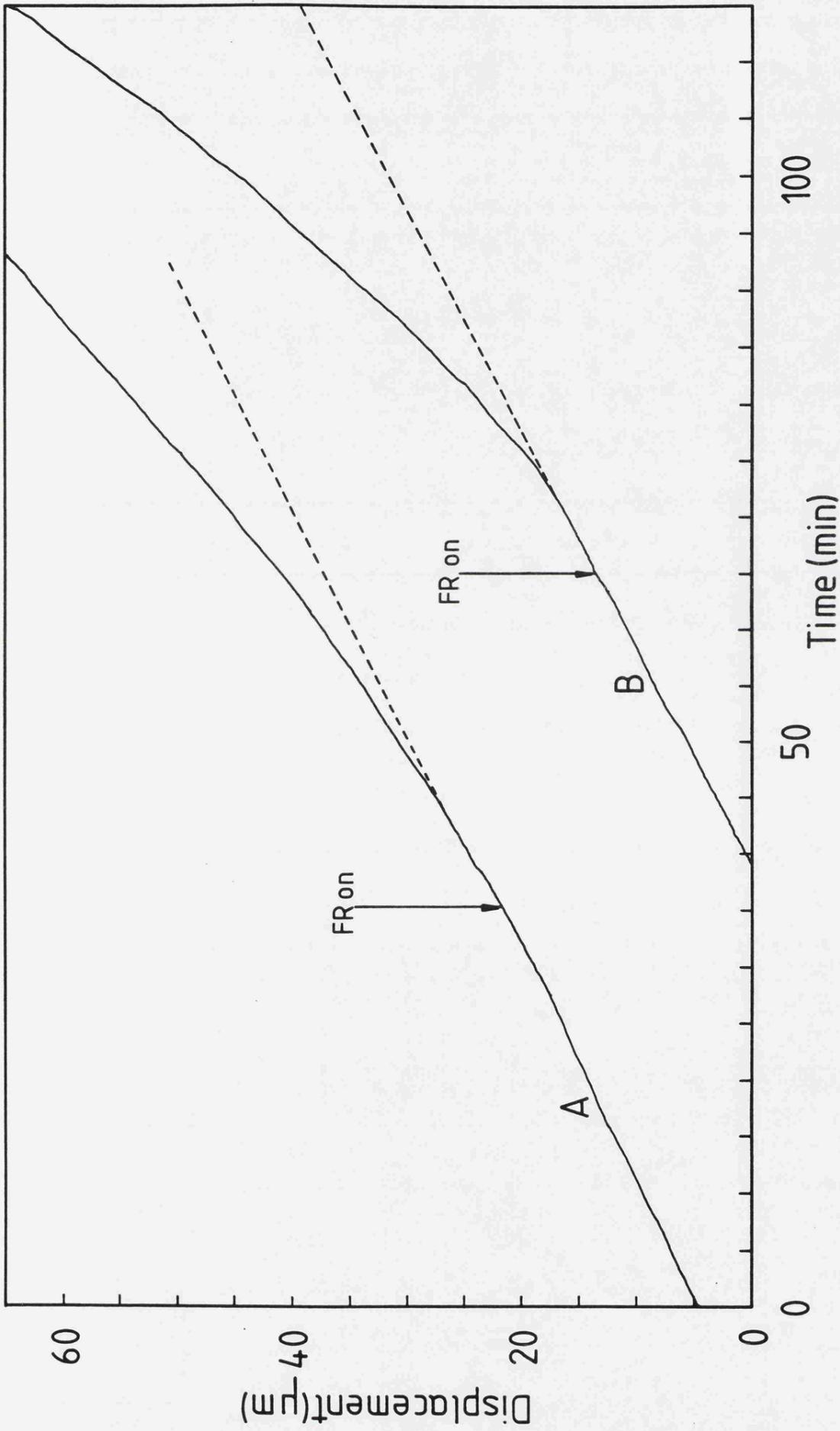


Figure 33

The effect of medium composition on elongation in internode segments from Sinapis alba.

The values are for the means of at least 5 segments (the bars are \pm S.E.).
Background WL was $25 \mu\text{mol/m}^2 \text{ s}$ and the elongation rates were measured over 120 min, 4 h after excision.

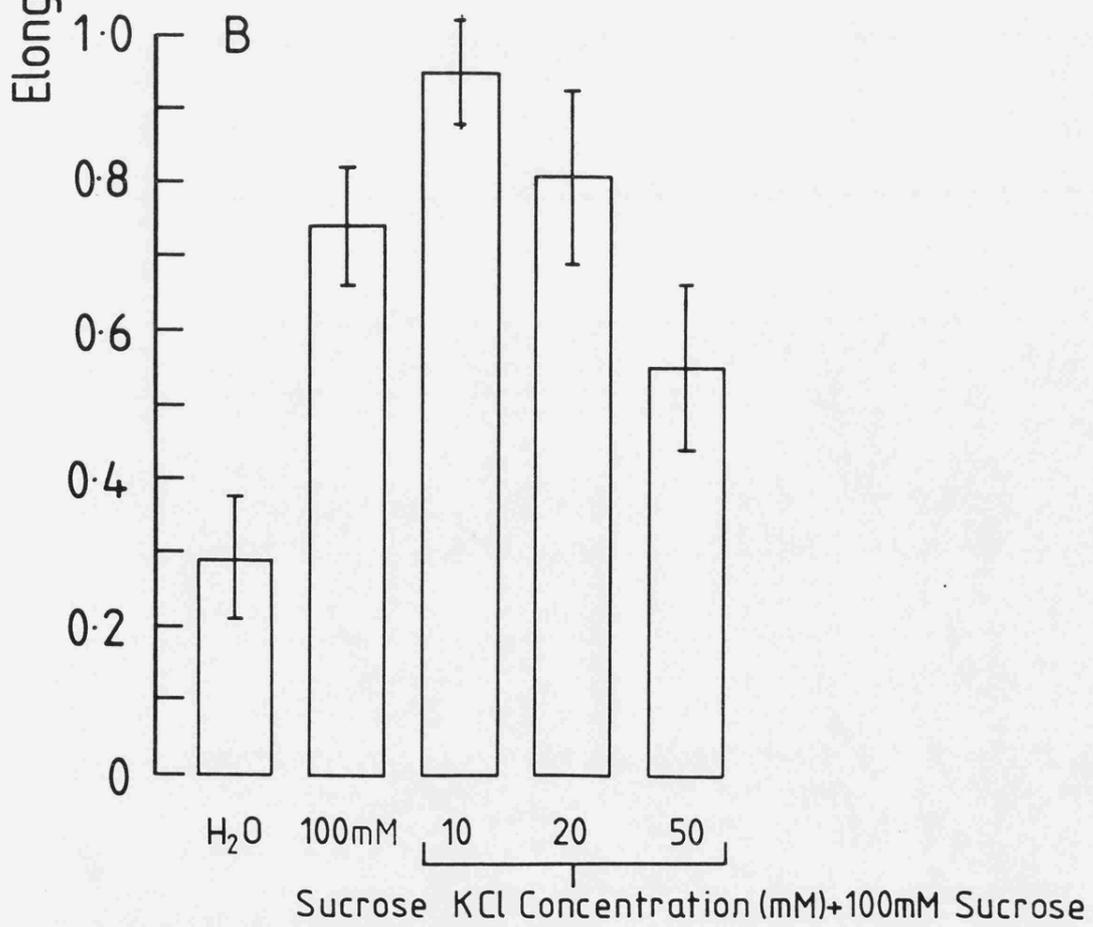
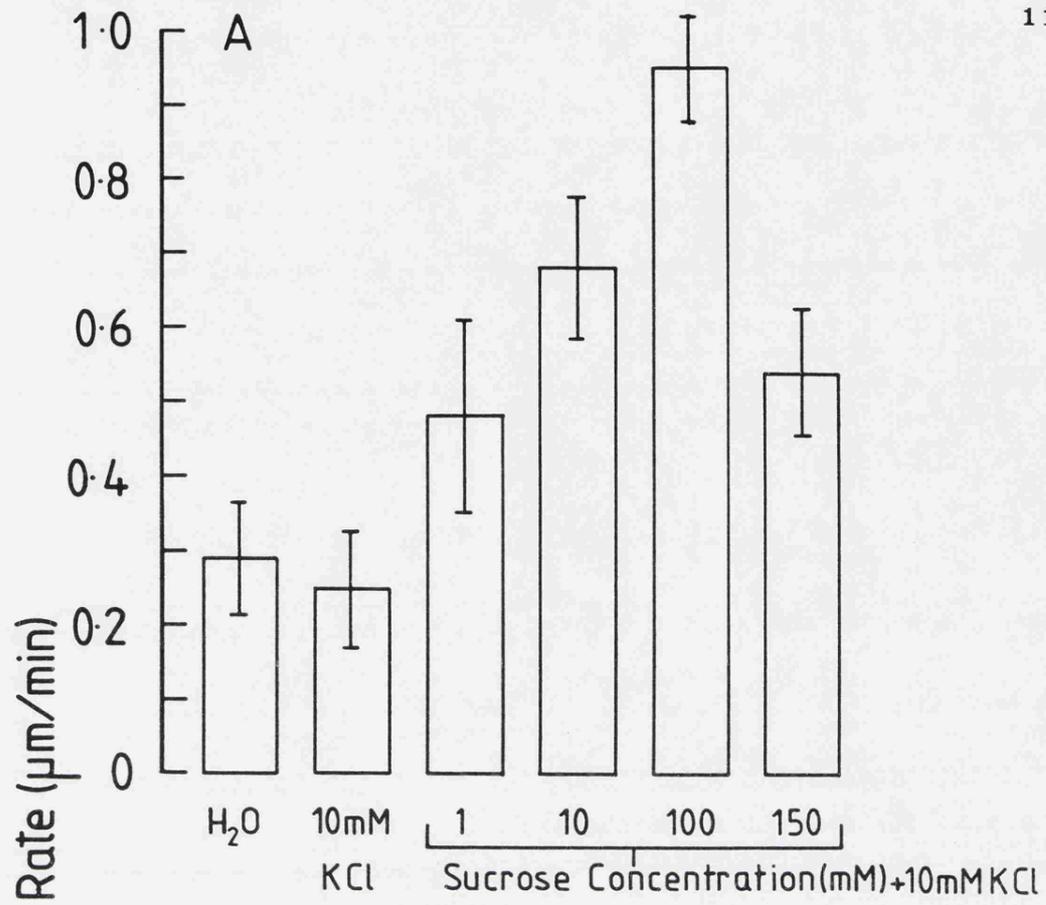
A: the effect of sucrose concentration. All treatments contain 10 mM KCl except the distilled water control.

B: the effect of KCl concentration. All treatments contain 100 mM sucrose except the distilled water control.

Note: the mean rate in 100 mM Sucrose = $0.74 \pm 0.06 \mu\text{m/min}$.

the mean rate in 100 mM Sucrose + 10 mM KCl = $0.95 \pm 0.07 \mu\text{m/min}$.

$t = 2.02^*$ (d.f. = 50)



changed, and the most rapid and extensive elongation rates were exhibited upwards of 150 min after excision - that is, when the endogenous elongation rate is increasing. These observations have prompted Hanson & Trewavas (1982) to conclude that much of the experimentally-realised stimulation of growth by auxin (and fusicoccin) is little more than accelerated recovery from excision injury. They propose that the trauma of excision impairs the function of the plasma-membrane ion pumps. These pumps recover during incubation and this process may be enhanced by growth factors (such as auxin), but a full restoration of growth rate may never occur. This could explain why excised segments rarely achieve the same growth rates as the intact plant. There is evidence that trauma induces a dysfunction in plasma-membrane ATPase systems. Gronewald & Hanson (1980) observed that the excision of maize root segments resulted in a depolarisation of the cell membranes, accompanied by a net loss of K^+ from the cells in the initial 30-60 min. Washing the roots brought about a spontaneous recovery of the tissue, involving restoration of K^+ influx and H^+ efflux, and cell membrane potential. Repolarisation of the cell membranes begins immediately after excision, but full recovery depends on the tissue and may take from 3-16h (see, Hanson & Trewavas, 1982). In maize roots H^+ efflux shows a rapid rate of recovery, and exceeds K^+ influx until this stabilises after 180 min, at which time H^+/K^+ becomes unity again (Gronewald & Hanson, 1980). Shock also causes a rapid, passive influx of Ca^{2+} from the apoplast which may impair the activity of certain H^+ ATPases (Balke et al, 1974; Zocchi & Hanson, 1983). The Ca^{2+} balance can be restored by a calmodulin directed transport of Ca^{2+} from the cell, and an antiport coupled to a fusicoccin-stimulated ATPase (Zocchi & Hanson, 1983).

It is possible that the spontaneous changes in elongation rate which occur in excised segments represent the recovery of membrane located

ion pumps from excision trauma. The ability of fusicoccin to assist the restoration of the Ca^{2+} balance may, in part, explain its capacity to stimulate elongation, but no such function has been demonstrated for auxin. Moreover, it would be expected for auxin to be more effective at stimulating proton efflux and elongation, in tissues where the activity of ion pumps has been restored; a point apparently not appreciated by Hanson & Trewavas (1982).

The Role of Sucrose and KCl in Elongation Growth

The highest elongation rates in WL were obtained in medium containing sucrose (optimum concentration 100 mM) and KCl (optimum concentration 10 mM) (Figure 33). Sucrose alone supported relatively high elongation rates and may have several roles: it may act as an osmoticum, an energy supply and a substrate for wall synthesis.

Auxin-induced elongation in oat coleoptiles is stimulated by sucrose (Ordin et al, 1956), and sucrose is also necessary for gibberellin-induced elongation in light-grown oat stem segments (Adams et al, 1973). In oat coleoptiles sucrose is probably required in the short-term primarily as an osmoticum. Ray (1962) showed that in the absence of sugar oat coleoptiles were still able to elongate for some time, but that the cell walls became thinner than those provided with sugar. Under these conditions the coleoptiles maintain the α -cellulose component of their cell walls at the expense of matrix polysaccharide synthesis. S. alba internode segments may not be capable of preserving the integrity of their cell walls in this way, and possibly require a constant supply of cell wall substrates.

Stevenson & Cleland (1981; 1982) found that the stimulation of

auxin-induced elongation by sucrose in oat coleoptiles was related to solute uptake and that this uptake was dependent upon the amount of elongation, rather than upon auxin concentration per se. KCl and NaCl were also found to be effective at stimulating auxin-induced elongation and they concluded that permeant solutes can enhance elongation by enabling the tissue to sustain a constant osmotic concentration.

In the internode segments used in these experiments, KCl alone had no significant effect on elongation rate (Figure 33); so it is unlikely that its ability to enhance elongation in the presence of sucrose is due to it acting as an osmoticum. Sucrose itself could fulfil this role, as well as being a substrate for respiration and wall synthesis. It is possible that KCl might provide K^+ as counter ions for proton efflux in the elongating internodes (Cleland & Lomax, 1977).

The Effect of R:FR on Elongation Rate

Isolated internode segments responded to added FR when the medium contained sucrose, but the largest response was in the presence of sucrose and KCl (Table 6). This response was exhibited in segments 4h after excision (Figures 32B, 34, 35 and 36) and also occurred in segments incubated for 24h (Figure 32). The latent period for this response was about 6 min (Table 7) which is 4.2 min shorter than the equivalent response in the intact plant. Unlike the response in intact plants, however, the latent period was not followed by a large, transient increase in growth (phase-1); instead the elongation rate increased gradually over 35 min before levelling out (Figure 36). This plateau is equivalent to phase-2 of the response in intact plants and could be sustained for as long as the FR was on. Switching off the FR caused the elongation rate to decline

Table 6

The effect of medium composition on the response to supplementary FR in internode segments from Sinapis alba.

Supplementary FR (λ max 719 nm, $50 \mu\text{mol}/\text{m}^2 \text{ s}$) was from fibre-optic probes. Background WL was $25 \mu\text{mol}/\text{m}^2 \text{ s}$. The elongation rates were measured over 60 min before the FR was given and over 60 min at the steady-state WL + FR level. All measurements were made 4 h after excision. The values are means \pm S.E.

Δ ER = the change in elongation rate.

Treatment	Elongation Rate ($\mu\text{m}/\text{min}$)		ΔER	Reps.
	WL	WL+FR		
Distilled H ₂ O	0.29±0.09	0.34±0.08	+0.05±0.03 N.S.	10
10mM KCl	0.25±0.08	0.38±0.12	+0.13±0.06 N.S.	10
100mM Sucrose	0.67±0.09	0.95±0.11	+0.29±0.05 *	10
100mM Sucrose+10mM KCl	0.79±0.10	1.22±0.11	+0.44±0.10 *	12

Figure 34

The effect of supplementary light on elongation in internode segments from Sinapis alba.

Representative segments incubated for at least 4 h in 100 mM Sucrose + 10 mM KCl.

Supplementary FR (λ max 719 nm, $50 \mu\text{mol}/\text{m}^2 \text{s}$) and R (λ max 640 nm, $50 \mu\text{mol}/\text{m}^2 \text{s}$) was from fibre-optic probes. Background WL was $25 \mu\text{mol}/\text{m}^2 \text{s}$.

A: Supplementary FR.

B: Supplementary R + FR followed by switching off the R.

C: Supplementary R.

○————○ = elongation rate ($\mu\text{m}/\text{min}$).

———— = elongation on chart trace.

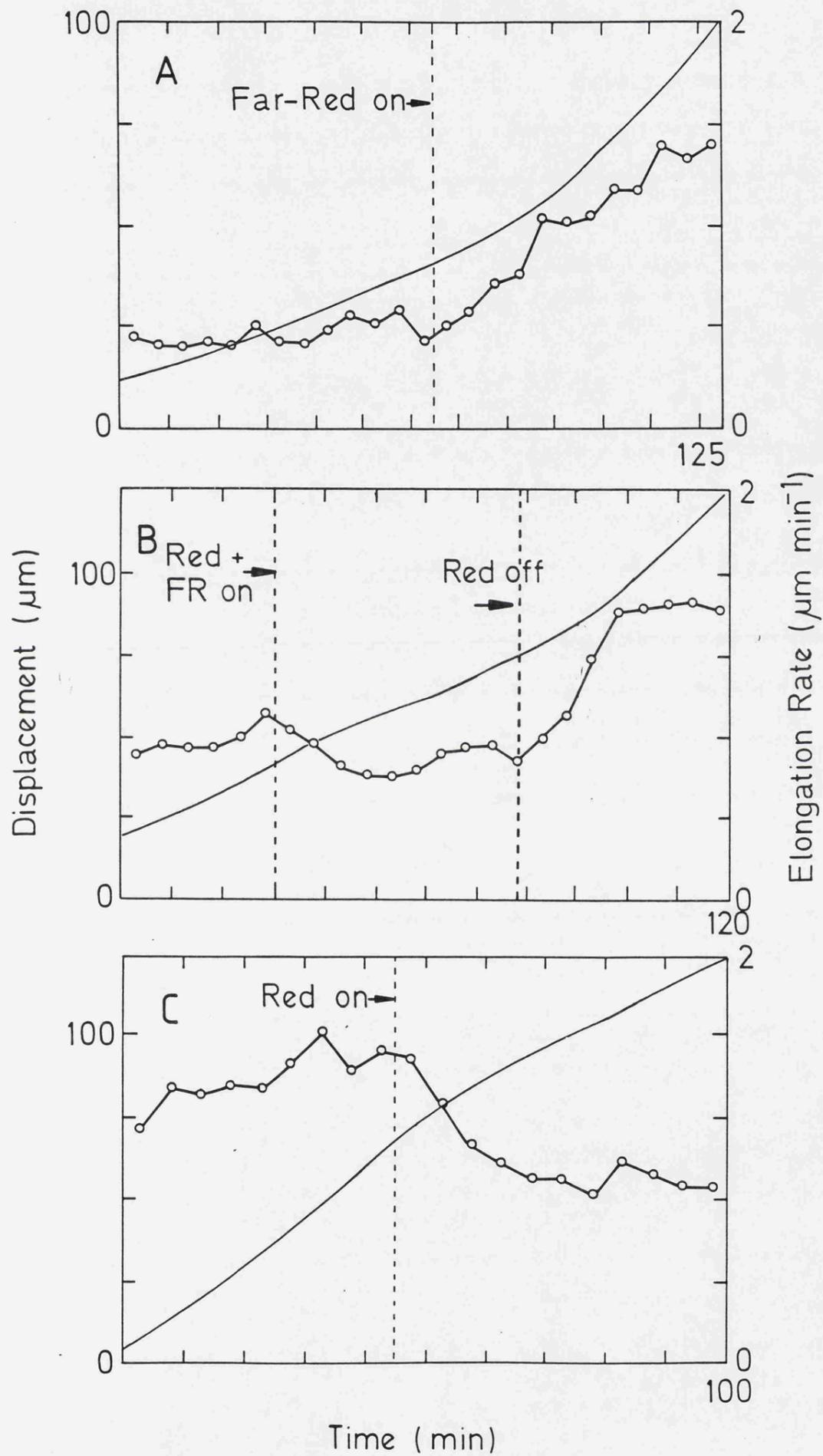


Figure 35

The effect of supplementary FR on elongation in internode segments from Sinapis alba.

A representative segment incubated for 4 h in 100 mM Sucrose + 10 mM KCl.

Supplementary FR (λ max 719 nm, $50 \mu\text{mol}/\text{m}^2 \text{ s}$) was from fibre-optic probes.

Background WL was $25 \mu\text{mol}/\text{m}^2 \text{ s}$.

○————○ = elongation rate ($\mu\text{m}/\text{min}$).

———— = elongation on chart trace.

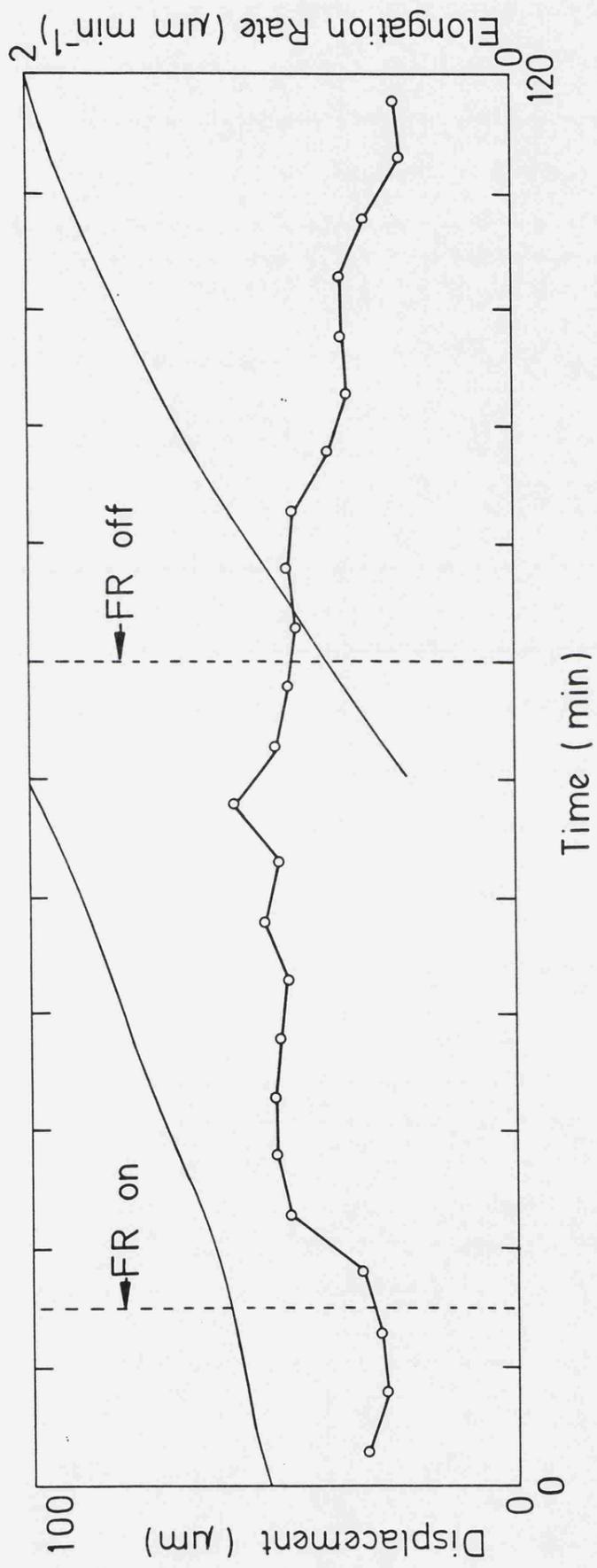


Figure 36

The effect of supplementary FR on elongation in internode segments from Sinapis alba.

Each point is the mean of 10 segments incubated for 4 h in 100 mM Sucrose + 10 mM KCl.

Supplementary FR (λ max 719 nm, $50 \mu\text{mol}/\text{m}^2\text{s}$) was from fibre-optic probes.

Background WL was $25 \mu\text{mol}/\text{m}^2\text{s}$.

The bar is \pm S.E.

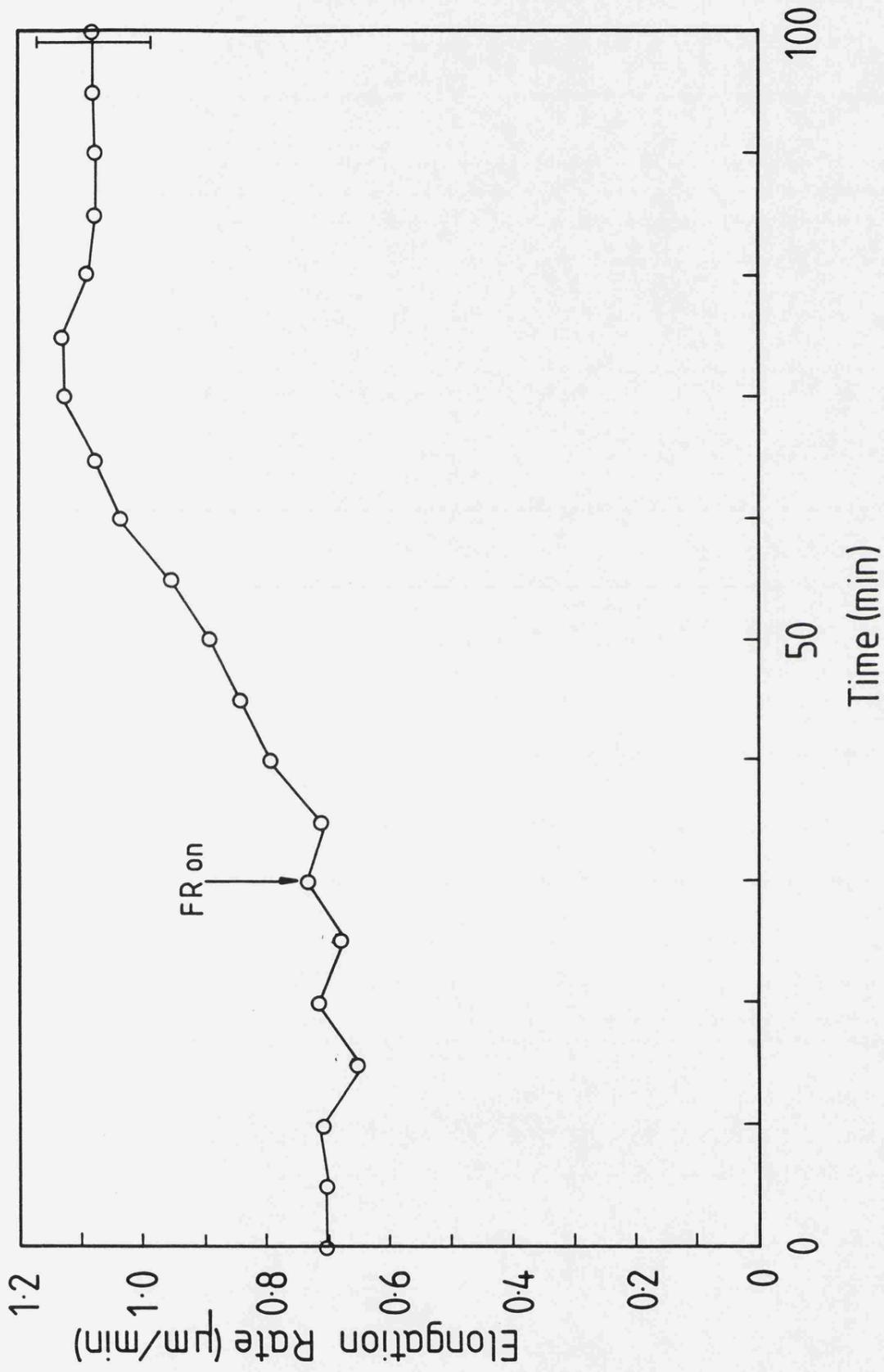


Table 7

The effect of supplementary light on elongation in internode segments from Sinapis alba.

Supplementary FR (λ max 719 nm, $50 \mu\text{mol}/\text{m}^2 \text{s}$) and R (λ max 640 nm, $50 \mu\text{mol}/\text{m}^2 \text{s}$) was from fibre-optic probes. Background WL was $25 \mu\text{mol}/\text{m}^2 \text{s}$.

All segments were incubated in 100 mM sucrose + 10 mM KCl and the measurements were made 4 h after excision. The elongation rates were measured over 60 min before the change in light treatment and over 60 min at the new steady-state level. ΔER = the change in elongation rate.

The values are means \pm S.E.

Treatment	Elongation Rate ($\mu\text{m}/\text{min}$)		ΔER	Lag Time (min)	Reps.
	Before	After			
WL \rightarrow WL+FR	0.79 \pm 0.10	1.22 \pm 0.11	+0.44 \pm 0.10 *	6.1 \pm 0.4	12
WL \rightarrow WL+R	1.06 \pm 0.14	0.82 \pm 0.09	-0.24 \pm 0.06 *	5.6 \pm 0.4	9
WL \rightarrow WL+FR+R	1.06 \pm 0.12	0.94 \pm 0.10	-0.12 \pm 0.07 N.S.	6.8 \pm 0.7	12
WL+FR+R \rightarrow WL+FR	0.94 \pm 0.10	1.22 \pm 0.13	+0.27 \pm 0.07 *	8.5 \pm 1.1	12

after about 7 min (Figure 35) and the original WL rate was attained after about 16 min, which is the same as the decay kinetics for intact plants. The response to added FR in internode segments, therefore, differs from that in intact plants in two features:

- (i) The latent period is approximately 40% shorter in excised segments.
- (ii) Excised segments exhibit no transient increase in growth rate, i.e., no first phase.

The shorter latent period may be due to the excised segments having direct access to a large supply of sucrose. Stems are usually net importers of sugar and in intact plants it could take a certain time to supply the important tissues with sufficient sugar for a large increase in cell elongation rate (see, Peel, 1974).

The transient phase-1 in intact plants could be the manifestation of a release of growth potential that has built up during the prolonged period of WL. Once this potential has been expressed, subsequent growth would be dependent upon the re-synthesis of substances required for cell elongation. In the excised segments, excision may cause the loss of this growth potential, perhaps through leakage of substances from the tissues or by proteolysis. Alternatively, the capacity to express this potential might be reduced, because of impaired enzyme activity; particularly of the plasma membrane ATPases.

The response to FR was negated by the simultaneous addition of R (Table 7, Figure 34). Removing the R allowed the FR response to be exhibited, with similar kinetics to FR given alone (Table 7, Figure 34). This shows that the FR response is reversed by R and that it

is independent of fluence rate, as the same response was obtained when the fluence rate was increased (by adding FR alone) or decreased (by removing R). R light alone caused a decrease in elongation rate which was detectable within 6 min (Table 7, Figures 34 and 37) and completed after about 20 min (Figure 37). These kinetics are similar to those shown when the FR was switched off (Figure 35).

These results are consistent with the regulation of rapid elongation responses by phytochrome acting via photoequilibrium, despite the FR ($\lambda_{\max} 719$ nm) being in the range which stimulates photosynthetic electron transport. Table 8 shows the photoequilibrium set-up by each light treatment; the addition of R to the fluorescent light caused a small increase in photoequilibrium, even in the presence of added FR. This increase may be related to the age of the tubes, because old fluorescent tubes have a higher FR output. R light is more effective at photoconverting phytochrome than FR; so when they are added at equal fluence rates R could more than counteract the effect of FR. Figure 38 demonstrates that the changes in elongation rate can be inversely related to the photoequilibrium under these light regimes.

Additional IR gave a steady-state increase in elongation rate about 50% less than that obtained with supplementary FR (Table 9). The temperature rise was 3.8°C, compared with a maximum rise of 0.3°C with FR, and 1.1°C for FR+R (a treatment which actually resulted in a small decrease in elongation rate). Furthermore, the kinetics of the IR and FR induced changes in elongation rate were different (Figure 39) and it seems improbable that the temperature changes associated with these light treatments make any significant contribution to the observed changes in elongation rates.

Figure 37

The effect of supplementary R on elongation in internode segments from Sinapis alba.

Each point is the mean of 7 segments incubated for 4 h in 100 mM Sucrose + 10 mM KCl. Supplementary R (λ max 640 nm, $50 \mu\text{mol}/\text{m}^2 \text{ s}$) was from fibre-optic probes. Background WL was $25 \mu\text{mol}/\text{m}^2 \text{ s}$.

The bar is \pm S.E.

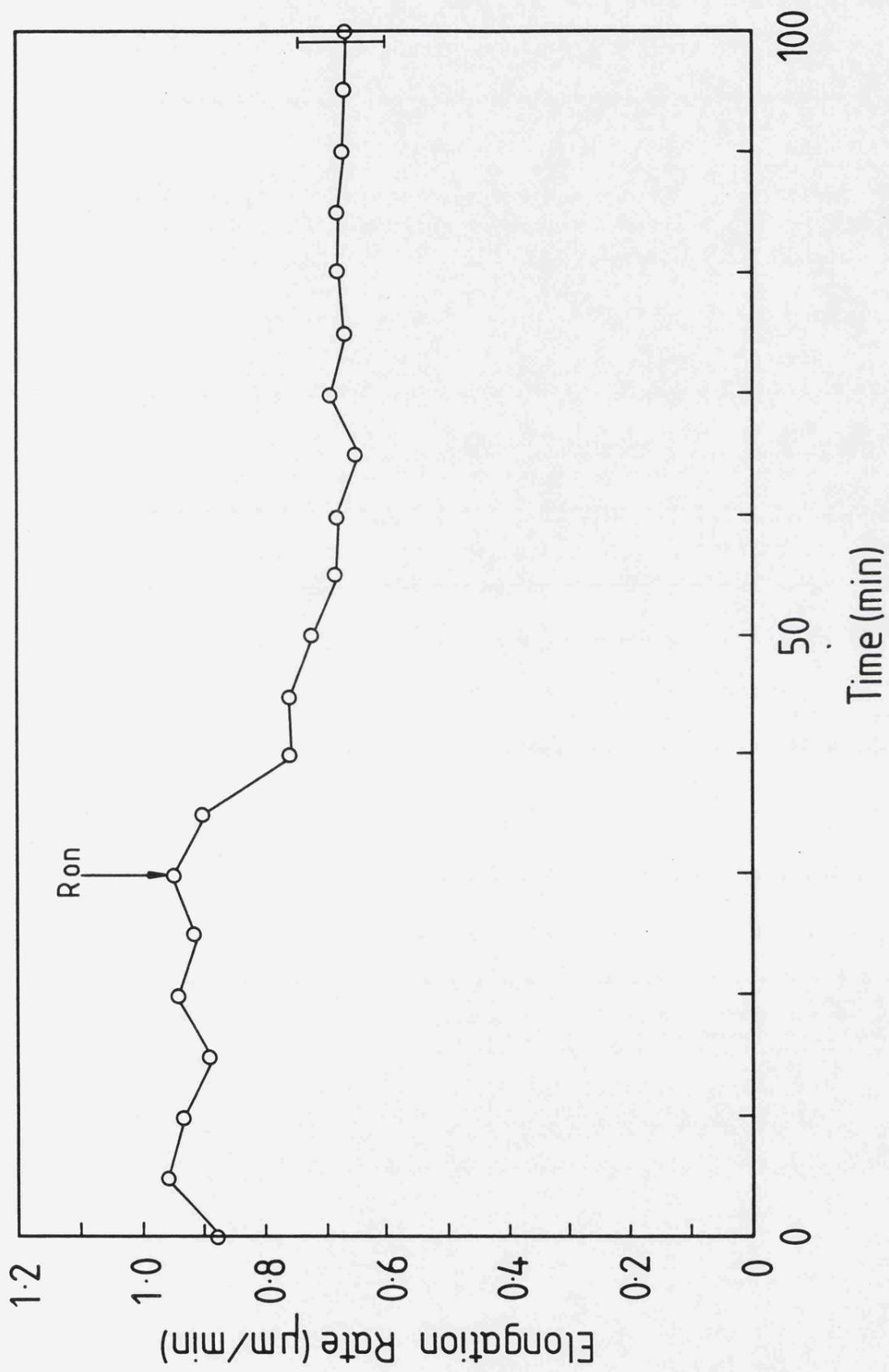


Table 8

Details of the light treatments for the experiments with internode segments from Sinapis alba.

Treatment	Total Fluence Rate ($\mu\text{mol}/\text{m}^2\text{s}$)	ϕ_m
WL	25	0.60
WL+FR	75	0.42
WL+R	75	0.65
WL+FR+R	125	0.63

Figure 38

The relationship between elongation rate and ϕ_m in internode segments from Sinapis alba.

Each point is the mean percentage change in elongation rate ($\% \Delta$ elongation rate) for at least 9 segments. Details of the elongation rates are shown in Table 7, the light treatments are described in Table 8.

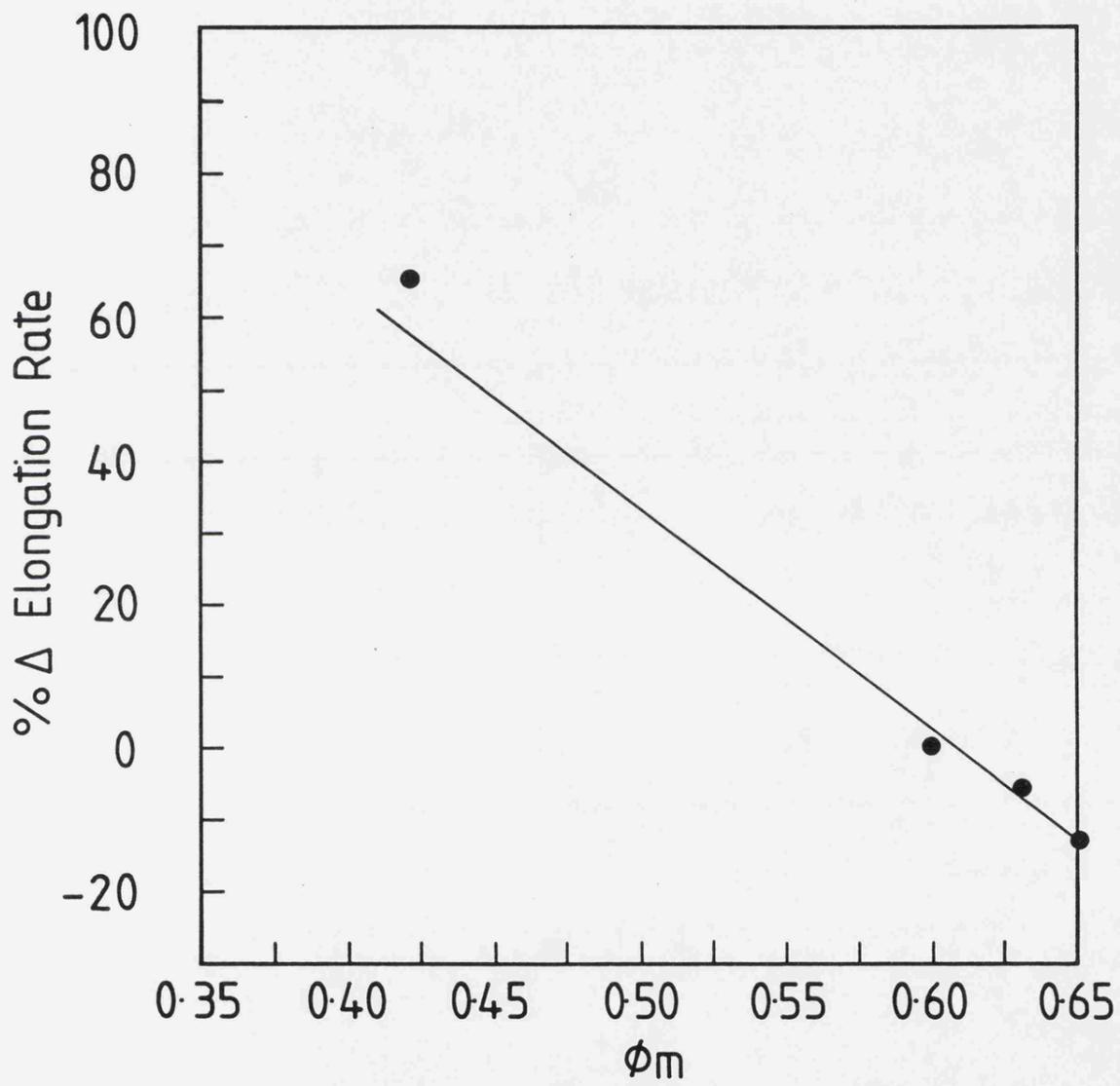


Table 9

The effect of IR on elongation rate in internode segments from Sinapis alba.

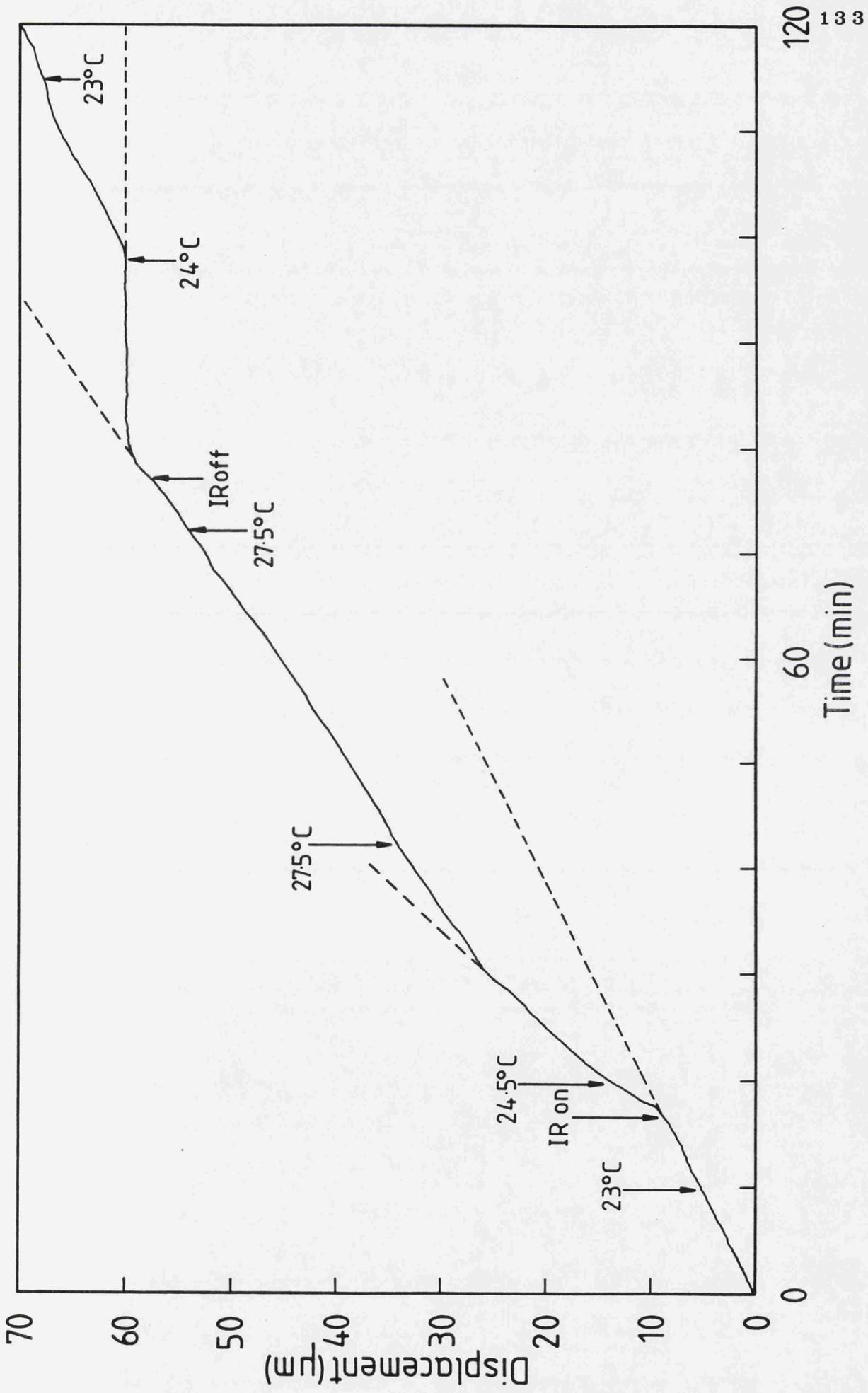
Segments were incubated in 100 mM sucrose for 4 h before measurements were made. The WL elongation rate was measured over 60 min before the IR was given. The initial rise in elongation rate is the rate over the 15 min following the addition of IR (the lag period for the response was about 1 min). The steady-state elongation rate was measured over 60 min after the initial rise. The temperature rise is the maximum recorded for 10 segments. Reference should be made to Figure 39 to clarify these details.

ΔER = the change in elongation rate between the WL and steady-state rates. The values are the mean \pm S.E.

	Elongation Rate ($\mu\text{m}/\text{min}$)	ΔER	Temperature Rise $^{\circ}\text{C}$	Reps.
WL	Initial Rise	Steady-State		
	1.99 ± 0.14	0.97 ± 0.09	3.8 ± 0.08	10

Figure 39

The effect of IR on elongation in internode segments from Sinapis alba.
A copy of the chart trace of a representative segment incubated in
100 mM Sucrose for 4 h and given IR from an electric dry-mounting iron.
The temperature changes indicated were measured at the surface of the
segment by a miniature bead thermistor. Background WL was $25 \mu\text{mol}/\text{m}^2 \text{ s}$.



The Effect of Medium pH on Elongation Rate

Internode segments from S. alba responded to a decrease in pH from 6.3 to 5.5 with an increase in elongation rate detectable within 1 min (Figure 40). The rate reached a maximum of about 5.5 $\mu\text{m}/\text{min}$ after 30 min, before declining over the next 160 min (Figure 41). The decline in rate was steep for 50 min and then more gradual for a further 110 min, until it reached a steady level 180 min after the change in pH. These kinetics are similar to those reported for segments of etiolated and green tissues from a number of species (e.g., Rayle, 1973; Barkley & Leopold, 1972; Cleland & Rayle, 1975; Pope, 1978).

A feature of acid-induced growth is that, unlike auxin-stimulated elongation, which can continue for up to 24h (Pope, 1978), it is usually exhausted after about 6h (e.g., Bonner, 1934). The transience of acid-growth has not been fully explained, although several theories have been put forward.

One of the first theories was that at pH 3.0, which originally was determined as the optimum pH for acid growth, membrane disruption would occur, resulting in a loss of turgor (Rayle & Cleland, 1970; Evans, 1974b). This explanation was supported by observations in frozen-thawed oat coleoptile segments, where turgor was replaced by a constant applied force. In these conditions acid-induced elongation could be maintained at a high rate for more than 10h (Rayle et al, 1970; Cleland, 1971b). Subsequent experiments, however, demonstrated that the pH optimum of 3.0 was an underestimate. Protons have difficulty in penetrating cuticles; particularly in plants, such as oats, which have relatively thick

Figure 40

The effect of a decrease in pH on elongation in internode segments from Sinapis alba.

A copy of the chart trace of a representative segment incubated in citrate phosphate buffer pH 6.3 (50 mM) for 4h; the pH was then reduced to pH 5.5 by flushing through citrate phosphate buffer pH 5.5 (50 mM).

Background WL was $25 \mu\text{mol/m}^2 \text{s}$.

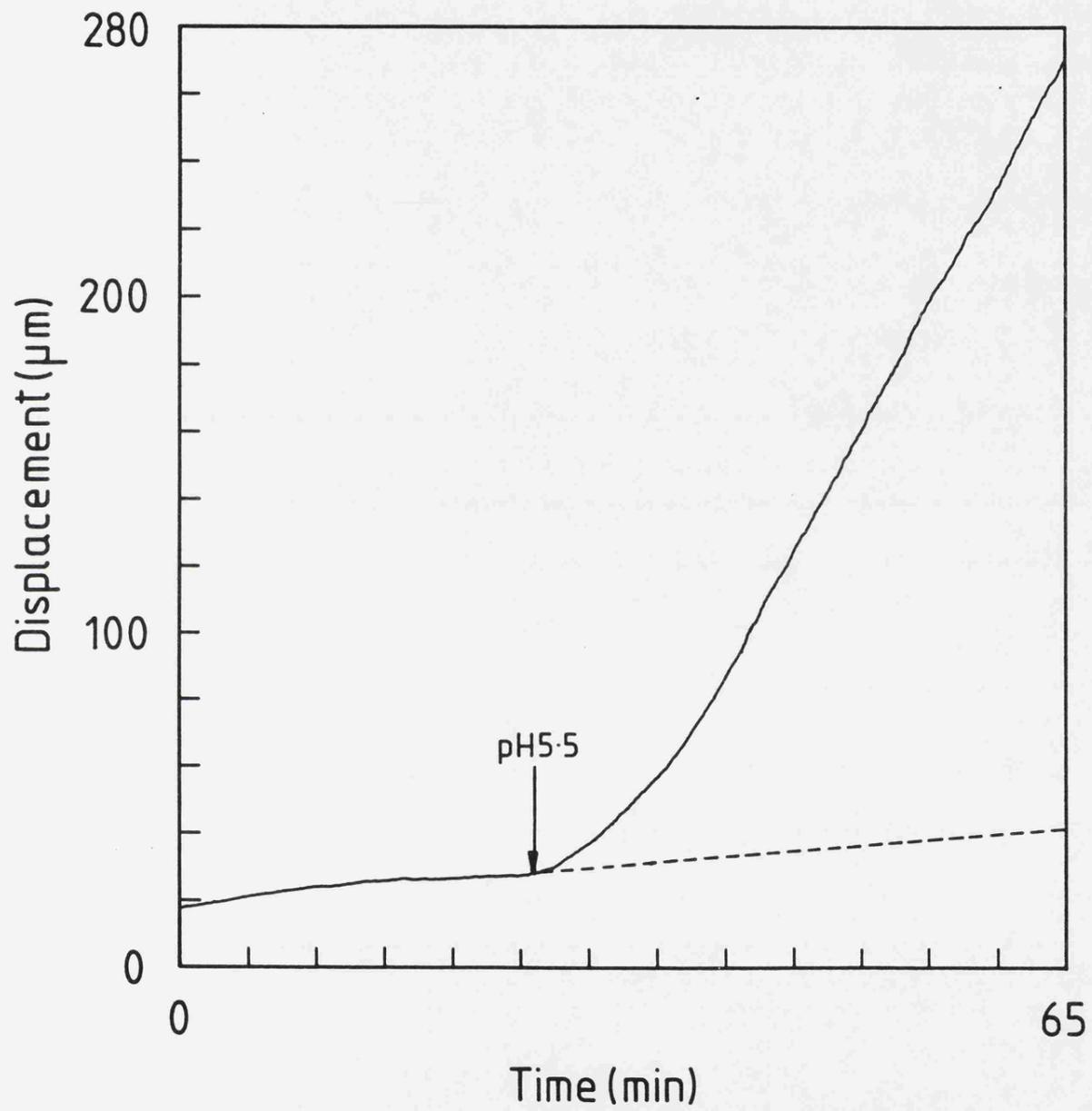
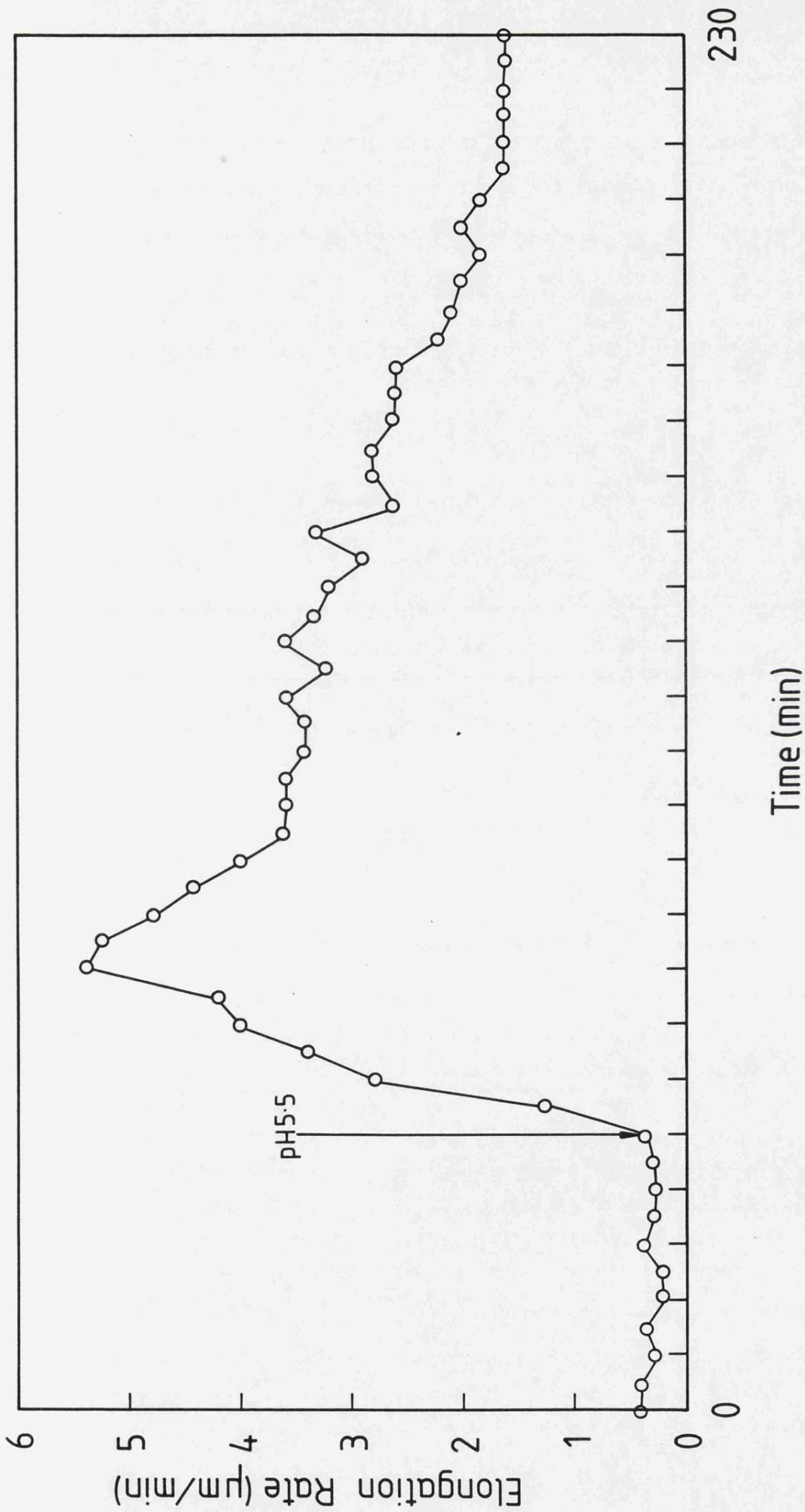


Figure 41

The effect of a decrease in pH on elongation in internode segments from Sinapis alba.

A representative segment incubated in citrate phosphate buffer pH 6.3 (50 mM) for 4h; the pH was then reduced to pH 5.5 by flushing through citrate phosphate buffer pH 5.5 (50 mM).

Background WL was $25 \mu\text{mol/m}^2 \text{s}$.



230

Time (min)

pH 5.5

Elongation Rate (µm/min)

cuticles. When the cuticle was either removed or abraided, the optimum pH for acid growth was found to be about 5.0; a value which is within the physiological range (Rayle, 1973; Marre et al, 1973a,b). The optimum pH for acid growth in S. alba internode segments was pH 5.5 (Figure 42). At this pH the segments remained turgid and elongated for several hours at a level above that of segments in unbuffered medium. This suggests that no membrane damage occurred. In buffered medium at pH 4.0, however, the segments became flaccid within 30 min. Further evidence that acidic pH within the physiological range is not deleterious is provided by Pope (1978), who showed that wheat coleoptiles were still capable of responding to auxin after they had become refractory to further acid treatment. A second explanation has been ventured by Goring (see, Taiz, 1984). He suggests that in segments with the cuticle intact, protons normally penetrate via the cut ends; but that these cuts are eventually sealed by wound reactions, thus, reducing access to the protons. Goring has apparently demonstrated that these segments can respond repeatedly to acid if the damaged cells near the cut surface are removed. The cuticle of S.alba, however, is thin and in these experiments it was gently abraided; so it is unlikely that protons would have difficulty in penetrating the tissues. A third possibility, and the most plausible, is that although acidic buffers are non-toxic up to 50 mM (Rayle & Cleland, 1970), they suppress proton efflux by the segments. Cleland (1975) established that in oat coleoptiles, incubated in low concentrations of buffers (1 mM) to stabilise the pH, active proton efflux was stimulated by external pH values above pH 5.7. Below pH 5.7 proton uptake occurred and active efflux was suppressed. At pH 5.7 active efflux and passive uptake were in equilibrium (under these conditions there is only limited elongation) (Rayle, 1973). Thus, in the absence of any

Figure 42

The effect of pH on elongation rate in internode segments from Sinapis alba.

The values are the means of at least 5 segments (the bars are \pm S.E.).

Background WL was $25 \mu\text{mol/m}^2 \text{ s}$ and the elongation rates were measured over 120 min, 4 h after excision.

They are, from left to right: pH 5.0, 50 mM succinate + 100 mM sucrose.

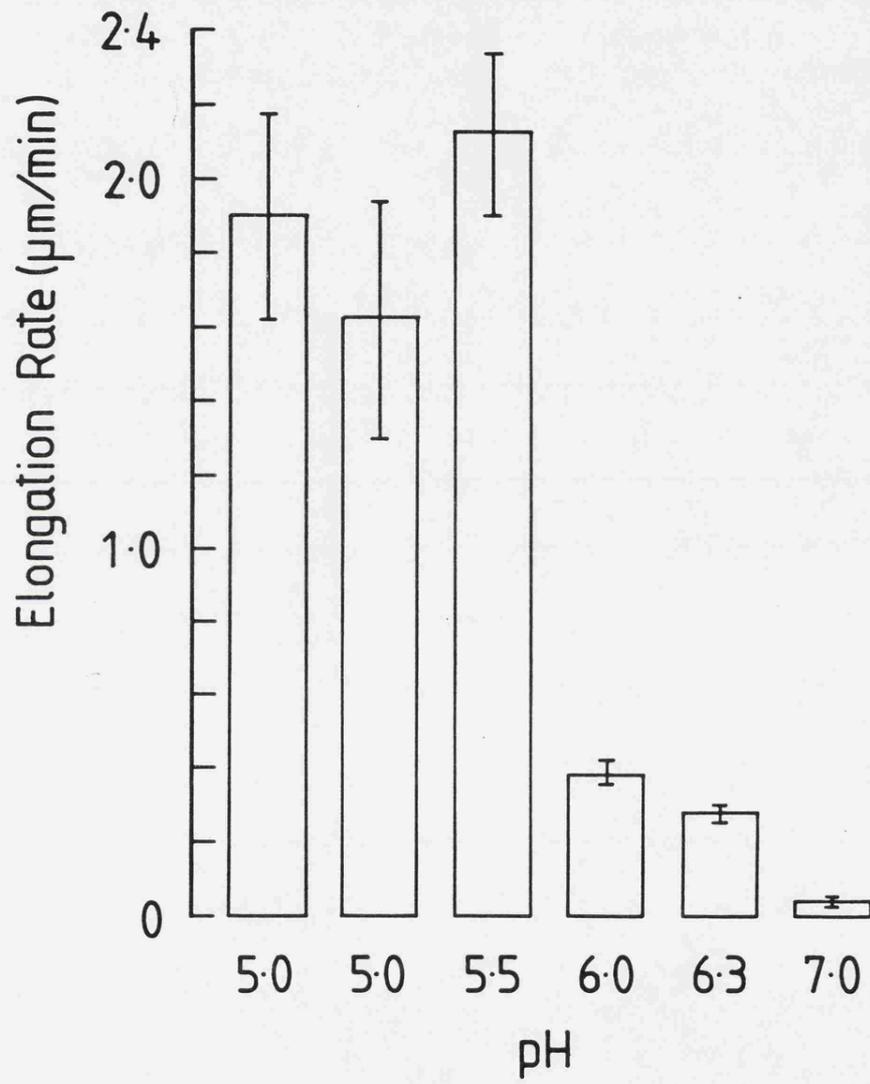
pH 5.0, 50 mM citrate phosphate.

pH 5.5, 10 mM succinate + 100 mM sucrose.

pH 6.0, 50 mM succinate + 100 mM sucrose.

pH 6.3, 50 mM citrate phosphate.

pH 7.0, 50 mM phosphate.



stimulus (e.g., auxin) segments secrete sufficient protons to maintain the cell wall at pH 5.7. Consequently, buffers below pH 5.7 may suppress the segment's own proton secretion and the other phenomena (e.g., plasma-membrane hyperpolarisation) which normally accompany this process. There are reports that these associated phenomena might be important in sustaining the cell's ability to respond to wall acidification. For example, plasma-membrane hyperpolarisation has been linked with the regulation of wall polysaccharide biosynthesis (Bacic et al, 1980) and this will be necessary for prolonged wall extension (Rayle et al, 1970). There was no evidence that the elongation of S. alba segments in acidic media was limited by the availability of substrates for wall synthesis as the inclusion of sucrose did not have a significant effect on elongation rate (Figure 42).

The Interaction Between pH and FR

In medium buffered to around the optimum pH for elongation, cell wall extensibility (M) should be high; so if FR stimulates elongation by changing the magnitude of other parameters of cell enlargement (i.e., by reducing Y, or increasing L or $\Delta\pi$), then it should be synergistic with acid, and increase the elongation rate. Figure 43A shows that at pH 5.5 (with 100 mM sucrose), added FR did not have a significant effect on elongation.

If FR-promoted growth is mediated by an increase in proton secretion, then any adjustment of the external pH should reduce this response. The pH of the distilled water used in these experiments was stable at $\text{pH } 5.8 \pm 0.1$, and was unaffected by the solutes sucrose, KCl and CaCl₂. At pH 5.8 active proton secretion and

Figure 43

The interaction between supplementary FR and medium pH in internode segments from Sinapis alba.

The values are the means of at least 5 segments (the bars are \pm S.E.).

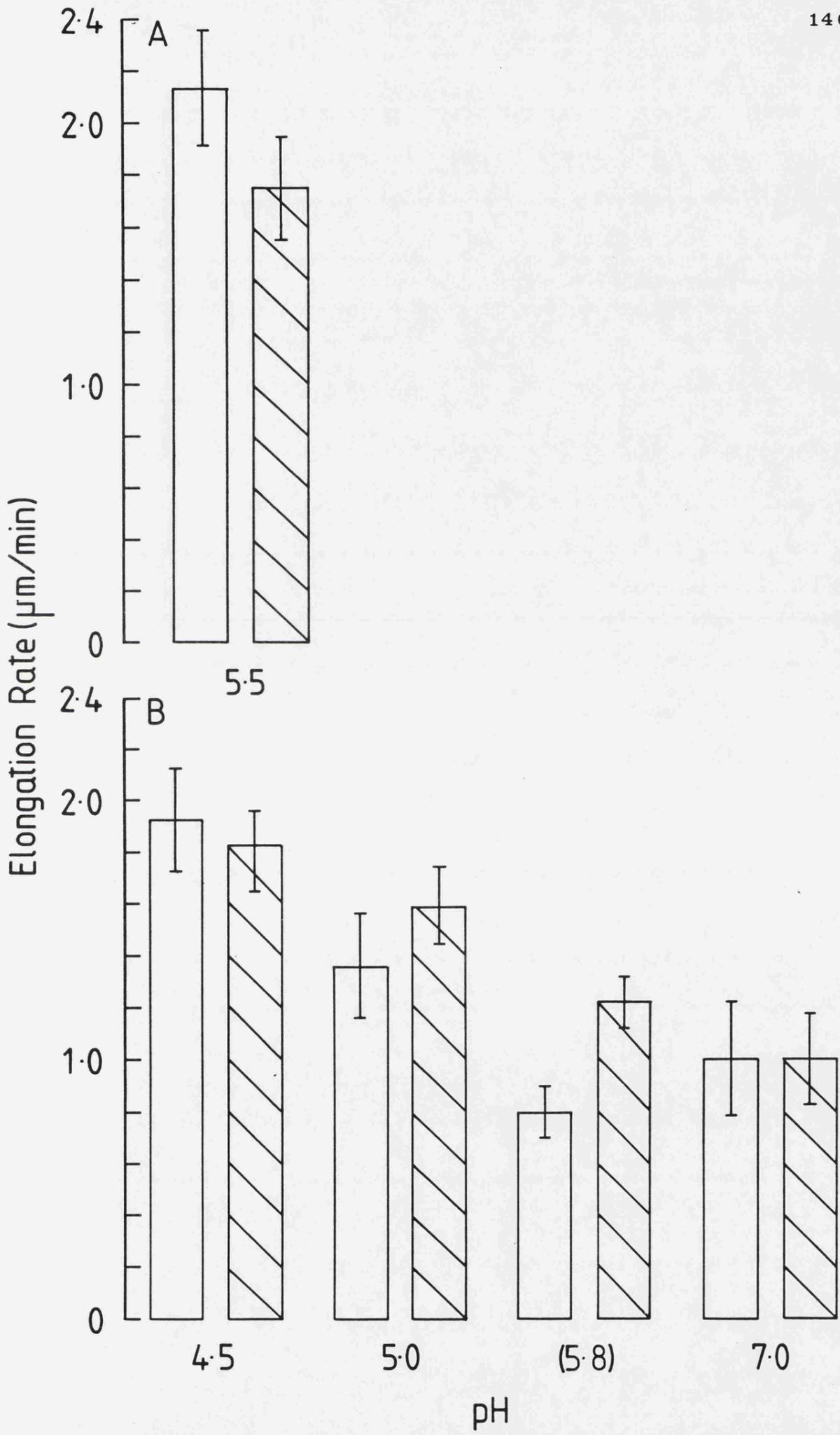
Supplementary FR (λ max 719 nm, $50 \mu\text{mol}/\text{m}^2 \text{ s}$) was from fibre-optic probes and background WL was $25 \mu\text{mol}/\text{m}^2 \text{ s}$. The elongation rates were measured over 120 min, 4 h after excision.

The open columns are WL.

The hatched columns are WL + FR.

A: Segments incubated in 10 mM succinate + 100 mM sucrose pH 5.5.

B: Segments incubated in 100 mM sucrose + 10 mM KCl with the pH adjusted using a few drops of dilute HCl or KOH. The value for pH 5.8 is for unadjusted distilled H₂O.



passive uptake should be equilibrium (Cleland, 1975) and this was the optimum condition for FR promoted elongation (Figure 43B). At values below pH 5.8, the effect of FR was reduced in relation to the lowering of the pH (Figure 43B). This would be expected if acid was suppressing active proton secretion. In contrast, at pH 7 proton efflux should be stimulated, and the results show that in the unbuffered medium the segments were able partly to counteract the neutral solution (Figure 43; compare with buffered medium at pH 7, Figure 43A). FR, however, did not raise the elongation rate and this may be because proton efflux was maximal.

These results must not be over interpreted, as there is wide statistical variability, but they are indirect evidence to support the working hypothesis that phytochrome controls stem elongation by rapidly modulating proton secretion into the cell wall.

A useful test of this hypothesis would be to monitor the pH of the apoplast during changes in the light regime. This could be achieved by inserting microelectrodes into intact stems or segments (see, Jacobs & Ray, 1976). A problem with this method is that it is difficult to know the exact location of the microelectrode.

Alternatively, proton efflux into the bathing medium can be measured. This approach was used in a pilot experiment based on the methods of Evans & Vesper (1980). Twenty segments were irradiated in a specially-constructed clear plastic water-bath, but because the segments were spaced (to avoid shading) the volume of the container was high. A large volume of medium dilutes pH changes and no proton efflux was measured with a miniature pH electrode. Cleland (1976c) has used a surface-electrode in direct contact with the segments, but although this requires only a small volume of medium it will

shade the tissue and may not be suitable for experiments involving light treatments.

The Effect of Ca^{2+} and EDTA on Elongation

The addition of 10 mM CaCl₂ to elongating segments reduced the growth rate to a low level within 10 min (Figure 44A) and inhibited the response to added FR (Figure 45).

The ability of low concentrations of Ca^{2+} to inhibit elongation growth has long been recognised (see references in introduction). The calcium-sensitive sites of membrane located ATPases are on the cytoplasmic side of the plasma-membrane (Balke et al, 1974); so the inhibitory effect is probably not due to a reduction in proton secretion. Additional Ca^{2+} is believed to have only a small effect on the strength of the pectic polysaccharide component of cell walls (Jarvis, 1984). This is because the number of possible cation-pectate bonds is probably maximal under normal circumstances. The inhibitory effect may, therefore, be due to the postulated ability of Ca^{2+} to compete with protons for specific binding sites on cell wall enzymes, enzyme substrates (Cleland & Rayle, 1977; Tepfer & Cleland, 1979), or structural glycoproteins (Kauss & Glaser, 1974).

A comparison between Ca^{2+} inhibition of acid-induced elongation in living and dead stem segments would allow the contributions of biochemical and non-biochemical wall loosening to be assessed. For example, dead cell wall preparations of S. alba segments could be made by boiling in methanol (see, Cleland & Rayle, 1977). If these segments were able to respond to acid when held under tension, then

Figure 44

The effect of CaCl_2 and EDTA on elongation in internode segments from Sinapis alba.

Copies of chart traces of representative segments incubated in the appropriate medium for 4 h after excision.

- A: the effect of CaCl_2 . Segment incubated in 100 mM sucrose + 10 mM KCl. 100 mM sucrose + 10 mM KCl + 10 mM CaCl_2 was flushed through where indicated.
- B: the effect of EDTA. Segment incubated in 100 mM sucrose + 10 mM KCl at pH 6.0 (phosphate buffer, 10 mM). 100 mM sucrose + 10 mM KCl + 10 mM EDTA at pH 6.0 (phosphate buffer, 10 mM) was flushed through where indicated.

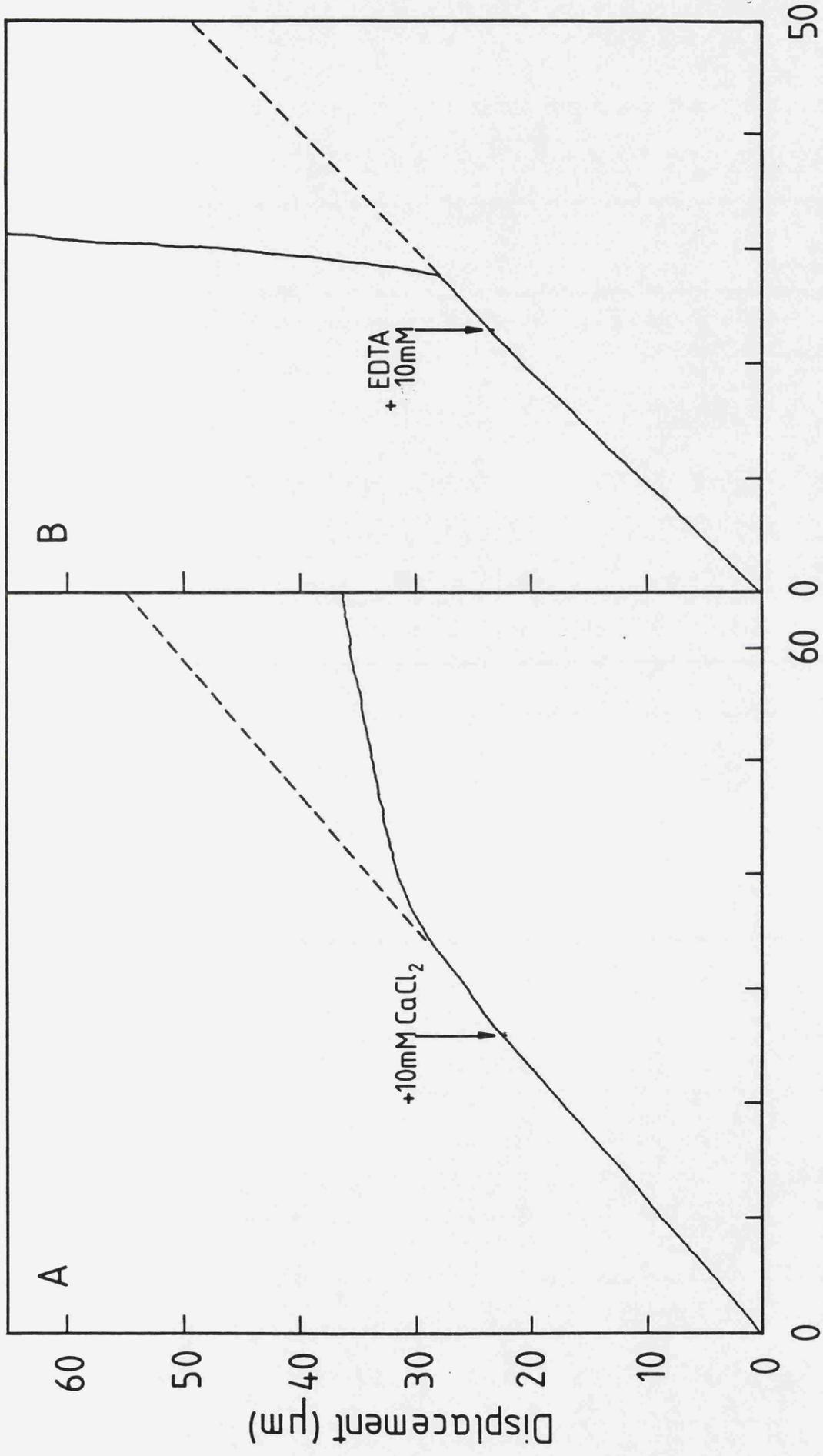


Figure 45

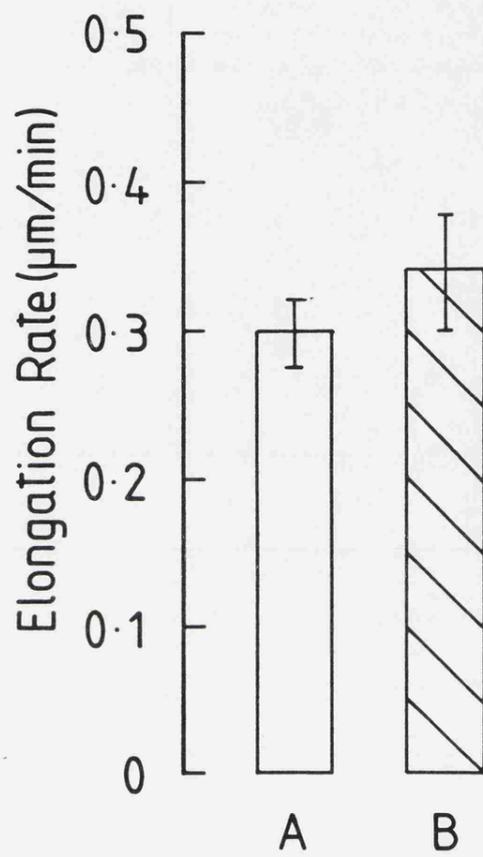
The interaction between supplementary FR and CaCl_2 in internode segments from Sinapis alba.

The values are the means of 5 segments (the bars are \pm S.E.).

Supplementary FR (λ max 719 nm, $50 \mu\text{mol}/\text{m}^2 \text{ s}$) was from fibre-optic probes and background WL was $25 \mu\text{mol}/\text{m}^2 \text{ s}$. The elongation rates were measured over 120 min, 4 h after excision, in 100 mM sucrose + 10 mM KCl + 10 mM CaCl_2 .

The open column is WL.

The hatched column is WL + FR.



the degree of inhibition caused by Ca^{2+} would indicate the importance of non-biochemical $\text{Ca}^{2+}/\text{H}^+$ interactions. Figure 44B shows that 10 mM EDTA (buffered at pH 6.0) rapidly stimulated elongation in the internode segments. The latent period of 5 min was longer than that of acid-induced elongation, but this difference may represent the time taken for EDTA to penetrate the tissue. The results are only preliminary, but an interesting feature of this EDTA-stimulated elongation is that the initial rate was about double that at pH 5.5; so a reduction in the concentration of Ca^{2+} in the cell wall may be more important than a decrease in pH per se. Furthermore, in species where non-biochemical wall loosening is predominant, EDTA is generally more effective than acid at stimulating wall extension (Tepfer & Cleland, 1979; Soll & Bottger, 1982) and this may be the case in S. alba.

The Role of Growth Regulators in the Response to Lowered R:FR

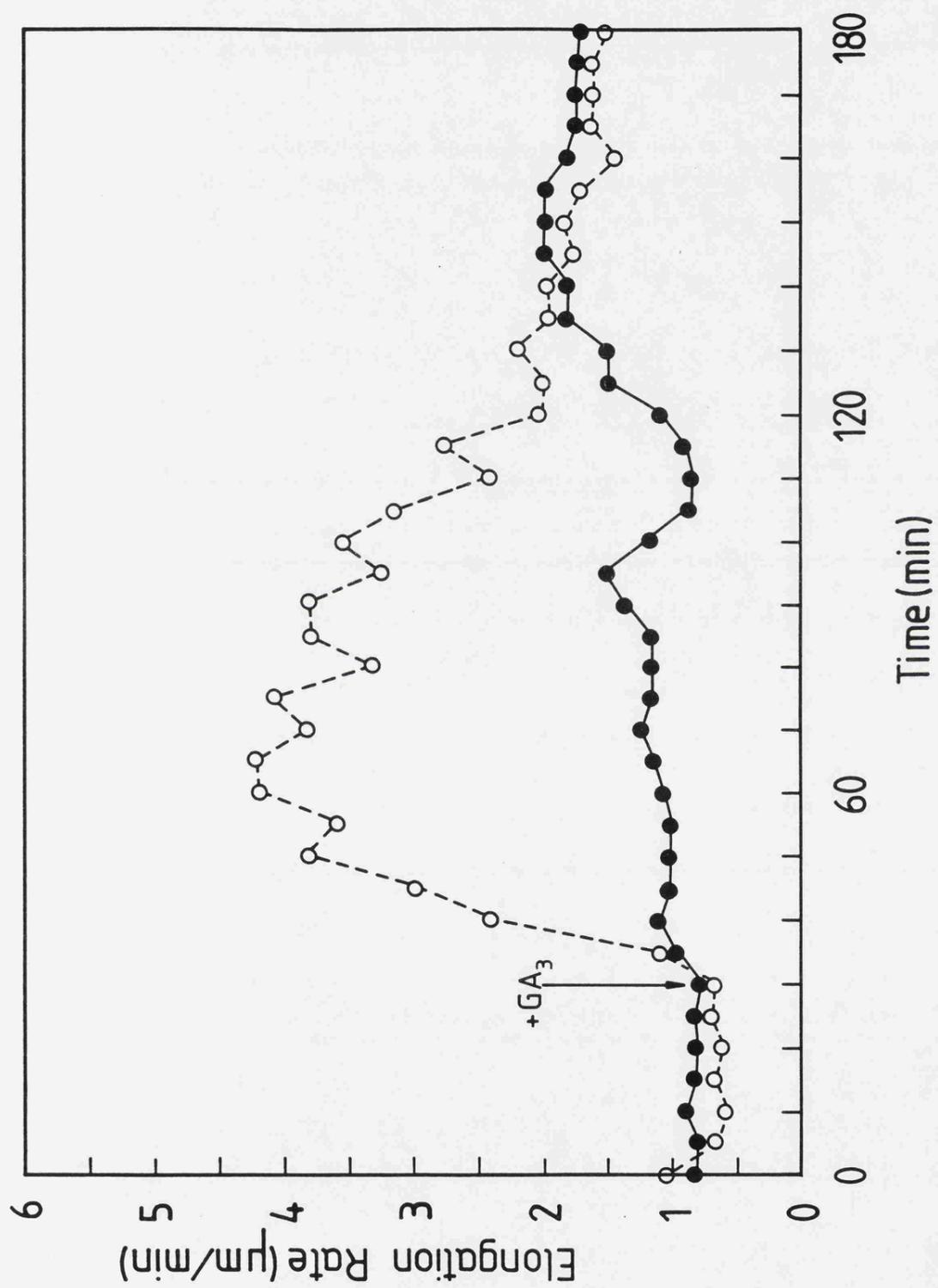
The internode segments from S. alba did not require exogenous growth regulators in order to respond to added FR. There is some evidence, however, that R:FR affects the levels of gibberellins in stems. In Chenopodium album, O'Brien (1980) reported that after seven days, stems of plants transferred to a low R:FR had higher levels of extractable GA_1 and GA_3 than plants grown continually in WL. Phytochrome controls the release of gibberellins from cereal etioplasts within 10 min (Cooke et al, 1975; Evans & Smith, 1976) and Moll & Jones (1981a) have shown that a number of gibberellins are capable of stimulating elongation in lettuce hypocotyl segments with latent periods of less than 20 min. The latent period with GA_3 was under 5 min, but unfortunately their method gives the impression that none of the gibberellin solutions were buffered. Figure 46

Figure 46

The effect of GA_3 on elongation in internode segments from Sinapis alba. Representative segments incubated in 100 mM sucrose for 4 h and medium containing $75 \mu M GA_3$ was flushed through where indicated. Background WL was $25 \mu mol/m^2 s$.

○-----○ 100 mM sucrose + $75 \mu M GA_3$, unbuffered.

●————● 100 mM sucrose + $75 \mu M GA_3$, pH adjusted to pH 6.0 with dilute KOH.



shows that unbuffered GA₃ rapidly stimulated elongation in S. alba internode segments, whereas GA₃ maintained at pH 6 only slightly increased elongation after 90 min; so it is possible that the responses reported by Moll & Jones are acid-growth effects, rather than specific gibberellin-stimulated responses. Adams et al (1973) have also observed gibberellin-stimulated elongation in light-grown oat internode segments, but the lag time was more than 40 min; so it is unlikely that GA release is involved in the rapid response to FR.

Auxin has also been implicated in the photocontrol of stem elongation. Fletcher & Zalik (1964) found that R and FR affected the amount of endogenous auxin in the stem of Phaseolus and that these levels were related to elongation. The lag time for auxin-induced elongation in excised segments is at least 12 min (see, introduction) which is 6 min longer than the latent period for the response of S. alba internode segments to FR. Firn (1982) has shown that in sunflower hypocotyl segments, auxin enters the responsive tissues radially across the cuticle and penetration of these tissues is rapid. Furthermore, the responsive tissues are believed to be the peripheral cell layers (see, Firn, 1982) and this raises the important question of whether exogenous auxin takes 6 min to reach its site of action. If penetration is quicker than 6 min then the inference must be that phytochrome action is closer to the locus of proton secretion than are the auxin sensitive sites; so rapid FR stimulated elongation could not be due to an increase in auxin levels.

CONCLUSIONS

The results presented in this thesis support the view that light-grown plants of the arable weed Sinapis alba L. perceive vegetational shade - expressed as a reduction in R:FR - by phytochrome. The stem itself is capable of detecting fluctuations in R:FR and modifies its elongation rate accordingly.

In these experiments the R:FR was reduced by adding FR to background white fluorescent light. Supplementary FR given directly to the first internode elicited an increase in elongation rate with a latent period of about 10 min. Removing the FR caused the elongation rate to decline to the original WL level within about 16 min. The first internode was found to be sensitive to brief pulses of FR (as low as 1 min) and could respond to successive pulses. This suggests that the plant is able to integrate numerous short periods of shade into a substantial overall increase in stem elongation. The fine adjustment of stem elongation to light quality will allow the plant to synchronise its growth with the light environment, and maximise the efficient deployment of resources in a competitive habitat.

The rapid response to supplementary FR was inversely and linearly related to ϕ over the range 0.17-0.63 - a much wider range than previously has been reported - and phytochrome cycling rate does not play any significant part in controlling the response. Furthermore, neither WL fluence rate nor B had any important short-term effect on elongation rate, which suggests that photosynthetic photosystems and B-photoreceptors are not involved in the rapid modulation of

non-directional stem elongation.

At the fluence rates used in these experiments ϕ should not be affected by the thermal reactions of the phytochrome system, and this obviates the problems encountered by other workers, notably Wall & Johnson (see, introduction to section 1), in relating stem elongation and ϕ at low fluence rates. Johnson (1981), however, claims that his light regimes - which comprise of FR added to background green light of about $13 \text{ umol/m}^2\text{s}$ - are a fair representation of vegetational shade. In fact, they mimic continuous deep shade and, in addition to the difficulty in achieving a true photoequilibrium, he admits that the PAR is below the level required for S. alba to attain its photosynthetic compensation point. This means that the plants could be unable to provide their stems with sufficient carbohydrates to respond to reductions in ϕ , and in normal circumstances they would perish.

The light treatments in these experiments were designed to be as natural as possible, but were not definitive representations of shadelight in herbaceous stands, because it is clear from the studies of Holmes & Smith (see, section 1 introduction) that these habitats are complex and change continually (e.g., the wind causes leaves and stems to move and alters the pattern of sunflecks and shade). The nature of the light regime under a plant canopy emphasises the selective advantage bestowed on plants that are able to adjust rapidly to any changes.

Although internode elongation rate can be related to ϕ , it was not possible to determine whether the ratio of Pfr:Pr, or the concentration of Pfr, is the physiologically active parameter of the

phytochrome system. In the experiments where the levels of phytochrome theoretically were modified by giving plants end-of-day treatments, there were significant differences in elongation rates between plants in the three treatments, both in darkness and in the following light period. The plants which were given an end-of-day FR pulse and no subsequent R pulse had the highest elongation rates, but this was probably due to an effect after the initial phytochrome action. If, however, the amount of phytochrome in the pool which is responsible for controlling stem elongation is affected by these end-of-day treatments, then the results are not compatible with the hypothesis of Pfr as the active form of phytochrome. Ideally, phytochrome levels should be measured in parallel with the monitoring of stem elongation.

A comparison of the rapid response of internode elongation to supplementary FR in intact S. alba, with the response of excised stem segments to auxin (as reported by others), indicated that light quality and auxin may alter cell enlargement in a similar way; i.e., by regulating proton secretion into the cell wall. This idea was tested using excised segments from the first internode of S. alba. The results show that when these segments were supplied with sucrose, they were capable of elongating for at least 24h; and responded to added FR within about 6 min, with an increase in elongation rate. The elongation rate of these segments was related to ϕ in the same way as in the intact plant. Sucrose may be required as an energy supply and a substrate for cell wall synthesis; while KCl, which enhanced elongation rate in the presence of sucrose, could provide K^+ as counter ions for proton efflux. The optimum pH for the elongation of these segments was about pH 5.5, and at this value supplementary FR had no additional effect on

elongation rate; so it appears that FR does not cause an increase in water uptake (by altering membrane conductivity) or solute uptake into the cells. Furthermore, adjustment of the external pH reduced the FR response; presumably by either suppressing or stimulating proton secretion by the tissue, and overriding the effect of light. On the basis of these observations I propose as a working hypothesis, that phytochrome modulates cell enlargement by regulating proton secretion into the cell wall. This hypothesis could be tested directly by measuring proton efflux in isolated segments or intact plants.

The kinetics of auxin and gibberellin-induced elongation in isolated segments have been analysed and I have concluded that the initial action of phytochrome need not involve an increase in the level of these growth regulators. If this is correct, then another mechanism of phytochrome control must be sought.

Because FR pulses of 1 min elicit increases in elongation rate in intact plants, and the FR response in excised segments has a lag of about 6 min, phytochrome action must be rapid, with few steps between the perception of a change in R:FR and the modification of cell enlargement. An important consideration is, therefore, whether the site of photoperception is separate from the cells which govern stem elongation. There is evidence that the peripheral cell layers of stems (i.e., the epidermis and subepidermal layers) control elongation in the organ as a whole by restricting cell enlargement in the cortex and pith (see, Mentze et al, 1977; Firn, 1982). If the peripheral cell layers are unable to perceive changes in R:FR, then they must receive information from other tissues. The time taken for the transmission of this information would reduce the possible number of steps between perception and response within the

stem. A knowledge of the location of phytochrome within the stem would allow this question to be answered and could also enable estimations of the effects of tissue absorption and refraction on ϕ to be made.

Most models of phytochrome action envisage the molecule as a membrane effector (e.g., Hendricks & Borthwick, 1967; Smith, 1975; Johnson & Tasker, 1979), but the evidence for the physiologically active phytochrome being associated with membranes is equivocal (see, Watson & Smith, 1982; Saunders et al, 1983). Moreover, despite the many instances of phytochrome-controlled transmembrane ion fluxes (see, Racusen & Galston, 1983), Raven (1981) has established that the cellular concentrations of phytochrome are too low to bring about directly the pumping of protons. The phytochrome signal must, therefore, be amplified by a second messenger which could then activate a membrane porter. An attractive contender for the role of second messenger is calcium. The regulatory function of calcium in animals is well documented: nerve excitation, muscular contraction and virtually all cellular secretory processes require calcium. In plants calcium may regulate enzyme activity directly, as in the H^+/K^+ ATPase of oat roots (Balke et al, 1974), or via calmodulin. Calmodulin is a protein found in animals and plants which binds calcium and changes conformation; in this changed form it alters the activity of certain enzymes (see, Dieter, 1984). The cytosolic concentrations of calcium are micromolar or less, so that small changes in calcium levels could have a large effect on cellular processes. There is good evidence that phytochrome modulates calcium transport (see, Roux, 1984; Dieter, 1984), so a small number of phytochrome molecules could regulate metabolism by adjusting calcium levels. Furthermore, calmodulin antagonists

stimulate proton secretion and plasma-membrane hyperpolarisation in maize coleoptiles and roots, and pea stems (Lado et al, 1981); and the effect of these antagonists is enhanced by suboptimal levels of auxin or fusicoocin. It is possible, therefore, that phytochrome and auxin-regulated proton secretion could be mediated by calcium. Phytochrome may also regulate the secretion of calcium into the apoplast. An increase in the extracellular concentration of calcium probably would not affect the rigidity of pectic gels in the cell wall (Jarvis, 1984), but it may alter the binding properties of structural lectins (Kauss & Glaser, 1974). EDTA was found to be very effective at promoting elongation of internode segments from S. alba and this could be associated with the removal of calcium from calcium-requiring lectins.

In addition to the rapid response of internode elongation to changes in R:FR perceived by the stem, stem elongation is also affected by R:FR detected by the leaves (Morgan et al, 1980). This response is slower, but more persistent than the response to FR given to the stem and must involve the transmission of a chemical stimulus. The response would be an advantage to plants which are completely overtopped by vegetation.

In the long-term experiments, low R:FR seemed to accelerate the start of internode elongation. However, this did not lead to an increase in the number of internodes, and the overall effect could mainly be due to R:FR modifying cell enlargement (Nakata & Lockhart, 1966; Le Noir, 1967; Child et al, 1981a). For example, if low R:FR causes an increase in the rate of cell enlargement, then the elongation of an internode will be measurable earlier than in high R:FR; and internode elongation should cease when the cells achieve the maximum

length possible - assuming there is a limit to cell length. R:FR had little effect on specific internode length and this implies that the pattern of differentiation was not altered (i.e., there is no difference in the degree of secondary thickening) and that the structure of cell walls was maintained.

In contrast to the long-term effects of R:FR, the WL fluence rate affected the rate of production of internodes and specific internode length. At high fluence rates more internodes were produced than at low WL levels, but their development was accelerated, with the results that after 20 days the plants at a high fluence rate had more, shorter and thicker internodes than those at a low WL fluence rate. The plants at the high WL level grew tallest.

The photoreceptor for these long-term effects of WL fluence rate is not known. It could be a B-photoreceptor, phytochrome acting via cycling rate, photosynthesis, or a combination of these. The effect of photosynthesis could be related to the translocation of sugar to the internodes (see, introduction to section 1) because, after transfer to darkness stem elongation was rapidly inhibited in intact plants, and the isolated internodes required sucrose to support elongation.

There are several reports of a synergism between certain wavelengths which suggest the interaction of photoreceptors: B and yellow together inhibit the elongation of light-grown peas (Elliot, 1979); and in the complex inhibition of internode elongation in Vigna sinensis by WL, there is a co-action between R and B which might involve phytochrome and a B-photoreceptor (Lechary & Jacques, 1980). Alternatively, there may be a fourth photoreceptor which has

yet to be characterised (see, Gaba & Black, 1983; for discussion).

Hypocotyl elongation was followed in different R:FR, and different WL fluence rates. An important contrast between hypocotyl and internode elongation was that the majority of hypocotyl elongation occurred within a few days, while internode elongation was indeterminate. Most of the hypocotyl elongation took place during the 3-4 days following emergence, and the duration of this period was unaffected by R:FR, but was reduced at high fluence rates. In this period, the elongation rate was inversely related to both R:FR and fluence rate. It is believed that hypocotyl growth is not dependent upon photosynthesis, but on the stored reserves in the seed (Grime & Jeffrey, 1965; Holmes et al, 1982). If this is the case, then the WL fluence rate cannot influence elongation via photosynthesis and the photoreceptor may be phytochrome (acting by cycling rate) or a B-photoreceptor. Hypocotyl elongation could be analysed using linear displacement transducers in the same way as internode elongation, but there are a number of difficulties: it is important that measurements should be made in the 3-4 days after emergence, when the seedlings are very delicate; and the differential elongation which occurs in zones of the hypocotyl must be considered when positioning fibre-optic probes to deliver supplementary light.

A WORKING HYPOTHESIS FOR THE PHOTOCONTROL OF STEM ELONGATION

Stem elongation in Sinapis alba initially is brought about by hypocotyl elongation; followed by internode elongation.

Hypocotyl elongation is inversely related to the fluence rate and R:FR of incident light. Fluence rate modifies elongation probably through a B-photoreceptor and/or phytochrome; but not by photosynthesis, because most hypocotyl elongation occurs before photosynthesis is established. Internode elongation is affected by fluence rate in a complex way, but this is unlikely to involve either a B-photoreceptor or phytochrome. Instead, internode development may be influenced by variations in the synthesis and partitioning of assimilates associated with the rate of photosynthesis. Internode elongation is inversely related to R:FR perceived, via phytochrome, by the leaves and internodes. The detection of changes in R:FR by the internodes themselves is through modulations of either Pfr levels or the ratio of Pr:Pfr. If the population of phytochrome which regulates internode elongation is not a constant size, then phytochrome control cannot be through Pfr concentration.

Phytochrome-controlled internode elongation is, in the first instance, mediated by changes in cell enlargement. Cell enlargement might be adjusted by phytochrome regulating the secretion of protons into the cell wall through the activity of calmodulin or calcium-dependent plasma-membrane H⁺ATPase pumps. The lowering of cell wall pH may bring about the displacement of Ca²⁺ from molecules involved in maintaining the rigidity of the cell wall.

Bibliography

- ADAMS, P.A., KAUFMAN, P.B and IKUMA, H. (1973)
Effects of gibberellic acid and sucrose on the growth of oat stem segments. Plant Physiol, 51, 1102-1108.
- ARNON, D.I. (1977)
Photosynthesis 1950-75: changing concepts and perspectives, in Photosynthesis I, Encyclopedia of Plant Physiology Vol 5, Ed Trebst, A. and Avron, M., Berlin, Springer-Verlag, 7-56.
- BACIC, A., KLEIN, A.S. and DELMER, D.P. (1980)
Stimulation of glucan synthesis in vitro by induction of membrane potentials, Plant Physiol. 655, 579.
- BAILEY, N.T.J. (1959)
Statistical methods in biology, Hodder and Stoughton.
- BALKE, N.E., SZE, H., LEONARD, R.T. and HODGES, T.K. (1974)
Cation sensitivity of the plasma membrane ATPase of oat roots. in Membrane Transport in Plants. Eds Zimmerman, U. and Dainty, J., Berlin, Springer-Verlag, 301-306.
- BARANETZKY, J. (1897)
in Plant Physiology by Palladin, V.I., authorized English edition (1923), Ed Livingston, B.E., Philadelphia, P. Blakiston's Son & Co.
- BARBER, J., HORLER, D.N.H. and CHAPMAN, D.J. (1981)
Photosynthetic pigments and efficiency in relation to the spectral quality of absorbed light, in Plants and the Daylight Spectrum, Ed Smith, H., Academic Press, 341-354.
- BARKLEY, G.M. and LEOPOLD, A.C. (1973)
Comparative effects of hydrogen ions, carbon dioxide, and auxin on pea stem segment elongation. Plant Physiol. 52, 76-78.
- BARTLEY, M.R. and FRANKLAND, B. (1982)
Analysis of dual role of phytochrome in the photoinhibition of seed germination. Nature, 300, 750-752.
- BATES, G.W. and RAY, P.M. (1981)
pH dependent interactions between pea cell wall polymers possibly involved in wall deposition and growth. Plant Physiol. 68, 158-164.
- BEGGS, C.J., HOLMES, M.G., JABBEN, M. and SCHAFER, E. (1980)
Action spectra for the inhibition of hypocotyl growth by continuous irradiation in light and dark-grown Sinapis alba L seedlings. Plant Physiol. 66, 615-618.

- BENNET-CLARK, T.A. (1956)
Salt accumulation and mode of action of auxin: a preliminary hypothesis. in The Chemistry and Mode of Action of Growth Substances. Eds Wain, R.L. and Wightman, F., New York, Academic Press, 284-291.
- BJORKMAN, O. (1981)
Responses to different quantum flux densities. in Physiological Plant Ecology I, Encyclopedia of Plant Physiology Vol 12A, Eds Lange, O.L., Nobel, P.S., Osmond, C.B., and Ziegler, H., Berlin, Springer-Verlag, 57-107.
- BJORKMAN, O., BOARDMAN, N.K., ANDERSON, J.M., THORNE, S.W., GOODCHILD, D.J. and PYLIOTIS, N.A. (1972)
Effect of light intensity during growth of Atriplex patula on the capacity of photosynthetic reactions, chloroplast components and structure. Carnegie Inst Ann Rep Dep Biol, 115-134.
- BLACK, M. and SHUTTLEWORTH, J.E. (1974)
The role of the cotyledons in the photocontrol of hypocotyl extension in Cucumis sativus L. Planta, 117, 57-66.
- BLACKMAN, G.E. and WILSON, G.L. (1951)
Physiological and ecological studies in the analysis of plant environment VIII, an analysis of the differential effects of light intensity on the net assimilation rate, leaf-area and relative growth rate of different species. Ann Bot, 15, 373-408.
- BOARDMAN, N.K. (1977)
Comparative photosynthesis of sun and shade plants. Ann Rev Plant Physiol. 28, 355-377.
- BONNER, J. (1934)
The relation of hydrogen ions to the growth rate of the Avena coleoptile. Protoplasma, 21, 406-423.
- BORTHWICK, H. (1972)
History of phytochrome. in Phytochrome, Eds Mitrakos, K. and Shropshire, W., Academic Press, 3-23.
- BOSE, J.C. (1929)
Growth and Tropic Movements of Plants. Longmans Green & Co.
- BOUCHET, M-H., PRAT, R. and GOLDBERG, R. (1983)
Kinetics of indole acetic acid-induced growth in hypocotyl sections of Vigna radiata. Physiol Plant. 57, 95-100.
- BOYER, J.S. and WU, G. (1978)
Auxin increases the hydraulic conductivity of auxin-sensitive hypocotyl tissue. Planta, 139, 227-237.
- BRIGGS, W.R. and CHON, H.P. (1966)
The physiological versus the spectrophotometric status of phytochrome in corn coleoptiles. Plant Physiol. 41, 1159-1166.

- BRIGGS, W.R. and IINO, M. (1983)
Blue-light-absorbing photoreceptors in plants. in Photoreception by Plants. Eds Wareing, P.F. and Smith, H., Royal Society.
- BUTLER, W.L., HENDRICKS, S.B. and SIEGELMAN, H.W. (1964)
Action spectra of phytochrome in vitro. Photochem Photobiol. 3, 521-528.
- CARR, D.J. and NG, E.K. (1959)
Residual effects of auxin, chelating agents, and metabolic inhibitor in cell extension. Aust J Biol Sci. 12, 373-387.
- CHILD, R., MORGAN, D.C. and SMITH, H. (1981a)
Control of development in Chenopodium album L. by shadelight: the effect of light quality (red:far-red ratio) on morphogenesis. New Phytol. 89, 545-555.
- CHILD, R., MORGAN, D.C. and SMITH, H. (1981b)
Morphogenesis in simulated shadelight quality. in Plants and the Daylight Spectrum. Ed Smith, H., Academic Press, 409-420.
- CLELAND, R.E. (1967a)
Extensibility of isolated cell walls: measurement and changes during cell elongation. Planta, 74, 197-209.
- CLELAND, R.E. (1967b)
A dual role of turgor pressure in auxin-induced cell elongation in Avena coleoptiles. Planta, 77, 182-191.
- CLELAND, R.E. (1968)
Auxin and wall extensibility: reversibility of auxin-induced wall-loosening process. Science, 160, 192-194.
- CLELAND, R.E. (1971a)
Instability of the growth-limiting proteins of the Avena coleoptile and their pool size in relation to auxin. Planta, 99, 1-11.
- CLELAND, R.E. (1971b)
The mechanical behaviour of isolated Avena coleoptile walls subjected to constant stress. Plant Physiol. 47, 805-811.
- CLELAND, R.E. (1975)
Auxin-induced hydrogen ion excretion: correlation with growth, and control by external pH and water stress. Planta, 127, 233-242.
- CLELAND, R.E. (1976a)
Fusicoccin-induced growth and hydrogen ion excretion of Avena coleoptiles: relation to auxin responses. Planta, 128, 201-206.
- CLELAND, R.E. (1976b)
Rapid stimulation of K⁺-H⁺ exchange by a plant growth hormone. Biochemical and Biophysical Research Communications, 69 No 2, 333-338.

- CLELAND, R.E. (1976c)
Kinetics of hormone-induced H⁺ excretion. Plant Physiol. 58, 210-213.
- CLELAND, R.E. (1977)
The control of cell enlargement. Symp Soc Exp Biol. 31, 101-116.
- CLELAND, R.E. (1982)
The mechanism of auxin-induced proton efflux. in Plant Growth Substances 1982, Ed Wareing, P.F., Academic Press, 23-31.
- CLELAND, R.E. and LOMAX, T. (1977)
Hormonal control of H⁺ -excretion from oat cells. in Regulation of Cell Membrane Activities in Plants, Eds Marre, E. and Ciferri, O, Amsterdam, Elsevier/North Holland Biomedical Press, 161-171.
- CLELAND, R.E., PRINS, H.B.A., HARPER, J.R. and HIGINBOTHAM, N. (1977)
Rapid hormone-induced hyperpolarization of the oat coleoptile transmembrane potential. Plant Physiol. 59, 395-397.
- CLELAND, R.E. and RAYLE, D.L. (1975)
Hydrogen ion entry as a controlling factor in the acid-growth response of green pea stem sections. Plant Physiol. 55, 547-549.
- CLELAND, R.E. and RAYLE, D.L. (1977)
Re-evaluation of the effect of calcium ions on auxin-induced elongation, Plant Physiol. 60, 709-712.
- CLINE, M.G. (1979)
The stability of the postulated wall-loosening enzyme in acid-induced growth. Planta, 145, 389-391.
- CLINE, M.G., EDGERTON, M. and REHM, M.M. (1974)
Accelerated endogenous growth in Avena coleoptile segments. Planta, 120, 213-214.
- COHEN, J.D. and NADLER, K.D. (1976)
Calcium requirement for indoleacetic acid-induced acidification by Avena coleoptiles, Plant Physiol. 57, 347-350.
- COLBERT, J.T., HERSHEY, H.P. and QUAIL, P.H. (1983)
Autoregulatory control of translatable phytochrome mRNA levels, Proc Natl Acad Sci USA. 80, 2248-2252.
- COOIL, B. and BONNER, J. (1957)
Effects of calcium and potassium ions on the auxin-induced growth of Avenacoleoptile sections. Planta, 48 696-723.
- COOKE, R.J., SAUNDERS, P.F. and KENDRICK, R.E. (1975)
Red light-induced production of GA-like substances in homogenates of etiolated wheat leaves and in suspension of intact etioplasts, Planta, 125, 319-328.

- COSGROVE, D.J. (1981)
Rapid suppression of growth by blue light, occurrence, time course, and general characteristics. Plant Physiol. 67, 584-590.
- COSGROVE, D.J. (1982)
Rapid inhibition of hypocotyl growth by blue light in Sinapis alba L, Plant Science Letters, 25, 305-312.
- COSGROVE, D.J. and CLELAND, R.E. (1983)
Osmotic properties of pea internodes in relation to growth and auxin action. Plant Physiol. 72, 332-338.
- COSGROVE, D.J. and GREEN, P.B. (1981)
Rapid suppression of growth by blue light: biophysical mechanism of action. Plant Physiol. 68, 1447-1453.
- DELROT, S. (1981)
Proton fluxes associated with sugar uptake in Vicia faba leaf tissues. Plant Physiol. 68, 706-711.
- DIETER, P. (1984)
Calmodulin and calmodulin-mediated processes in plants. Plant Cell Environ. 7, 371-380.
- DOWNS, R.J., HENDRICKS, S.B. and BORTHWICK, H.A. (1957)
Photoreversible control of elongation in pinto beans and other plants under normal conditions of growth. Bot Gaz. 118, 199-208.
- DRUMM, H. and MOHR, H. (1974)
The dose response curve in phytochrome-mediated anthocyanin synthesis in the mustard seedling. Photochem Photobiol. 20, 151-157.
- DURAND, H. and RAYLE, D.L. (1973)
Physiological evidence for auxin-induced hydrogen ion secretion and the epidermal paradox. Planta, 114, 185-193.
- ELLIOT, W.M. (1979)
Control of leaf and stem growth in light-grown pea seedlings by two high irradiance responses. Plant Physiol. 63, 833-836.
- EVANS, A. and SMITH, H. (1976)
Localisation of phytochrome in etioplasts and its regulation in vitro of GA levels. Proc Natl Acad Sci USA. 73, 138-142.
- EVANS, M.L. (1973)
Rapid stimulation of plant cell elongation by hormonal and non-hormonal factors. Bio Science, 23 No 12, 711-718.
- EVANS, M.L. (1974a)
Evidence against the involvement of galactosidase or glucosidase in auxin- or acid-stimulated growth. Plant Physiol. 54, 213-215.
- EVANS, M.L. (1974b)
Rapid responses to plant hormones. Ann Rev Plant Physiol. 25, 195-223.

- EVANS, M.L. and HOKANSON, R. (1969)
Timing of the response of coleoptiles to the application and withdrawal of various auxins. Planta, 85, 85-95.
- EVANS, M.L. and RAY, P.M. (1969)
Timing of the auxin response in coleoptiles and its implications regarding auxin action. Journal of General Physiology, 53, 1-20.
- EVANS, M.L., RAY, P.M. and REINHOLD, L. (1971)
Induction of coleoptile elongation by carbon dioxide, Plant Physiol. 47, 335-341.
- EVANS, M.L. and SCHMITT, M.R. (1975)
The nature of spontaneous changes in growth rate in isolated coleoptile segments. Plant Physiol. 55, 757-762.
- EVANS, M.L. and VESPER, M.J. (1980)
An improved method for detecting auxin-induced hydrogen ion efflux from corn coleoptile segments. Plant Physiol. 66, 561-565.
- FIRN, R.D. (1982)
Auxin uptake and the rapid auxin-induced growth in isolated sections of Helianthus annuus. Plant Physiol. 70, 1568-1570.
- FLETCHER, R.A. and ZALIK, S. (1964)
Effect of light quality on growth and free indoleacetic acid content in Phaseolus vulgaris. Plant Physiol. 39, 328-331.
- GABA, V. and BLACK, M. (1979)
Two separate photoreceptors control hypocotyl growth in green seedlings. Nature 278, 51-54.
- GABA, V. and BLACK, M. (1983)
The control of cell growth by light. in Photomorphogenesis, Encyclopedia of Plant Physiology Vol 16 A, Eds Shropshire, W. and Mohr, H., Berlin, Springer-Verlag, 358-392.
- GARDNER, G. and GRACEFFO, M.A. (1982)
The use of a computerized spectroradiometer to predict phytochrome photoequilibria under polychromatic irradiation. Photochem Photobiol. 36, 349-354.
- GOTTMANN, K. and SCHAFER, E. (1982)
In vitro synthesis of phytochrome apoprotein directed by mRNA from light and dark-grown Avena seedlings. Photochem Photobiol. 35, 521-525.
- GRIME, J.P. (1979)
Plant Strategies and Vegetation Processes, Chichester, John Wiley & Sons.
- GRIME, J.P. and JEFFREY, D.W. (1965)
Seedling establishment in vertical gradients of sunlight. J Ecol. 53, 621-642.

GRONEWALD, J.W. and HANSON, J.B. (1980)
Sensitivity of the proton and ion transport mechanisms of corn roots to injury. Plant Science Letters, 18, 143-150.

HAGER, A., MENZEL, H. and KRAUSS, A. (1971)
Versuche und Hypothese zur Primarwirkung des Auxins beim Streckungswachstum. Planta, 100, 47-75.

HAHN, T-R., SONG, P-S., QUAIL, P.H. and VIERSTRA, R.D. (1984)
Tetranitromethane oxidation of phytochrome chromophore as a function of spectral form and molecular weight. Plant Physiol. 74, 755-758.

HANSON, J.B., and TREWAVAS, A.J. (1982)
Regulation of plant cell growth: the changing perspective. New Phytol. 90, 1-18.

HARTMANN, K.M. (1966)
A general hypothesis to interpret "high energy phenomena" of photomorphogenesis on the basis of phytochrome. Photochem Photobiol. 5, 349-366.

HARTMANN, K.M. (1967)
Ein Wirkungsspektrum der Photomorphogenese unter Hohenenergiebedingungen und seine Interpretation auf der Basis der Phytochroms (Hypokotylwachstumhemmung bei Lactuca sativa L.). Zeitschrift für Naturforschung, 22, 1172-1175.

HARTT, C.E. (1965)
Light and translocation of ^{14}C in detached blades of sugar cane. Plant Physiol. 40, 718-724.

HARTT, C.E. (1966)
Translocation in coloured light. Plant Physiol. 41, 369-372.

HASCHKE, H-P. and LUTTGE, U. (1975)
Interaction between IAA, potassium and malate accumulation, and growth in Avena coleoptile segments. Z Pflanzenphysiol. 76, 450-455.

HEATHCOTE, L., BAMBRIDGE, K.R. and MCLAREN, J.S. (1979)
Specially constructed growth cabinets for simulation of the spectral photon distributions found under natural vegetation canopies. J Exp Bot. 30, 347-353.

HEBARD, F.V., AMATANGELO, S.J., DAYANANDAN, P. and KAUFMAN, P.B. (1976)
Studies on acidification of media by Avena stem segments in the presence and absence of gibberellic acid. Plant Physiol. 58, 670-674.

HEIM, B., JABBEN, M. and SCHAFER, E. (1981)
Phytochrome destruction in dark- and light-grown Amaranthus caudatus seedlings. Photochem Photobiol. 34, 89-93.

- HENDRICKS, S.B. and BORTHWICK, H.A. (1967)
The function of phytochrome in regulation of plant growth. Proc Natl Acad Sci USA. 58, 2125-2130.
- HERSHEY, H.P., COLBERT, J.T., LISSEMORE, J.L., BARKER, R.F. and QUAIL, P.H. (1984)
Molecular cloning of cDNA for Avena phytochrome. Proc Natl Acad Sci USA. 81, 2332-2336.
- HIGINBOTHAM, N. and ANDERSON, W.P. (1974)
Electrogenic pumps in higher plant cells. Can J. Bot. 52, 1011-1021.
- HOLMES, M.G. (1981)
Spectral distribution of radiation within plant canopies. in Plants and the Daylight Spectrum, Ed Smith, H., Academic Press, 147-158.
- HOLMES, M.G., BEGGS, C.J., JABBEN, M. and SCHAFER, E. (1982)
Hypocotyl growth in Sinapis alba L: the roles of light quality and quantity. Plant Cell Environ. 5, 45-51.
- HOLMES, M.G. and FUKSHANSKY, L. (1979)
Phytochrome photoequilibria in green leaves under polychromatic radiation, a theoretical approach. Plant Cell Environ. 2, 59-64.
- HOLMES, M.G. and SMITH, H. (1977a)
The function of phytochrome in the natural environment I. Characterisation of daylight for studies in photomorphogenesis and photoperiodism. Photochem Photobiol. 25, 533-538.
- HOLMES, M.G., and SMITH, H. (1977b)
The function of phytochrome in the natural environment. II. The influence of vegetation canopies on the spectral distribution of natural daylight. Photochem. Photobiol. 25, 539-545.
- HOLMES, M.G. and WAGNER, E. (1980)
A re-evaluation of phytochrome involvement in time measurement in plants. J Theor Biol. 83, 255-265.
- HOLMES, M.G. and WAGNER, E. (1981)
Phytochrome control of hypocotyl extension in light-grown Chenopodium rubrum. Physiol Plant. 53, 233-238.
- HUGHES, A.P. and EVANS, G.C. (1962)
Plant growth and the aerial environment II. Effects of light intensity on Impatiens parviflora. New Phytol. 61, 154-174.
- HUNT, R.E. and PRATT, L.H. (1980)
Radioimmunoassay of phytochrome content in green, light-grown oats. Plant Cell Environ. 3, 91-95.
- HUTCHINGS, V.M. (1978a)
Sucrose and proton co-transport in Ricinus cotyledons I, H⁺ influx associated with sucrose uptake. Planta, 138, 229-235.

HUTCHINGS, V.M. (1978b)

Sucrose and proton co-transport in Ricinus cotyledons II, H⁺ efflux and associated K⁺ uptake. Planta, 138, 237-241.

IDLE, D.B. (1956)

Studies in extension growth I, a new contact auxanometer. J Exp Bot. 7, 347-361.

ILAN, I. (1973)

On auxin-induced pH drop and on the improbability of its involvement in the primary mechanism of auxin-induced growth promotion. Physiol Plant. 28, 146-148.

JABBEN, M., BEGGS, C. and SCHAFER, E. (1982)

Dependence of Pfr/Ptot-ratios on light quality and light quantity. Photochem Photobiol. 35, 709-712.

JABBEN, M. and HOLMES, M.G. (1983)

Phytochrome in light-grown plants. in Photomorphogenesis, Encyclopedia of Plant Physiology Vol 16 B, Eds Shropshire, W. and Mohr, H., Berlin, Springer-Verlag, 704-722.

JACOBS, M. and RAY, P.M. (1976)

Rapid auxin-induced decrease in free space pH and its relationship to auxin-induced growth in maize and pea. Plant Physiol. 58, 203-209.

JARVIS, M.C. (1984)

Structure and properties of pectin gels in plant cell walls. Plant Cell Environ. 7, 153-164.

JOHNSON, C.B. (1981)

How does phytochrome perceive light quality?. in Plants and the Daylight Spectrum, Ed Smith, H., Academic Press, 481-497.

JOHNSON, C.B. and TASKER, R. (1979)

A scheme to account quantitatively for the action of phytochrome in etiolated and light-grown plants. Plant Cell Environ. 2, 259-265.

JOHNSON, K.D., DANIELS, D., DOWLER, M.J. and RAYLE, D.L. (1974)

Activation of Avena coleoptile cell wall glycosidases by hydrogen ions and auxin. Plant Physiol. 53, 224-228.

JOHNSON, K.D. and RAYLE, D.L. (1976)

Enhancement of CO₂ uptake in Avena coleoptiles by fusaric acid. Plant Physiol. 57, 806-811.

JONES, A.M. and VANDERHOEF, L.N. (1981)

Effect of abrading the cuticle using emery. J Exp Bot. 32, 405-410.

JOSE, A.M. and VINCE-PRUE, D. (1977)

Light-induced changes in the photoresponses of plant stems; the loss of a high irradiance response to far-red light. Planta. 135, 95-100.

- KAUSS, H. and GLASER, C. (1974)
Carbohydrate-binding proteins from plant cell walls and their possible involvement in extension growth. FEBS Letters. 45 No 1, 304-307.
- KEEGSTRA, K., TALMADGE, K.W., BAUER, W.D. and ALBERSHEIM, P. (1973)
The structure of plant cell walls III. Plant Physiol. 51, 188-196.
- KENDRICK, R.E. and SPRUIT, C.J.P. (1972)
Light maintains high levels of phytochrome intermediates. Nature. 237, 281-282.
- KENDRICK, R.E. and SPRUIT, C.J.P. (1973)
Phytochrome intermediates in vivo I, effects of temperature, light intensity, wavelength and oxygen on intermediate accumulation. Photochem Photobiol. 18, 139-144.
- KHAN, A.A. and TAO, L.K. (1978)
Phytohormones and seed dormancy, in Phytohormones and Related Compounds II, Eds Letham, D.S., Goodwin, P.B. and Higgins, T.J.V., Amsterdam, Elsevier/North Holland Biomedical Press, 371-422.
- KILSBY, C.A.H. and JOHNSON, C.B. (1981)
The influence of light quality on the phytochrome content of light-grown Sinapis alba L and Phaseolus aureus Roxb. Planta, 153, 109-114.
- KILSBY, C.A.H. and JOHNSON, C.B. (1982)
The in vivo spectrophotometric assay of phytochrome in two mature dicotyledonous plants. Photochem Photobiol. 35, 255-260.

- LABAVITCH, J.M. and RAY, P.M. (1974a)
Turnover of cell wall polysaccharides in elongating pea stem segments. Plant Physiol. 53, 669-673.
- LABAVITCH, J.M. and RAY, P.M. (1974b)
Relationship between promotion of xyloglucan metabolism and induction of elongation by indoleacetic acid. Plant Physiol. 54, 499-502.
- LADO, P., PENNACHIONI, A., RASI-CALDOGNO, F., RUSSI, S. and SILANO, V. (1972)
Comparison between some effects of fusicochin and indole-3-acetic acid on cell enlargement in various plant materials. Physiological Plant Pathology, 2, 75-85.
- LADO, P., RASI-CALDOGNO, R., COLOMBO, R., DE MICHELIS, M.I. and MARRE, E. (1976) Effects of monovalent cations on IAA- and FC-stimulated proton-cation exchange in pea stem segments. Plant Science Letters, 7, 199-209.
- LADO, P., REVANA, R., BONETTI, A., MARRE, M.T. and MARRE, E. (1981) Effects of calmodulin inhibitors in plants I. Synergism with fusicochin in the stimulation of growth and H⁺ secretion and in the hyperpolarisation of the transmembrane electric potential. Plant Sci Letters, 23, 253-262.
- LAMPORT, D.T.A. and CATT, J.W. (1981)
Glycoproteins and enzymes of the cell wall. in Plant Carbohydrates II, Encyclopedia of Plant Physiology Vol 13 B, Eds Tanner, W. and Loewus, F.A., Berlin, Springer-Verlag, 133-165.
- LECHARNY, A. and JACQUES, R. (1980)
Light inhibition of internode elongation in green plants, a kinetic study with Vigna sinensis L. Planta, 149, 384-388.
- LE NOIR, W.C. (1967)
The effects of light on the cellular components of polarised growth in bean internodes. Am J Bot. 54, 876-887.
- LOCKHART, J.A. (1960)
Intracellular mechanism of growth inhibition by radiant energy. Plant Physiol. 35, 129-135.
- LOCKHART, J.A. (1965)
An analysis of irreversible plant cell elongation. J. Theoret Biol. 8, 264-275.
- LOMAX-REICHERT, T., HERTEL, R. and BRIGGS, W.R. (1982)
Characterization and localization of membrane vesicles which transport ¹⁴C IAA in vitro. Plant Physiol. 69 Sup, 12.
- LUCAS, W.J. and SMITH, F.A. (1973)
The formation of alkaline and acid regions at the surface of Chara corallina cells. J Exp Bot. 24, 1-14.
- LUTTGE, U. and HIGINBOTHAM, N. (1979)
Transport in Plants. New York, Springer-Verlag.

- MAHMOUD, A. and GRIME, J.P. (1974)
A comparison of negative relative growth rates in shaded seedlings. New Phytol. 73, 1215-1219.
- MALONE, M. and RIDGE, I. (1983)
Ethylene-induced growth and proton excretion in the aquatic plant Nymphoides peltata. Planta, 157, 71-73.
- MARME, D., MARCHAL, B. and SCHAFER, E. (1971)
A detailed analysis of phytochrome decay and dark reversion in mustard cotyledons. Planta, 100, 331-336.
- MARRE, E. (1977)
Effects of fusicoccin and hormones on plant cell membrane activities: observations and hypotheses. in Regulation of Cell Membrane Activities in Plants, Eds Marre, E. and Ciferri, O., Amsterdam, Elsevier/North Holland Biomedical Press, 185-202.
- MARRE, E., LADO, P., RASI-CALDOGNO, F. and COLOMBO, R. (1973a)
Correlation between cell enlargement in pea internode segments and decrease in the pH of the medium of incubation I. Plant Science Letters, 1, 179-184.
- MARRE, E., LADO, P., RASI-CALDOGNO, F. and COLOMBO, R. (1973b)
Correlation between cell enlargement in pea internode segments and decrease in the pH of the medium of incubation II, Plant Science Letters, 185-192.
- MARRE, E., LADO, P., RASI-CALDOGNO, F., COLOMBO, R. and DE MICHELIS, M.I. (1974)
Evidence for the coupling of proton extrusion to K⁺ uptake in pea internode segments treated in fusicoccin or auxin. Plant Science Letters, 3, 365-379.
- MASUDA, Y. (1980)
Auxin-induced changes in noncellulosic polysaccharides of cell walls of monocot coleoptiles and dicot stems. in Plant Growth Substances 1979, Ed Skoog, F., Berlin, Springer-Verlag, 79-89.
- MASUDA, Y., PJON, C-J. and FURUYA, M. (1970)
Phytochrome action in Oryza sativa L. V. Planta, 90, 236-242.
- MASUDA, Y. and YAMAMOTO, R. (1970)
Effect of auxin on B-1, 3-glucanase activity in Avena coleoptile. Development, Growth and Differentiation, Vol 11 No 4, 287-296.
- MEIJER, G. (1968)
Rapid growth inhibition of gherkin hypocotyls in blue light. Acta Bot Neerl. 17, 9-14.
- MENTZE, J., RAYMOND, B., COHEN, J.D. and RAYLE, D.L. (1977)
Auxin-induced H⁺ secretion in Helianthus and its implications. Plant Physiol. 60, 509-512.
- METRAUX, J-P. and TAIZ, L. (1977)
Cell wall extension in Nitella as influenced by acids and ions. Proc Natl Acad Sci USA. 74 No 4, 1565-1569.

- MOHR, H. (1972)
Lectures in Photomorphogenesis, Berlin, Springer-Verlag.
- MOLL, C. and JONES, R.L. (1981a)
 Short-term kinetics of elongation growth of gibberellin-responsive lettuce hypocotyl sections. Planta, 152, 442-449.
- MOLL, C. and JONES, R.L. (1981b)
 Calcium and gibberellin-induced elongation of lettuce hypocotyl sections. Planta, 152, 450-456.
- MOORBY, J., EBERT, M. and EVANS, N.T.S. (1963)
 The translocation of ^{14}C -labelled assimilate in the soybean. J Exp Bot. 14, 210-220.
- MORGAN, D.C. and SMITH, H. (1976)
 Linear relationship between phytochrome photoequilibrium and growth in plants under simulated natural radiation. Nature, 262, 210-212.
- MORGAN, D.C. and SMITH, H. (1978a)
 Simulated sunflecks have large, rapid effects on plant stem extension. Nature, 273, 534-536.
- MORGAN, D.C. and SMITH, H. (1978b)
 The relationship between phytochrome photoequilibrium and development in light-grown Chenopodium album L. Planta, 142, 187-193,
- MORGAN, D.C. and SMITH, H. (1979)
 A systematic relationship between phytochrome-controlled development and species habitat for plants growing in simulated natural radiation. Planta, 145, 253-258.
- MORGAN, D.C. and SMITH, H. (1981)
 Control of development in Chenopodium album L by shadelight: the effect of light quantity (total fluence rate) and light quality (red: far-red ratio), New Phytol. 88, 239-248.
- MORGAN, D.C., CHILD, R. and SMITH, H. (1981)
 Absence of fluence rate dependency of phytochrome modulation of stem extension in light-grown Sinapis alba L.. Planta, 151, 498-498.
- MORGAN, D.C., O'BRIEN, T. and SMITH, H. (1980)
 Rapid photomodulation of stem extension in light-grown Sinapis alba L.: studies on kinetics, site of perception, and photoreceptor. Planta, 150, 95-101.
- MCCREE, K.J. (1981)
 Photosynthetically active radiation. in Physiological Plant Ecology I, Encyclopedia of Plant Physiology Vol 12A, Eds Lange, O.L., Nobel, P.S., Osmond, C.B. and Ziegler, H., Berlin, Springer-Verlag, 41-55.

- MCLAREN, J.S. and SMITH, H. (1978)
Phytochrome control of the growth and development of Rumex obtusifolius under simulated canopy light environments, Plant Cell Environ. 1, 61-67.
- MACROBBIE, E.A.C. (1965)
The nature of the coupling between light energy and active ion transport in Nitella translucens. Biochim Biophys Acta. 94, 64-73.
- NAKATA, S. and LOCKHART, J.A. (1966)
Effects of red and far-red radiation on cell division and elongation in the stem of pinto bean seedlings. Am J Bot. 53, 12-20.
- O'BRIEN, T. (1980)
The control of plant development by light quality. PhD thesis Leicester University.
- OELZE-KAROW, H-M. and MOHR, H. (1973)
Quantitative correlation between spectrophotometric phytochrome assay and physiological response. Photochem Photobiol. 18, 319-330.
- ORDIN, L., APPLEWHITE, T.H. and BONNER, J. (1956)
Auxin-induced water uptake by Avena coleoptiles sections. Plant Physiol. 31, 44-53.
- PALLADIN, V.I. (1914)
Plant Physiology. authorized English edition (1923), Ed Livingston, B.E., Philadelphia, P. Blakiston's Son & Co.
- PEEL, A.J. (1974)
Transport of Nutrients in Plants. Butterworths.
- PENNY, P. (1969)
The rate of response of excised stem segments to auxins. N.Z. J Bot. 7, 290-301.
- PENNY, P. (1971)
Growth-limiting proteins in relation to auxin-induced elongation in lupin hypocotyls. Plant Physiol. 48, 720-723.
- PENNY, D. (1972)
Multicompartment analysis of the response of lupin hypocotyl segments to the addition and removal of IAA. Can J Bot. 50, 2015-2026.
- PENNY, P. and PENNY, D. (1978)
Rapid responses to phytohormones. in Phytohormones and Related Compounds, A Comprehensive Treatise, Vol II, Eds Letham, Y., Goodwin, P. and Higgs, T., Amsterdam, Elsevier/North Holland Biomedical Press, 537-597.
- PERLEY, J.E. and PENNY, D. (1974)
Effect of some aldonolactones on IAA-induced growth of lupin hypocotyl segments. N.Z. J Bot. 12, 503-512.

- PIKE, C.S. (1976)
Lack of influence of phytochrome on membrane permeability to tritiated water. Plant Physiol. 57, 185-187.
- PLAUT, Z. and REINHOLD, L. (1969)
Concomitant photosynthesis implicated in the light effect on translocation in bean plants. Aust J Biol. 22, 1105-1111.
- POPE, D.G. (1978)
Does indoleacetic acid promote growth via cell wall acidification?. Planta, 140, 137-142.
- POPE, D. and BLACK, M. (1972)
Effect of IAA on coleoptile extension growth in the absence of protein synthesis. Planta, 102, 26-36.
- POPP, H.W. (1926)
A physiological study of the effect of light of various ranges of wavelength on the growth of plants. Am J Bot. 13, 706-735.
- PRATT, L.H. (1982)
Phytochrome: the protein moiety. Annu Rev Plant Physiol. 33, 557-582.
- PRESTI, D. and DELBRUCK, M. (1978)
Photoreceptors for biosynthesis, energy storage and vision. Plant Cell Environ. 1, 81-100.
- PRESTON, R.D. (1974)
The Physical Biology of Plant Cell Walls. Chapman & Hall.
- PRESTON, R.D. (1979)
Polysaccharide conformation and cell wall function, Annu Rev Plant Physiol. 30, 55-78.
- QUAIL, P.H., SCHAFER, E. and MARME, D. (1973)
Turnover of phytochrome in pumpkin cotyledons. Plant Physiol. 52, 128-131.
- RACUSEN, R.H. and GALSTON, A.W. (1983)
Developmental significance of light-mediated electrical responses in plant tissues. in Photomorphogenesis, Encyclopedia of Plant Physiology Vol 16, Eds Shropshire, W. and Mohr, H., Berlin, Springer-Verlag, 687-703.
- RANSON, S.L. and HARRISON, A. (1955)
Experiments on growth in length of plant organs I, a recording auxanometer. J Exp Bot. 6, 75-79.
- RAVEN, J.A. (1976)
Transport in algal cells. in Transport in Plants II, Part A, Cells, Eds Luttge, U. and Pitman, P. G., New York, Springer-Verlag, 129-188.
- RAVEN, J.A. (1981)
Light quality and solute transport. in Plants and the Daylight Spectrum, Ed Smith, H., Academic Press, 375-390.

- RAY, P.M. (1962)
Cell wall synthesis and cell elongation in oat coleoptile tissue. Am J Bot. 49, 928-939.
- RAY, P.M. (1977)
Auxin-binding sites of maize coleoptiles are localized on membranes of the endoplasmic reticulum. Plant Physiol. 59, 594-599.
- RAY, P.M. and BAKER, D.B. (1965)
The effect of auxin on synthesis of oat coleoptile cell wall constituents. Plant Physiol. 40, 353-360.
- RAYLE, D.L. (1973)
Auxin-induced hydrogen-ion secretion in Avena coleoptiles and its implications. Planta. 114,63-73.
- RAYLE, D.L. and CLELAND, R.E. (1970)
Enhancement of wall loosening and elongation by acid solutions. Plant Physiol. 46, 250-253.
- RAYLE, D.L. and CLELAND, R.E. (1980)
Evidence that auxin-induced growth of soybean hypocotyls involves proton excretion. Plant Physiol. 66, 433-437.
- RAYLE, D.L., HAUGHTON, P.M. and CLELAND, R.E. (1970)
An in vitro system that simulates plant cell extension growth. Proc Natl Acad Sci USA, 67 No 4, 1814-1817.
- RITTER, A., WAGNER, E. and HOLMES, M.G. (1981)
Light quantity and quality interactions in the control of elongation growth in light-grown Chenopodium rubrum L seedlings. Planta, 153, 556-560.
- ROUX, S.J. (1984)
Calcium and phytochrome action in plants. Bioscience, 25-29.
- SACHS, J. (1882)
Lectures on the Physiology of Plants. translated by Ward, H.M., Oxford, Clarendon Press.
- SATTER, R.L. and WETHERELL, D.F. (1968)
Photomorphogenesis in Sinningia speciosa cv Queen Victoria. II, Stem elongation: interaction of a phytochrome-controlled process and a red-requiring energy dependent reaction. Plant Physiol. 43, 961-967.
- SAUNDERS, M.J., CORDONNIER, M-M., PALEVITZ, B.A. and PRATT, L.H. (1983)
Immunofluorescence visualization of phytochrome in Pisum sativum epicotyls using monoclonal antibodies. Planta, 159, 545-553.
- SCHAFER, E. (1975)
A new approach to explain the "high irradiance responses" of photomorphogenesis on the basis of phytochrome. J Math Biol. 2, 41-56.

- SCHAFER, E. (1981)
Phytochrome and daylight. in Plants and the Daylight Spectrum, Ed Smith, H., Academic Press, 461-480.
- SCHMIDT, R. and MOHR, H. (1982)
Evidence that a mustard seedling responds to the amount of Pfr and not to the Pfr/Ptot ratio. Plant Cell Environ. 5, 495-499.
- SERVAITES, J. and GEIGER, D.R. (1974)
Effects of light intensity and oxygen on photosynthesis and translocation in sugar beet. Plant Physiol. 54, 575-578.
- SHIMAZAKI, Y., CORDONNIER, M-M. and PRATT, L.H. (1983)
Phytochrome quantitation in crude extracts of Avena by enzyme-linked immunosorbant assay with monoclonal antibodies. Planta, 159, 534-544.
- SILK, W.K. and JONES, R.L. (1975)
Gibberellin response in lettuce hypocotyl sections. Plant Physiol. 56, 267-272.
- SMITH, H. (1975)
Phytochrome and Photomorphogenesis. McGraw-Hill.
- SMITH, H. (1981)
Evidence that Pfr is not the active form of phytochrome in light-grown maize. Nature, 293, 163-165.
- SMITH, H. (1982)
Light quality, photoperception, and plant strategy. Ann Rev Plant Physiol. 33, 481-518.
- SOKAL, R.R., and ROHLF, F.J. (1969)
Biometry. W.H. Freeman & Co., San Francisco.
- SOLL, H. and BOTTGER, M. (1982)
The mechanism of proton-induced increase of cell wall extensibility. Plant Science Letters, 24, 163-171.
- SPANSWICK, R.M. (1974)
Hydrogen ion transport in giant algal cells, Can J Bot. 52, 1029-1034.
- STEVENSON, T.T. and CLELAND, R.E. (1981)
Osmoregulation in the Avena coleoptile in relation to auxin and growth. Plant Physiol. 67, 749-753.
- STEVENSON, T.T. and CLELAND, R.E. (1982)
Osmoregulation in the Avena coleoptile. Plant Physiol. 69, 292-295.
- TAIZ, L. (1984)
Plant cell expansion: regulation of cell wall mechanical properties. Ann Rev Plant Physiol. 35, 585-657.

- TAGAWA, T. and BONNER, J. (1957)
Mechanical properties of the Avena coleoptile as related to auxin and ionic interactions. Plant Physiol. 34, 207-212.
- TASKER, R. and SMITH, H. (1977)
The function of phytochrome in the natural environment. V. Seasonal changes in the radiant energy quality in woodlands. Photochem Photobiol. 26, 487-491.
- TEPFER, M. and CLELAND, R.E. (1979)
A comparison of acid-induced cell wall loosening in Valonia ventricosa and in oat coleoptiles. Plant Physiol. 63, 898-902.
- THIMANN, K.V. and SCHNEIDER, C.L. (1938)
The role of salts, hydrogen ion concentration and agar in the response of the Avena coleoptile as related to auxin. Am J Bot. 25, 270-280.
- THOMAS, B. (1981)
Specific effects of blue light on plant growth and development. in Plants and the Daylight Spectrum, Ed Smith, H., Academic Press, 443-459.
- THOMAS, B., PENN, S.E., BUTCHER, G.W., and GALFRE, G. (1984)
Discrimination between the red and far-red-absorbing forms of phytochrome from Avena sativa L by monoclonal antibodies. Planta, 160, 382-384.
- THOMSON, B.F. (1950)
The effect of light on the rate of development of Avena seedlings. Am J Bot. 37, 284-291.
- THROWER, S.L. (1962)
Translocation of labelled assimilates. II. The pattern of translocation in intact and defoliated plants. Aust J Biol Sci. 15, 629-649.
- VANDERHOEF, L.N. (1981)
Auxin-regulated elongation. What's New in Plant Physiology, 12 No 7.
- VANDERHOEF, L.N. and BRIGGS, W.R. (1978)
Red light-inhibited mesocotyl elongation in maize seedlings I: the auxin hypothesis. Plant Physiol. 61, 534-537.
- VANDERHOEF, L.N. and DUTE, R.R. (1981)
Auxin-regulated wall loosening and sustained growth in elongation. Plant Physiol. 67, 146-149.
- VANDERHOEF, L.N., QUAIL, P.H. and BRIGGS, W.R. (1979)
Red light-inhibited mesocotyl elongation in maize seedlings II: kinetics and spectral studies. Plant Physiol. 63, 1062-1067.
- VANDERHOEF, L.N. and STAHL, C.A. (1975)
Separation of two responses to auxin by means of cytokinin inhibition. Proc Natl Acad Sci USA. 72 No 5, 1822-1825.

- VAN VOLKENBURGH, E. and CLELAND, R.E. (1979)
Separation of cell enlargement and division in bean leaves. Planta, 146, 245-247.
- VAN VOLKENBURGH, E. and CLELAND, R.E. (1980)
Proton excretion and cell expansion in bean leaves. Planta, 148, 273-278.
- VAN VOLKENBURGH, E. and CLELAND, R.E. (1981)
Control of light-induced bean leaf expansion: role of osmotic potential, wall yield stress, and hydraulic conductivity. Planta, 153, 572-577.
- VESPER, M.J. and EVANS, M.L. (1978)
Time-dependent changes in the auxin sensitivity of coleoptile segments: apparent sensory adaptation. Plant Physiol. 61, 204-208.
- VEZINA, P.E. and BOULTER, D.W.K. (1966)
The spectral composition of near UV and visible radiation beneath forest canopies. Can J Bot. 44, 1267-1284.
- VINCE-PRUE, D. (1977)
Photocontrol of stem elongation in light-grown plants of Fuchsia hybrida. Planta, 133, 149-166.
- VOGELMANN, T.C. and BJORN, L.O. (1984)
Measurement of light gradients and spectral regime in plant tissue with a fibre optic probe. Physiol Plant. 60, 361-368.
- WALL, J.K. and JOHNSON, C.B. (1981)
Phytochrome action in light-grown plants: the influence of light quality and fluence rate on extension in Sinapis alba L. Planta, 153, 101-108.
- WALL, J.K. and JOHNSON, C.B. (1982)
The effect of temperature on phytochrome-controlled hypocotyl extension in Sinapis alba L. New Phytol. 91, 405-412.
- WALL, J.K. and JOHNSON, C.B. (1983)
An analysis of phytochrome action in the high irradiance response. Planta, 159, 387-397.
- WARNER, T. and ROSS, J.D. (1981)
Phytochrome control of maize coleoptile section elongation: the role of cell wall extensibility. Plant Physiol. 68, 1024-1026.
- WATSON, P.J. and SMITH, H. (1982)
Integral association of phytochrome with a membranous fraction from etiolated Avena shoots: red/far-red photoreversibility and in vitro characterization. Planta, 154, 128-134.
- WEINSTEIN, L.H., MEISS, A.M., UHLER, R.L. and PURVIS, E.R. (1956)
Growth promoting effect of ethylenediamine tetraacetic acid. Nature, 178, 1188.

- WENT, F.W. (1941)
Effect of light on stem and leaf growth. Am J Bot. 28, 83-95.
- WENT, F.W. (1974)
Reflections and speculations. Ann Rev Plant Physiol. 25, 1-26.
- WETMORE, R.H. and RIER, J.P. (1963)
Experimental induction of vascular tissues in callus of Angiosperms.
Am J Bot. 50, 418-430.
- WILDERMANN, A., DRUMM, H., SCHAFER, E. and MOHR, H. (1978)
Control by light of hypocotyl growth in de-etiolated mustard seedlings. I. Phytochrome as the only photoreceptor pigment.
Planta, 141, 211-216.
- WOOLLEY, J.T. (1971)
Reflectance and transmittance of light by leaves. Plant Physiol. 47, 656-662.
- WOOLMAN, J.A. (1980)
Studies on the Phytochrome Regulation of Membrane Permeability.
PhD thesis Leicester University.
- WORTMANN, J. (1889)
in Plant Physiology by Palladin. V.I.. authorized English edition (1923), Ed Livingston, B.E., Philadelphia, P. Blakiston's Sons & Co.
- YOCUM, C.S., ALLEN, L.H. and LEMON, E.R. (1964)
Photosynthesis under field conditions. VI. Solar radiation balance and photosynthetic efficiency. Agron J. 56, 249-253.
- ZOCCHI, G. and HANSON, J.B. (1983)
Calcium transport and ATPase activity in a microsomal vesicle fraction from corn roots. Plant Cell Environ. 6, 203-209.
- ZURFLUH, L.L. and GUILFOYLE, T.J. (1980)
Auxin-induced changes in the patterns of protein synthesis in soybean hypocotyl. Proc Natl Acad Sci USA. 77 No 1, 357-361.