

LEAF MOVEMENT OF LINUM USITATISSIMUM

A thesis submitted for the degree of Doctor of Philosophy  
in the Faculty of Science in the University of London

by

Shamim Akhtar

Department of Botany,  
Bedford College,  
Regent's Park,  
London NW1 4NS.

1974



ProQuest Number: 10098245

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.



ProQuest 10098245

Published by ProQuest LLC(2016). Copyright of the Dissertation is held by the Author.

All rights reserved.

This work is protected against unauthorized copying under Title 17, United States Code.  
Microform Edition © ProQuest LLC.

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

A B S T R A C T

A brief historical review and summary of recent work on leaf movement is presented. The leaf movements of Linum usitatissimum were studied by measurements of the angular position of the sub-opposite pair of leaves at the second node of the seedling. Oscillatory up and down movements of these leaves continue for about three days in normal light:dark cycles but cease as the leaves mature. In continuous bright light a rhythmic movement persists for two cycles. In prolonged darkness no further movement occurs after the first nyctinastic cycle. A rhythmic movement in darkness can be induced by short (4 h) periods of light and when the light is given in late subjective night the phase of the rhythmic movement is advanced; a delayed movement is induced by a short light period given in the early subjective night. These phase responses of the leaf movement rhythm are considered in relation to the mechanism of entrainment to a non-diurnal (20 h) cycle of 10 h light and 10 h darkness.

Evidence is presented for the involvement of the phytochrome system in the regulation of the leaf movement. Induction of rhythmic nyctinasty by short light periods in the early part of the subjective day can be reversed by short (15 min) treatment with far-red light, which is itself reversible by subsequent red irradiation. Red light breaks during darkness will induce rhythmic nyctinasty when given at appropriate times in the subjective day.

At the close of the day the leaves are not sensitive to red light which is perceived, or darkness, but blue light delays the rising leaf movement until the rhythmic control overrides this effect of light.

These results are discussed in relation to results recently published by other workers on movement of leaves possessing pulvini. Anatomical study of the basal part of the leaf of Linum confirms the absence of a pulvinus.

ACKNOWLEDGEMENTS

I would like to express my gratitude to Mr. D. W. Brett for his supervision and interest during the course of this research, and also to thank Professor D. C. Spanner for his many valuable suggestions, and Mr. R. Jones for his assistance during the electron microscopy work.

I am further greatly indebted to my research colleagues and in particular to Miss M. Zelwer for typing the thesis and Dr. F. A. Qureshi for his constant help and encouragement.

My thanks are due also to the British Council, Edwina Mountbatten Trust, Wali Mohammad Fellowship Trust and Bedford College for financial assistance.

Finally, I would like to mention the great kindness and understanding of Miss B. Stevens during my period in residence at Hanover Lodge.

C O N T E N T S

	page
Abstract .....	1
Acknowledgements .....	3
General Introduction	
Historical Review .....	8
Leaf movement and endogenous rhythm .....	11
Effects of light on leaf movements .....	17
Mechanisms of leaf movement .....	23
Aims and scopes of the present study .....	29
Materials and Methods	
The seeds .....	31
Growing techniques .....	31
Measurements of angular position .....	31
Colour filters .....	34
Statistical analysis .....	39
Preparation of the tissue for anatomical studies..	41

SECTION 1Nyctinastic movement in relation to previous illumination

Introduction .....	46
Results	
Nyctinastic movement in response to normal 24 h light:dark cycles .....	48
Nyctinastic movement following single short light periods .....	55

	page
Nyctinastic movement following an extended light period .....	76
Nyctinastic movement following light given after 24 h darkness .....	81
Nyctinastic movement following longer periods of continuous light .....	86
Comments .....	90

## SECTION 2

### Endogenous rhythm and nyctinasty

Introduction .....	93
Results	
Leaf movement under constant light .....	94
Shift in the night maximum .....	99
Entrainment to non-diurnal (20 h) cycle .....	102
Comments .....	107

## SECTION 3

### Leaf movement and the light spectrum

Introduction .....	112
Results	
Effect on nyctinastic movement of prior exposure to far-red radiation .....	114
Far-red effect following 10 h light period .....	117
Reversibility of the far-red effect .....	120
Far-red radiation following short light period ...	127

	page
Reversibility of far-red effect following short light period .....	130
Light quality and induction of rhythmic nyctinastic movement .....	133
Light given in the middle of established day .....	133
Induction of rhythmic nyctinastic movement by red light breaks during darkness .....	139
Reversibility of rhythmic nyctinastic response induced by red light .....	139
Induction of rhythmic nyctinastic response by 4 h white light and 15 min red in the middle of established light period .....	145
Phase shift by different wavelengths of light ....	151
Comments .....	157

#### SECTION 4

##### Anatomy and fine structure of the base of leaf

Introduction .....	161
Methods .....	161
Observations .....	162
Comments .....	163

#### SECTION 5

General Discussion .....	181
Bibliography .....	195



SECTION 6

## Appendix

Shadowgraph technique .....	207
Effect on leaf movement of excision of the shoot and application of Indole Acetic Acid .....	208
Effect on nyctinastic movement of painting the leaf with India ink .....	211

GENERAL INTRODUCTIONA. Historical Review.

The common observation that the leaves of many plants change their position, or "close", as night approaches provides probably the earliest recorded example of a rhythmic plant movement. Such movements were observed on legumes, especially Tamarindus indicus, by Androstenes accompanying Alexander the Great in the 4th century B.C.

Plinius, writing in the 1st. century A.D., described the closing in stormy weather of clover leaves (something which can be seen in summer thundery weather on English lawns) and Pfeffer (1875), in a historical review of the subject, says Plinius was also aware that changes in the attitude of leaves repeated themselves daily.

Pfeffer's account shows how interest in the subject developed from the 16th century. Among the earliest writers of that time were Valerius Cordus and Garcias de Horto, who described the daily periodic movements of the leaflets of various Leguminosae which over the years have remained the chief objects of research on leaf movements. To some extent the concentration of research on the legumes with few other species having been investigated gives a false impression of the occurrence of movements of leaves and cotyledons, but reference to the Darwins' book on the *Movements of Plants* (1880) shows how widespread the phenomenon is, with a catalogue of species from many families of flowering plants studied by him or previous workers.

Linnaeus wrote in 1751 and 1755 on the "sleep of plants" (*Somnus plantarum*) and through his writings the widespread occurrence of the sleep movements of leaves and flowers became generally known.

It was at the beginning of the 18th century that the first experimental observations were made by an astronomer, de Mairan, in 1729. He found that the movements continued in darkness and were therefore not simply a response to the alternation of night and day.

The causes of the periodic movements were sought either in external factors such as light and temperature or in some innate cause. De Mairan's observations suggested for the first time an innate cause. However, the observation by Hill (1757) that when the plants were darkened during the day the leaves responded by assuming the same sleep position as they did at night, led the investigator to conclude that it must be the daily alternation of light and darkness which caused the movements. But, as Zinn (1759) and others again confirmed, the sleep movements continued in the dark and also took place even if the plants were brought into light towards the evening, so such a simple explanation would not suffice. Some of Zinn's experiments were conducted in a cellar so he was also able to rule out temperature and humidity changes as causes of the movements and concluded that they were due to unknown innate causes.

De Candolle's investigations published in his *Physiologie vegetale* (1832) pointed to some definite relationship between the alternation of light and darkness and the daily periodic movements. He succeeded, for example, in inverting the normal rhythm of leaf movement by illuminating the plant artificially at night and placing the plant in darkness during the day. These and earlier investigations really brought out the question whether light was the actual cause of the movements or some kind of regulator, and this problem was seen clearly by Sachs and Pfeffer who made the most significant contributions to the subject in the latter half of the last century.

Some of Sach's investigations, as well as his own views and a general statement of the subject are to be found in his *Lectures on the Physiology*

of Plants (1882; English translation 1887). Much of the special terminology of plant movements was introduced by Sachs although some terms are not in use today. The effects of light which cause the leaves to "open" as light intensity increases, and to "close" as darkness comes on he called 'paratonic effects'; and he recognized that they only occur if the leaves are in the "normal vital condition", which he termed 'phototonus'. In prolonged darkness the well known dark rigor sets in and movements are only set up again by exposure to light and restoration of phototonus. Pfeffer (1875) recognized the daily periodicity as a combination of the direct paratonic action and its after-effects.

Sachs studied the effect of different parts of the spectrum using ruby and cobalt glass bell jars. Placing plants of Phaseolus and Oxalis in the blue jar (which excluded all the yellow, green and orange light) had no effect on the day position of the leaves, whereas the plants responded to the exclusion of blue light under the ruby bell-jar as to darkness. Plants transferred from red light in "sleep" condition were awakened in 30-60 min by the blue light. He thus established the importance of blue light in leaf movements as he had earlier shown these wavelengths to be active in heliotropic (phototropic) curvatures.

Pfeffer's later investigations bring us into the present century and to the modern phase of research on rhythmic leaf movements. In the light of the work of Semon (1905), which Pfeffer himself confirmed using his newly devised automatic recording apparatus (Pfeffer 1915), Pfeffer at last abandoned his long held view that the movements observed in continuous light or continuous dark were after-effects of the previous cycles of light and darkness. When light:dark cycles of less than 24 hours were given the movements may follow these but as soon as these cycles were stopped, the daily rhythm was restored in continuous light or

darkness. It was firmly established that a truly "inherited" daily periodicity existed in these plants.

#### B. Leaf Movement and Endogenous Rhythm.

Recognition of the endogenous rhythm.

It will be clear from the foregoing brief historical account that, from the time of the earliest scientific investigations of the subject, the study of the movements of leaves has been closely linked to the interpretation of the rhythmic nature of these movements. The final recognition of the existence of endogenous rhythmic movements was an important step even though they were still thought to be "an inherited impression ('memory') of the light:dark alternation" (Semon 1905). However, the realization and proof that there is an endogenous time-keeping system or biological clock controlling these movements came much later (e.g., see Bünning 1960). Bünning and Stern (1930) showed that the rhythms often deviated from a strictly 24-hours period length. They found the period for Phaseolus to have an average length of 25-26 hours. In fact, this had been noticed and reported before by De Candolle (1832) who found the time between the successive "open" phases of Mimosa was 22-23 hours in continuous light; Pfeffer (1915) mentions other examples. It was left to Bünning to see the significance of these deviations from the strictly diurnal periodicity. Later the term 'circadian' was introduced by Halberg et. al. (1959) to emphasize this aspect of the endogenous periodicity.

It is of interest to note that the study of leaf movements and to a lesser extent flower petal movements, really provided almost all the early information leading up to the modern (say, post 1930) investigations of circadian rhythms. A few other rhythmic phenomena

had been studied, such as fluctuations in growth rates, exudation of sap from the cut shoots and arthropod pigmentation cycles, but the fundamental work on these and other phenomena really date from the time of the last comprehensive investigations by Kleinhoonte (1929, 1932) of the leaf movements of Canavalia and the contemporaneous realization by Bünning and Stern (1930) of the endogenous nature of the oscillating system controlling the rhythms.

#### General properties of circadian rhythm.

A great deal of the modern knowledge and theoretical considerations of circadian rhythms has been built up on rhythmic phenomena other than leaf movements. The various rhythms involving a wide variety of physiological activities in plants and animals show many similarities and this has led to the conclusion that the basic "clock" oscillating system is similar in most if not all of the organisms that have been studied. Pittendrigh (1960) has compiled a Table of Empirical Generalizations about Circadian Rhythms which makes this clear. The properties of a rhythm such as leaf movement can therefore be discussed in the wider context of the general characteristics that have been found to be common to all rhythmic processes. It would, however, be inappropriate to attempt here any more general review of work on biological rhythms.

In an earlier paper, Pittendrigh (1954) proposed that five types of observations can demonstrate that the control of the 24-hour rhythm involves an endogenous clock. Although these specifically concern the demonstration of the endogenous nature of the clock system controlling the time at which Drosophila adults emerge from their pupae (the eclosion rhythm), these have been accepted in a modified form as five general criteria to establish that a rhythm is endogenous (Wilkins 1969).

It will be useful to discuss some of the recent work on leaf movements in the context of these five criteria. The order in which they are considered has been chosen to facilitate discussion.

(1) Persistence of rhythm in constant environment.

In most experiments this has meant the demonstration of leaf movement in continuous darkness and/or continuous light at a constant temperature.

Plants raised in normal light conditions do not generally continue their rhythm of leaf movement for many days in darkness but plants raised in dim light or darkness will continue a rhythm evoked under these conditions for much longer and this has been the practice for experiments with Phaseolus (Bünning 1959).

Bünning (1967) remarks that movements are often not evident at the time of emergence of a seedling in the constant environment and the young plants probably require a fluctuation in light or temperature to initiate the rhythm. Alford and Tibbitts (1970) have demonstrated a circadian rhythm of leaf movement in seedlings of Phaseolus angularis grown from seed in constant light, and controlled constant CO<sub>2</sub>, humidity, and temperature. The rhythm of movement of one plant was often quite out of phase with another and, as seen also by Hoshizaki and Hamner (1964), who demonstrated the persistence of rhythmic leaf movement in P. vulgaris var. Pinto for up to four weeks in constant light and temperature, the two leaves on one plant although usually closely in phase could be several hours out of phase. Probably the movements were initiated as the plant emerged from the soil or the leaves from the bud. The asynchrony results because the plants were never exposed to a synchronizing dark period or temperature change.

In most species studied the movements decrease in amplitude after

a few cycles in constant conditions - a damping of the rhythm occurs and a new signal is required to re-initiate the movements. This applies particularly to leaves with pulvini. In leaves without pulvini, e.g., Xanthium (Hoshizaki, Brest and Hamner 1969) in which the movements are rapidly damped after about 4 cycles in both light and dark, it is unlikely that movements can be evoked again in the same leaves since it is only the young expanding leaves which exhibit any large movements.

(II) Period of rhythm not exactly 24 hours.

A feature of the rhythm of leaf movements under constant conditions is that the period length deviates somewhat from 24 hours in contrast to the situation where there is a regularly occurring 24-hours cycle of light and darkness.

The rhythm is said to be free-running under these constant conditions and in Phaseolus multiflorus (Leinweber 1956) it was found that the free-running period was 28 hours. In P. vulgaris v. Pinto it was 26 hours (Hoshizaki and Hamner 1964).

Free-running period length is subject to alteration under different constant conditions, e.g., temperature, light intensity and light quality; these effects of light quality will be mentioned in more detail in the following part of the introduction.

(III) Rhythm initiated by a single stimulus.

As mentioned under (I) above, a fluctuation in light or temperature may initiate a rhythm in a previously aperiodic system. Likewise, a rhythm may be initiated in plants where leaf movements have been damped by prolonged exposure to uninterrupted darkness; the alternative is also found: a single period of darkness reinitiates a rhythm that has damped in constant light. As noted by Pittendrigh (1954), this is perhaps the



most remarkable single feature of a circadian system because following this unrepeated signal, applied at any clock hour (GMT) the organism immediately starts measuring off intervals of approximately 24 h without any extraneous experience of such a cycle.

(IV) Phase of rhythm may be shifted and new phase retained under constant conditions.

When a rhythm is initiated in a previously arrhythmic or damped system, the phase of the rhythm bears a fixed relationship to the signal given, e.g., the dark:light or light:dark transition.

This corresponds to the phase reference point for the cycle in nature when the repeatedly occurring light:dark cycles entrain the circadian system to the 24 hour periods of the natural environment.

Because of this relationship between the endogenous system and the external signal the phase of the rhythm is shifted if the signal is given at a phase (time) in the cycle when it would not normally be experienced. Thus it has been possible to completely invert the normal rhythm of leaf movement by illuminating the plants at night and placing them in darkness during the day, or indeed to entrain the rhythm to many artificial light:dark cycles in the controlled environment. Such discoveries were among the earliest (Bünning 1959) although they have been pursued since in only 2 or 3 species.

Further to this, however, is the observation that a free-running rhythm in darkness, for example, may be phase-shifted by a single, often very short, light signal. When the phase of the rhythm is changed in this way the new phase is retained in the dark following the signal, i.e., the dark free-running rhythm is reset to the new phase, although there may be one or more transient cycles of variable length before the new steady state is attained.

Whether the phase is advanced or delayed, and the magnitude of the shift, depends on the point in the cycle at which the signal falls. This variable response throughout the circadian cycle has been found to be similar in several circadian systems such that the phase response curves for Phaseolus (Moser 1962) and Coleus (Halaban 1968) leaf movement are not greatly different from that for the Drosophila eclosion rhythm and some others (Pittendrigh 1965). Light signals given during the first half of the subjective day and early subjective night delay the circadian rhythm of leaf movement; those given during the late subjective night advance it.

(V) Loss of time (phase delay) under hypoxia.

The endogenous clock controlling the rhythm is dependent on aerobic metabolism. Exposure to anaerobic conditions for some hours slows or stops the clock and this is reflected in a corresponding cessation of rhythmic activity which is restored with return to aerobic conditions. The leaf movement in Phaseolus is arrested in this way (Bünning, Kurras and Vielhaben 1965).

### C. Effects of Light on Leaf Movements.

Certain effects of light on leaf posture such as those responses generally considered as "phototropism" and "photonasty" (Branner L., 1959), while not strictly relevant to the present studies are nevertheless of some importance since they will determine the leaf angle in the light (Brett 1970). As a consequence of this the leaf angle at the start of the nyctinastic movement may be determined by the previous illumination.

The relationship to light intensity varies from species to species. In Plantago lanceolata the rosette leaves only become prostrate in light of sufficiently high intensity (Barber, Halsall and Palmer 1968) but the leaves of Phaseolus multiflorus (Brauner, M. 1932) and cotyledons of Linum usitatissimum (Brett 1970) are raised with increasing light intensity..

The intensity of illumination during the day has been shown to affect the subsequent nyctinastic movement of leaves in several species. Darwin noticed that the sleep (nyctinastic) movements of plants kept in the room away from the window were much weaker than the sleep movements of brightly lit plants (Darwin 1880). No further investigations of this phenomenon appear to have been made although it is perhaps predictable that the requirements for photosynthesis may be involved.

Under conditions of continuous illumination the leaf movements of Phaseolus vulgaris var. Pinto were found to persist for several weeks and were of uniform period length under different light intensities (Hoshizaki and Hamner 1964).

In Coleus, rhythmic movements persist for at least 6 to 7 cycles in both strong (13800 lux) or weak (107 lux and 320 lux) light (Halaban 1968a- I), but amplitude was greatest in darkness and damps with

increasing light intensity. Period length is longer in bright than in dim light. The rhythm of movement of Coleus leaves in dim light may be phase-shifted by a period of bright light (Halaban 1968b- II).

Thus it appears that a change in intensity of light may be as effective in phase setting the rhythm of movement as a transition from light to dark or from dark to light.

Burkholder and Pratt (1936) found that the speed of opening of dark closed Mimosa leaf pinna was proportional to the intensity of light given. They found very little variation in the time taken for leaves to close when darkened through the daylight hours (07.55, 17.31 h), although the time for closure or darkening decreased markedly after plants in the greenhouse had closed naturally (about 18.00 h). Plants remaining in artificial light after this time remained open for several hours, but irregular closing began at about 20.30 h. Thus, the closing movement can be postponed by light for a time but the endogenous rhythmic control determines the eventual leaf closure. In Albizzia, Hillman and Koukkari (1967) found rate of closing upon darkening decreased during the light period.

The experiments of Burkholder and Pratt (1936) further revealed a diurnal variation in photic sensitivity. When darkened Mimosa pinna were illuminated at this time through the 24 hours (during the daylight hours the plants were initially darkened for 45 min to close the leaf), their results showed a decrease in light sensitivity of darkened plants through the late afternoon and an increase towards dawn reaching a maximum opening when plants in the greenhouse were opening naturally; an hour later plants in darkness since the previous evening opened irregularly without light.

This is an important finding because it clearly demonstrates a rhythm of changing responsiveness in the plant apart from the overt rhythm of movement.

In another experiment to determine the effect of premature darkening on the rhythm of light sensitivity Burkholder and Pratt showed that placing plants in the dark at midday (12.00 h) resulted in the earlier 'awakening' as revealed by relative rates of leaf opening when exposed to light. The result (loc. cit., Fig. 3) in fact shows what we would now call a "phase advance" of about 2-3 hours compared to the phase of the plants darkened at the usual time (18.00 h), and is quite comparable to the phase changes of the actual rhythm of movement achieved by changing the illumination schedules as described by Kleinhoonte (1929, 1932) for Canavalia, who postulated a mechanism whereby the open phase of the endogenous leaf movement remains in constant relationship to the beginning of the light period.

As noted in the previous section of this Introduction, a steady state phase-change may not occur immediately when a single signal is given to a free-running rhythm. Complete change of a light:dark schedule, which is in any case entraining the rhythm, will, however, in the case of leaf movements generally result in a virtually immediate change of phase. Even so when a single span of darkness in a 16:8 light:dark schedule was shortened or lengthened by 4 hours to advance or delay the leaf movement rhythm in Albizia (Koukkari, Halberg and Gordon 1973), advance of the movement rhythm occurred more rapidly than a delay. This difference is clearly related to the "phase response" of the rhythm discussed earlier.

The relationship between the beginning of the light period ('dawn' signal) and the day maximum in leaf movement rhythm is not simple. The

time of maximal leaf opening or raising varies with length of photoperiod. The peak of the movement in Portulaca occurred successively later in the day as the length of photoperiod was increased showing thereby a closer correlation with the end of the photoperiod ('dusk' signal) than the beginning (Karve and Jigajinni 1965). This may be more apparent than real since the true relation may be to the overall length of light period the peak of the rhythm positioned at a constant phase. Although the day maximum in Phaseolus also varies with photoperiod the night maximum shows a much closer relationship to the beginning of the photoperiod, always occurring 17-18 hours after light-on through a range of photoperiod from 1 h (1:23) to 23 (23:1) hours (Flügel 1949).

Although not a simple matter, the effects of light on leaf movements may be separated into those that directly affect the opening and closing or raising and lowering of the leaf - the photonastic effects, and those which act via the endogenous oscillating system. However, the greatest complication arises because the endogenous rhythm also controls the sensitivity or responsiveness of the leaf to light. This was made clear by Burkholder and Pratt (1936) results described above. Palmer and Asprey (1958) have also demonstrated that the leaf of Samanea saman is not responsive to light at the end of the "open" phase of leaf movement since when the leaf is closed by a short period of darkness at this phase (late subjective day), illumination of the leaf will not cause it to re-open. Likewise, short periods of light during the dark-closed phase (subjective night) did not reopen the leaf.

Effects of different regions of the spectrum.

To some extent, the study of the activity of different spectral bands has resolved the two components of the leaf movement mechanism. Photonastic effects have been found to be brought about mainly by the

absorption of light in the blue spectral regions (Sachs 1882). Thus the photohyponastic response to light exhibited by leaves of Phaseolus (Brauner 1959) and cotyledons of Linum (Brett 1970) depends on the blue light as does the photo-epinastic response of Oryza leaves (Inada 1969).

The effectiveness of blue light in the leaflet opening response of Mimosa may be related to these findings, but in Mimosa the far-red wavelengths are also involved. Bukholder and Pratt (1936) found the blue and far-red regions to be effective and this has since been confirmed by Fondeville et al. (1967), who interpreted this in the light of other similar action spectra of the so-called high-energy photoresponse. There is, however, another petiole dropping movement in Mimosa involving the primary pulvinus which appears to be entirely blue-light dependent (Fondeville et al. 1967).

#### Participation of the phytochrome system.

The participation of the phytochrome system in the nyctinastic movement of Mimosa and Albizzia and some relative legumes has now been the subject of considerable study (Fondeville, Borthwick and Hendricks, 1966; Jaffe and Galston, 1967; Sweet and Hillman, 1969; Satter, Marinoff and Galston, 1970; Hillman and Koukkari, 1967; Koukkari and Hillman, 1968; Satter and Galston, 1971 a, b; Kadman and Zahavi 1972), since the initial discovery (Fondeville, Borthwick and Hendricks 1966) that the closing movement of Mimosa pinnae upon change from light to darkness depends on the presence of phytochrome in the far-red absorbing form ( $P_{fr}$ ). As in all other phytochrome systems the closing movement can be repeatedly potentiated and reversed by repeated alternations of red and far-red light.

Using Albizzia julibrissin, Hillman and Koukkari (1967) and Jaffe and Galston (1967) showed that the requirement for  $P_{fr}$  only appears in the early part of the daily light period, but later, towards the end of the light period there is little or no phytochrome control so that exposure to far-red light before darkening has little effect on the closing of the leaf. This is presumably due to interaction with the endogenous rhythm, and this interaction has been the subject of recent work by Satter and Galston (1971), in which they relate the rhythmic behaviour of the leaflets to a rhythmic increase in potassium efflux from the ventral motor cells.

#### Effects of wavelength on the rhythm.

The action of the different spectral bands on the rhythm of leaf movement has been studied by Bünning and Lörcher (1957) and Lörcher (1958) in Phaseolus multiflorus. Continuous irradiation with red light (610-690 nm) increased the period length while far-red light (690-850 nm) decreases the period length compared to the period observed in other wavelengths and in darkness. Furthermore, red light (600-700 nm) will initiate the rhythm of leaf movement in arrhythmic dark-grown plants, and this inductive effect can be nullified by subsequent exposure to far-red light (700-900nm) as in the phytochrome response. In Bauhinia, illumination with blue, far-red or green light through the late night had no effect on time of leaf closure in the following darkening, whereas similar illumination with white light caused leaves to close some 5 h earlier, and red about half this effect (Holdsworth 1960). Bünning (1960) reported that the phase of the rhythm of Phaseolus seedlings in darkness could be shifted by almost all regions of the spectrum. Bünning and Moser (1966) have since shown, however, that the phase shifting brought about by white light in Phaseolus is mainly due to the direct effect of red light on the leaf joint. Effects of far-red and blue are



due to light absorbed by the lamina and advances of phase are predominant.

Halaban (1969), in her studies of the leaf movement rhythm of Coleus found the period length under continuous red light to be shorter (20.5 h) than the length under blue light (24 h). Blue light signals delayed the phase of the rhythm of leaf movement when given at times when white light would have caused a delay, and red light advanced the phase when white light would have caused an advance. Far-red light had no effect on phase and also did not reverse the effects of previous exposure to red or blue light.

These results suggest that the circadian rhythm is periodically responsive to blue and red irradiation, each of which presumably induces a different photoreaction.

#### D. Mechanisms of Leaf Movement.

Recognition that the various types of movement exhibited by plants organs may be brought about either by reversible changes in turgor or by irreversible growth (Darwin 1880) has led to their separation in most general discussions of the subject into 'growth (nutational) movements' and 'variation movements'. The nastic movements of leaves are typically brought about by differential changes in cell volume affecting the upper or lower sides of special leaf joints called pulvini. Well-differentiated pulvini are, however, only found in a few families of plants and then not in every species. They are most common in the Leguminosae and Oxalidaceae among dicotyledons but also occur in a few monocotyledons (Marantaceae) and in the pteridophyte Marsilea.

The widespread occurrence of leaf movements throughout the flowering plants, most of which have no obvious pulvini suggests that the pulvini are specializations which modify a more fundamental plant movement mechanism. Pulvinate leaves have been the obvious choice for experiment and all the major studies of leaf movements have until recently been on the large leaves of the Leguminosae. A few studies have shown that rhythmic movements exist in non-pulvinate leaves e.g. Chenopodium amaranticolor (Könitz 1958), Coleus (Halaban 1968 I, II; 1969), Portulaca (Karve and Jigajinni 1965, 1966) and Xanthium (Hoshizaki, Brest and Hamner 1969). Whether such movements are due to periodic changes in cell volume or to growth has not been determined. According to Metzner (1934), the leaf movements of some tropical plants are due to a combination of reversible turgor changes and differential growth. If growth alone were responsible then the movements would be expected to cease once the leaves or petioles ceased to expand and this is probably the case in many leaves and cotyledons which exhibit movements for only a few days (Darwin 1880). Movements brought about by an alternation of epinastic and hyponastic growth have been studied by Yin (1941) in Carica papaya. Growth of the upper side of the petiole is stimulated towards the end of the day and the leaf bends down and assumes the night position. This rapid phase of epinasty soon ceases and the continued growth of the lower side of the petiole brings the leaf up once again to the day raised position. Stimulation of rapid epinastic growth was attributed by Yin to an accumulation of auxin during the day in the basal part of the leaf which has vascular connexions mainly to the upper side of the petiole.

The role of auxin in leaf epinasty has been the subject of several recent studies but these have dealt with relatively long-term effects and the relevance of these effects to the daily movements is not direct.

Lyon (1964) studied the epinasty induced by rotation on the horizontal clinostat. He found the petiole showed little or no epinastic reaction if the lamina was removed unless IAA was applied. Palmer (1964) induced epinasty in Helianthus leaves by placing the whole plant in a horizontal position or by bending the upper part of the shoot into a horizontal position. Palmer and Halsall (1969) demonstrated the inhibition of polar transport of IAA -  $^{14}\text{C}$  in Helianthus shoots following rotation on the horizontal clinostat or training the shoots in a horizontal position. Brett (1970) showed that the epicotyl shoot, or IAA applied to the decapitated stump, damped both epinastic and hyponastic responses of Linum cotyledons.

Unilateral applications of IAA to the pulvinus of Mimosa pudica were reported by Burkholder and Pratt (1936a) to result in movements such as would be caused by increase in size of the tissue at the point of application: auxin on the upper side caused the petiole to move downward, auxin on the lower side caused the petiole to move upward. Williams and Rhagavan (1966) found addition of IAA to the medium on which leaflets of M. pudica were floated increased the opening during the day, and at high concentrations closure of the leaflets at night was never complete.

The effect of applications of IAA to the pulvinus of Phaseolus multiflorus was investigated by von Guttenberg and Kröpelin (1947) following earlier studies by Portheim (1941), in which it was not clear whether the action was due to growth or turgor variation. As in Mimosa, application of IAA to the upper side of the pulvinus resulted in downward curvature and vice versa. Application of IAA to the midrib of the leaf, however, caused the leaf to rise, but downward movement was evoked when IAA was applied in a ring around the pulvinus. The

concentration used was  $10^{-3}$  M, but at lower concentrations ( $10^{-5}$  and  $10^{-6}$  M) they found the lower side of the pulvinus was more responsive than the upper side, thus resulting in a small upward curvature. They were able to show a certain amount of reversibility by measuring the angle again after killing the tissue in hot water but in general the upward curvatures were not reversed after death. Von Guttenberg and Kröpelin also found a diurnal periodicity in production of diffusible auxin by the leaf blade. Brauner and Arslan (1951) confirmed some of the earlier observations, although their results differed quantitatively from those of von Guttenberg and Kröpelin and some were contradictory as in the relative sensitivity of the upper and lower sides such that symmetrical applications of IAA caused marked upward movement. They found, however, that whereas application of IAA to the midrib of the leaf causes upward movement, application to the lateral veins at the base of the leaf induces downward movement. Their explanation of this involved the demonstration that abaxial parts of the pulvinus vascular tissue are continuous with the midrib while adaxial parts connect to the lateral veins. Brauner and Arslan concluded that the periodic movements of the leaf could be due to a periodicity in auxin production in the lamina and variation in concentration in the distal and basal parts of the leaf. This resembles the explanation suggested by Yin (1941) to account for the alternation of epinasty and hyponasty in Carica described above.

In the work quoted above, Brauner and Arslan also studied the movements of the pulvinus on release of turgor by killing the tissue in hot water at times throughout the nyctinastic cycle. During the hours the leaf was in the maximum daytime raised position turgor loss caused a downward movement, whereas during the evening and night it caused an upward movement, the inference being "that during the

culmination of the day position the pulvinus must be elastically deformed in the adaxial direction and that it is held in constraint by the turgor pressure of the abaxial flank. When the turgor is removed in both halves the abaxially directed elastic force is released and the pulvinus bends downwards. Throughout the night the signs of all tensions are reversed. The elastic deformation is then in the abaxial direction and the sleeping position is maintained by a turgor excess in the adaxial half. Thus release of pressure now must cause an upward movement." \*

Both adaxial and abaxial sides of the pulvinus are therefore seen to be active in the leaf movement cycle, alternately undergoing increase and decrease in turgor. This supports the hypothesis suggested on quite different evidence by Asprey and Palmer (1955) and further developed by Palmer and Asprey (1958), in which both halves of the pulvinus participate equally in the movement. Palmer and Asprey showed that the normal nyctinastic movements of the leaf of Samanea saman continued, although at reduced amplitude, when either the upper or the lower half of the pulvinus was removed. "The response of each half of the pulvinus to a given stimulus is fully complementary, suggesting that if the cells in one half of the pulvinus respond to a stimulus by gaining turgor, those in the opposite half respond by losing turgor." (loc. cit., p. 784).

Jaffe and Galston (1967) found a greater rate of electrolyte flux from the cut ends of pinnae of Albizzia julibrissin whose pinnules were closing than from those that were stationary. Pretreatment with red light

---

\* In this quotation the terms adaxial and abaxial have been substituted for the terms dorsal and ventral respectively since Brauner and Arslan used these in the sense opposite to that in which they are normally employed.

facilitated both closure and electrolyte flux.

Phytochrome control of nyctinastic movements has already been discussed in the previous part of the present thesis. Control is exerted rapidly following the pigment conversion and operates through changes in the volume of the motor cells of the pulvinule which involve water movements into and out of these cells. Satter, Marinoff and Galston (1970) have since shown that, during closure potassium is lost from ventral cells and enters dorsal cells. The changes in volume of pulvinule cells which cause leaflet movement are therefore controlled by K flux, and this was found to be the case whether the leaf movement was phytochrome controlled or entirely due to the control exerted by the endogenous rhythm (Satter and Galston 1971 a, b). The following table summarizes the control of movements of Albizzia pulvinules (based on Table IV, Satter and Galston 1971 b).

Leaflet Movement	Necessary Conditions	Prevented by Meta- bolic Inhibitors?	Chemical Basis *	
			K dorsal	K ventral
Nyctinastic Closure	Darkness	Yes	++	
	Darkness, P <sub>fr</sub>	Yes	+++	--
Rhythmic Closure	Prolonged light	?	++	--
Rhythmic Closure	Prolonged darkness	No	+	---
Rhythmic nycti- nastic Closure	Several hours of light followed by darkness	No	++	---
Light-promoted Opening	Blue light	?	---	+
Rhythmic Opening	Prolonged darkness	Yes	-	+++

\* + indicates influx and - indicates efflux.

Aims and scopes of the present study.

The aims of the study were conceived in general terms to investigate:

- i) the course of nyctinastic movement in relation to the light and dark periods previously experienced by the plant.
- ii) The conditions necessary for leaf movement to occur, i.e., the induction and "potentiation" of the movement.
- iii) The involvement of an endogenous oscillation controlling the movement.
- iv) The involvement of phytochrome system.
- v) The possibility of discriminating between the direct effects of light on the leaf movement mechanism and effects via the endogenous rhythm.

The plant differs greatly from any used in recent detailed studies of leaf movement and the technique involved sacrificing a population of plants for each measurement. Although laborious, this has the merit of providing adequate samples for statistical analysis. It was, however, intended that this should be supplemented by photographic recording whenever appropriate. In the event an automatic recording camera was not available for this purpose and the manual recording technique has proved adequate for the present investigations.

It soon became apparent that a circadian rhythm regulated leaf movement and that some preliminary study of rhythmic control would be possible despite the limitation imposed by the technique of measurements. With the discovery that leaf movement ceases altogether in prolonged darkness came the additional question whether the endogenous oscillation itself had ceased to function under the conditions. It has been possible to answer this question, at least insofar as the observations are restricted to the first 24 h cycle in darkness.

The investigations which follow are grouped into four main sections. In Section 1 are presented the results which refer specially to the nyctinastic leaf movement that occurs in 24 h cycles of alternating light and dark and the movement which follows such cycles when the length of the final light period is modified. Some of the evidence for rhythmic control emerges from these investigations and in Section 2 some additional results having a special bearing on the endogenous rhythm are presented.

In the third Section are grouped the investigations which refer to the action of specific regions of the light spectrum and evidence for phytochrome control is presented there.

A further Section is devoted to an anatomical study of the basal part of the leaf in an attempt to locate any special cells that might through periodical changes in turgor be responsible, wholly or in part, for the leaf movement observed.

Some additional results of a preliminary nature are included in the Appendix. These are concerned with the role of auxin from the apex above the measured leaves and also the effect of darkening the leaves by painting with India ink.



## MATERIALS AND METHODS

### 1. The seeds.

Seeds of Linum usitatissimum were obtained from agricultural seed merchants. The variety labelled 'Blue Flax' has been used.

### 2. Growing Techniques.

The seeds were sown on a moist tissue pad in a glass dish and left to germinate at laboratory temperature for two days. The germinated seeds were then planted into moist vermiculite or peralite in  $3\frac{1}{2}$ " pots. Each pot contained 15 germinated seeds. The pots were kept in the controlled environment cabinet at a constant temperature of 25°C. The plants were watered with half strength nutrient solution (Knop's solution, Hewitt 1952; sequestric acid, ferric sodium salt was used instead of  $\text{FePO}_4$ ) every other day. The plants were mainly grown on a 14:10 light:dark cycle unless otherwise mentioned in the text.

Lighting was provided by 'cool white' fluorescent tubes separated from the growth chamber by a plastic diffusing screen. Light intensity at the plant level was  $10^3$  lux. The same light source was used for short period light treatments.

### 3. Measurements of angular position.

Most of the experiments are carried out with 12-13 day old plants. The measurement of the leaf angle is taken on both of the second pair of leaves. The first pair of leaves are opposite and arise very close to the cotyledons with which they alternate in position. The second pair of leaves is sub-opposit 5-10 mm above the cotyledons. The angle measured was the angle enclosed by the stem and a line passing through the point of attachment of the leaf and the leaf tip. The scale used was ruled

on paper with unit intervals of  $5^{\circ}$ . On the scale (Fig. 1) a reading of 18 ( $= 90^{\circ}$ ) is the horizontal position. This method of angle measurement was verified by using more elaborate shadowgraph techniques. Details of this are given in the Appendix. The shadowgraphs obtained were projected on a scale and the accurate angle was found. The leaf angle measured by hand was then compared. The difference between the two measurements was found to be insignificant. As the shadowgraph method is much more tedious and time consuming, hand measurements were used in all the experiments. By this hand measurement method, plants are sacrificed for each treatment, a large number of plants are required for each experiment, and also late hours are involved. For avoiding this, photographic method was tried but later discarded because of the unavailability of the time lapse camera.

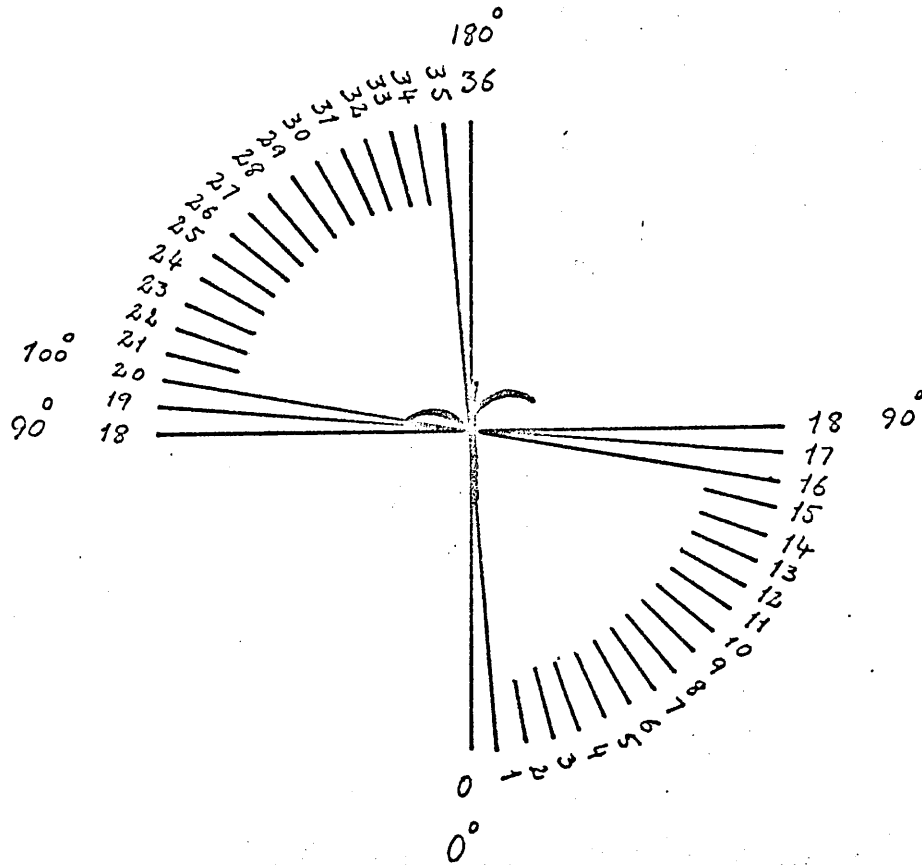


Fig.1. Measurement of leaf angle. The device is ruled in  $5^{\circ}$  intervals (in this diagram the centre is left blank for clarity). The leaf on the left scores  $100^{\circ}$ .

#### 4. Colour filters.

The following cinemoid filters (Strand Electric Co.) were used to get the specific ranges of light quality (Fig. 2). The source for all these filters was the cool white fluorescent tubes in the controlled growth cabinets used to eliminate the emission of wavelengths above 700 nm to which these filters are transparent.

Filter combination	Peak transmission	Incident energy
Dark blue (19)	450 nm	0.30 J m <sup>-2</sup>
Ruby (14) + Orange (5)	600 nm	0.43 J m <sup>-2</sup>
Dark green (24)*	530 nm	2.8 J m <sup>-2</sup>

\* - Safe light: a separate source was used for taking measurements in the dark and also for transferring the plants for various treatments. A green fluorescent tube of 20 watts was covered with three layers of dark green cinemoid (24). The incident energy did not exceed 2.8 J m<sup>-2</sup> at the bench where measurements were taken.

Wavelength in the far-red region was obtained by using one layer of blue (19) and one layer of ruby (14). The filter combination transmits only above 725 nm. The light source was six 100 watts incandescent flood lamps (Fig. 3). The white light was passed through a perspex trough filled with running water (4 cm deep), to reduce the transmitted spectrum of infra-red wavelengths.

Fig. 2 Transmission spectrum for cinemoid filters and combination.  
It is percent transmittance as a function of wavelength.  
Blue (————); green (- - - - -); red (- - - - -); far-red (----).  
The values were obtained on Beckman DB-G spectro-photometer.

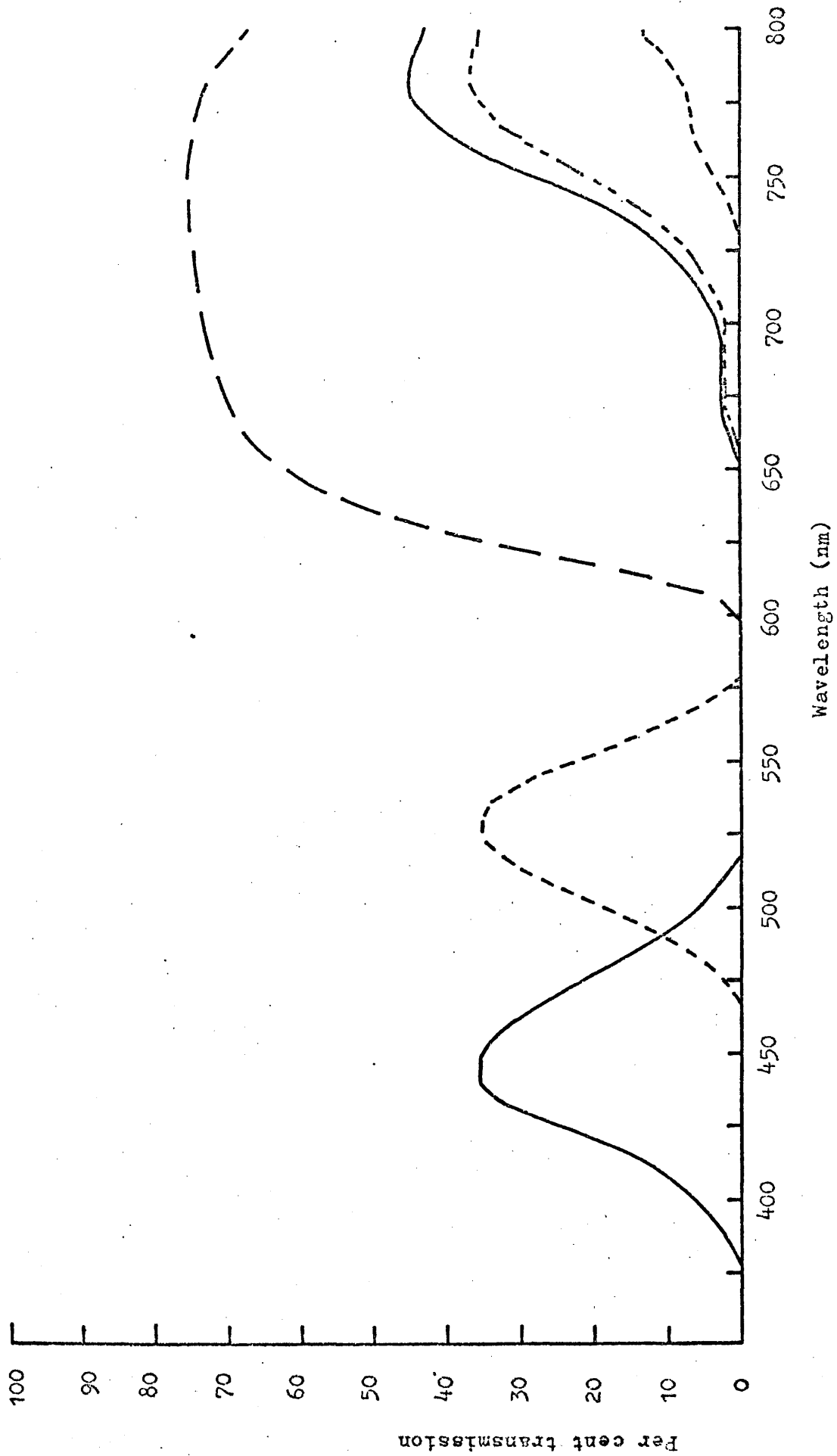
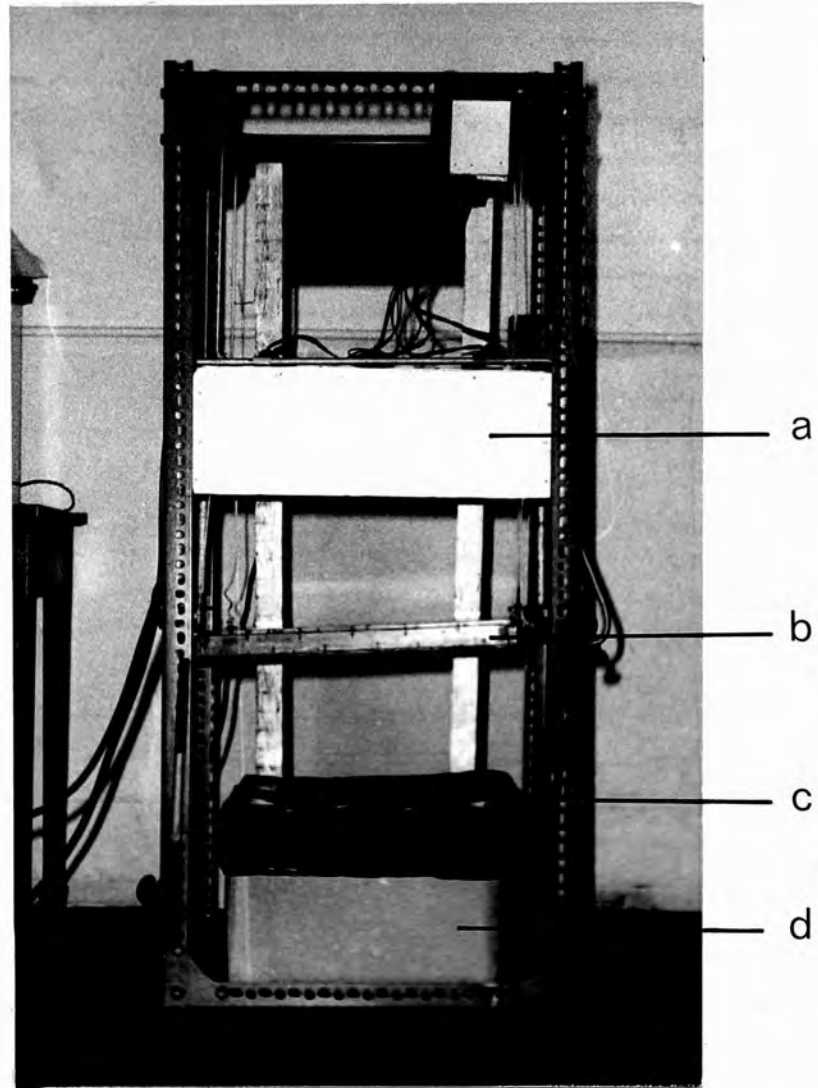


Fig. 3. The set up used for the far-red irradiation.

- a. Light source, six 100 watts reflector lamps.
- b. Perspex filter with running water.
- c. Far-red filter.
- d. Box containing plants.



Faint, illegible text, possibly bleed-through from the reverse side of the page.



## 5. Statistical Analysis.

The data obtained were subjected to various significance tests. The tables showing the analysis are given in the text.

The original data (obtained in units of 5°) were analysed and changed into degrees as mentioned below.

Confidence limits are calculated in a number of experiments by the following formula:

$$\text{Confidence limits} = \pm \frac{\text{S.D.} \times t \times 5}{\sqrt{n}} \quad \text{degrees}$$

where

S.D. = standard deviation calculated for each set of data;

t = value with (n-1) degrees of freedom obtained from student's t-distribution table for probability level specified;

n = number of observations on which the mean is based.

The factor 5 changes the limits into degrees. These values are shown in the table along with the means which are also expressed in degrees.

Certain experiments, when desirable, were subjected to an "analysis of variance" (Bailey 1959). When significant differences among the means were indicated by the analysis of variance, the significance of the difference between the individual means was further tested by Duncan's Multiple Range Test (Duncan 1955). The test was carried out on the original data and the analysis of variance table shows the values obtained from such data. The means shown in a separate table are expressed in degrees as usual.

The data for all the treatments were pooled and subjected to the analysis of variance. As the plant is sacrificed for every measurement, the values are considered independent.

6. Preparation of the tissue for anatomical studies.

a. Fixation.

Thin slices, 2 mm thick, cut at the point of insertion of the leaf with stem by two simultaneous transverse cuts were fixed in 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 3-4 h. Vacuum was applied to make the penetration easier and to eliminate any air bubbles. The slices were then thoroughly washed twice with buffer and left in buffer overnight at 4°C.

Light microscopy.

The fixed tissue was dehydrated through various grades of alcohol to tertiary butyl alcohol (TBA). The tissue was later embedded in wax after passing through 2:1, 1:2 mixtures of TBA and paraffin. Toluidene blue was used for staining.

Electron Microscopy.

Fixation was done as mentioned above. The following processes were then followed:

- b. Post fixation: 1% Osmium Tetroxide in 0.1 M phosphate buffer for one hour, followed by a rinse with water.
- c. Dehydration was carried out in graded acetone as shown:
- (1) 30% for 15 min.
  - (2) 50% " " "
  - (3) 70% " 20 min
  - (4) 95% " 25 "
  - (5) 100%, 3 changes of 30 min each.

d. Infiltration was performed through propylene oxide in the following order:

- (1) acetone + propylene oxide (1:1) for 20 min.
- (2) propylene oxide, 2 changes, 20 min each.
- (3) propylene oxide + resin (10%), 24 hours.
- (4) propylene oxide + resin (50%), 48 hours.
- (5) resin (100%), 2 changes, 24 hours each.

e. Embedding was in the resin of following composition in parts by weight:

TAAB embedding resin	10 g
DDSA (Dodecenyl Succinic Anhydride)	5 g
MNA (Methyl nadic anhydride)	5 g
BDMA (Benzyl dimethyl amine)	0.2 g.

f. Polymerization was carried out in small polythene lids in an oven at 60°C overnight.

g. Sectioning was done with glass knives on a Cambridge Huxley ultramicrotome. Sections were picked up on copper grids coated with formvar films.

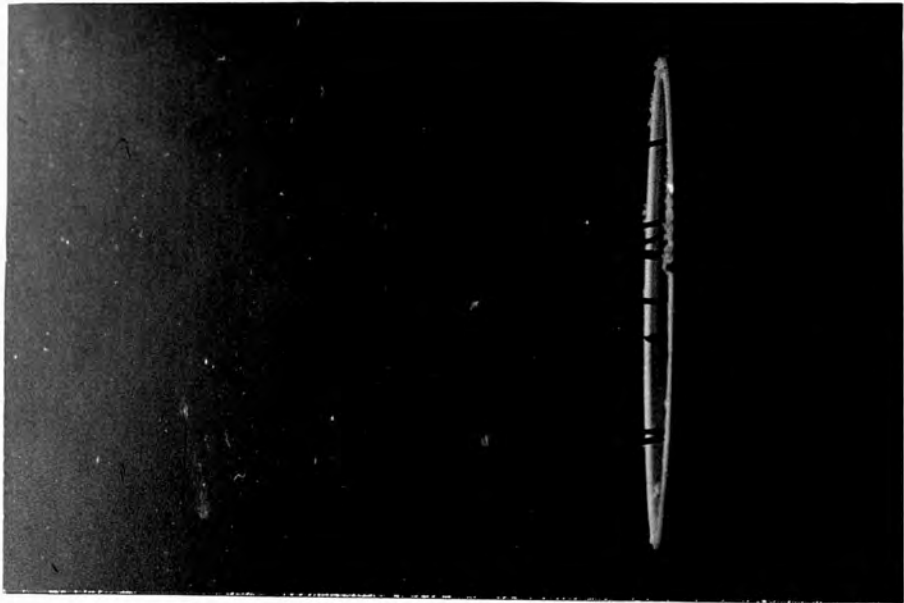
h. Staining was done with Reynolds lead citrate (1963) for 10 min.

Fig. 4. Pots showing plants in two different conditions.

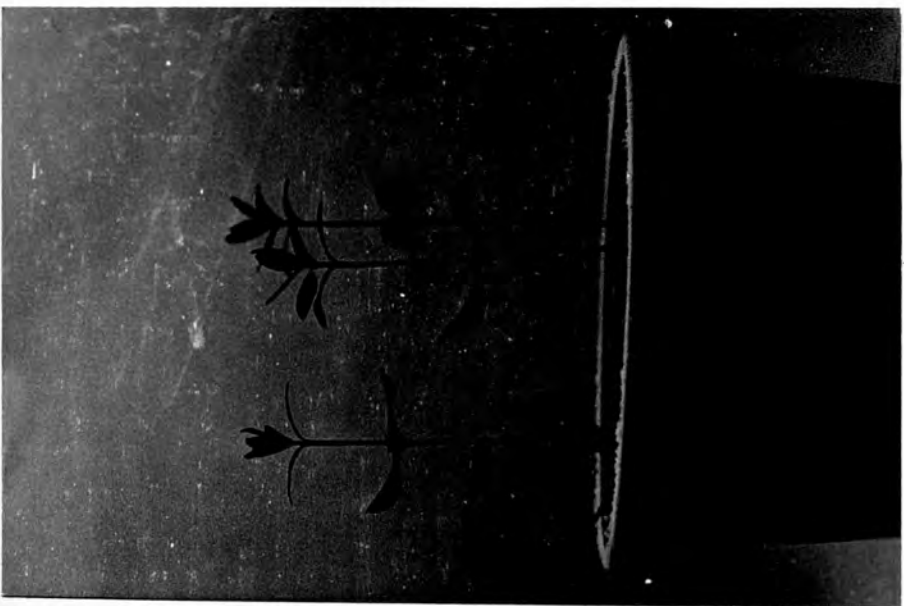
A. Plants in light.

B. Plants in dark.

Most of the plants were removed from the pots  
for taking photographs.



B



A

SECTION 1

NYCTINASTIC MOVEMENTS IN RELATION  
TO PREVIOUS ILLUMINATION

Nyctinastic movements in relation to previous illumination.

Introduction.

In this section the experiments are described under five headings:

1. Nyctinastic movements in response to normal 24-h light:dark cycles (14:10 or 12:12).
2. Nyctinastic movement following single short light periods.
3. Nyctinastic movement following an extended light period.
4. Nyctinastic movement following light given after 24 h dark.
5. Nyctinastic movement following longer periods of continuous light.

Under the first of these the course of leaf movement is traced through the 24 h of the routine 14:10 light:dark cycle on which the plants are raised to determine the general course of the nyctinastic movement, and also the time in the 24 h cycle of minimum (day) and maximum (night) leaf position.

The nyctinastic movement is also followed during the dark period of a 12:12 light:dark regime. Although the 14:10 regime was used routinely because the seedlings grew rather better under long days, the response during the 12:12 cycle should provide a standard of reference for the response following the shorter photoperiods considered in the second series and also for eventual comparison with other work on circadian rhythmic phenomena.

It has appeared in studies of nyctinastic opening and closing movements of pulvinate leaves that the speed and amount of leaf closure on sudden darkening varies with the moment in the cycle at which the treatment is given as discussed in the general introduction to this thesis. The present experiments involving short light periods are



therefore, expected to determine (a) the minimum length of light period that is required to potentiate a nyctinastic response; (b) whether this response varies with length of previous illumination; and (c) whether the same minimum length of light period is equally effective when applied at different times in the day.

This line of enquiry is continued in the third series which examines the effect of extending the established 14 h light period with the possibility of delaying the nyctinastic response until some hours of the subjective night have elapsed; and in the fourth series when light was given after a prolonged dark period.

Finally, an experiment is described in which a long period of uninterrupted light precedes the transfer to darkness. This was envisaged as a crucial test for any varying responsiveness to the light:dark transition through the circadian cycle.

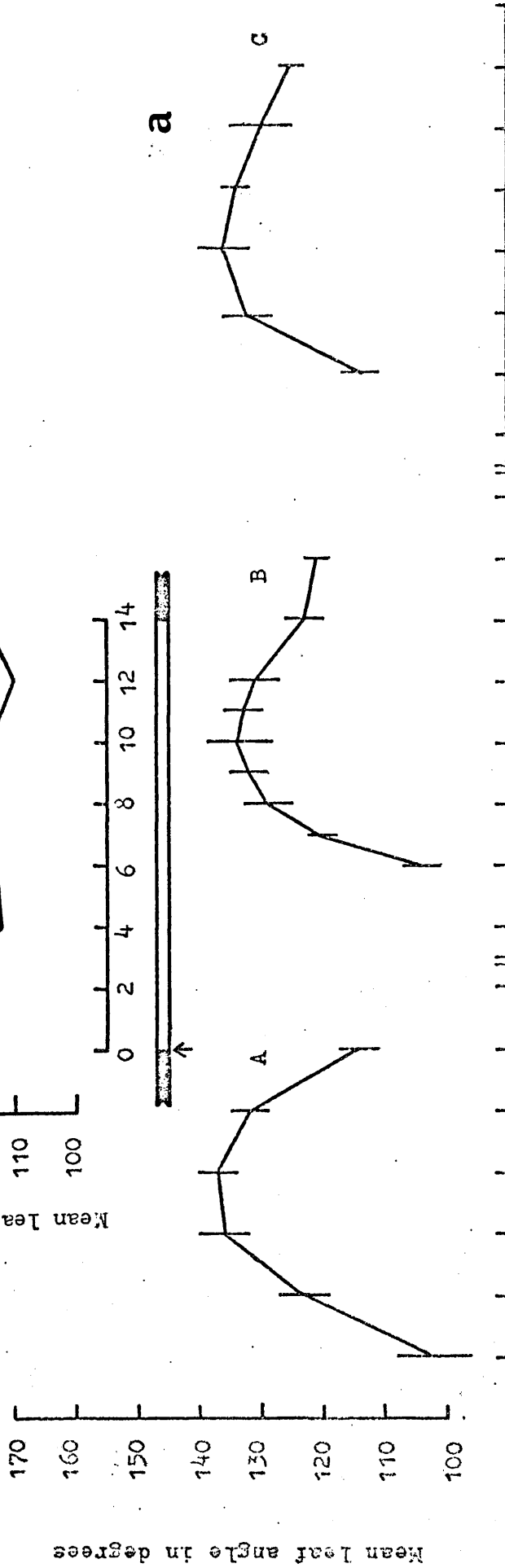
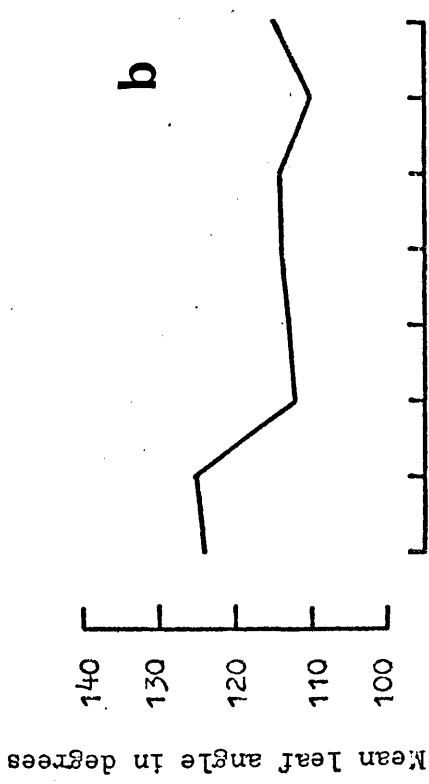
Results.Nyctinastic movement in response to normal 24-h light:dark cycles.

1 - 1. For most of the experiments in the following sections, plants were raised on a 14:10 light:dark cycle. Under these conditions, measurements are practicable from about the eleventh day after sowing. The graph (Fig. 1-1a) shows the movement during the 10 h dark period over three days. These leaves rise quite rapidly at first when the light period is terminated and reach the night maximum position in about four hours. The raised posture is not maintained for very long; within an hour or two the movement is reversed and the leaves are lowered to an approximation of the average day posture in the second half of the dark period. Table 1-1 shows that during the dark, leaves are raised much more rapidly and significantly within the first two hours, after which the movement is slower. Although the movement begins to reverse after 4-6 hours, the significant difference may not be observed until the 10th hour in the dark. It will be seen from the angles recorded at the beginning and end of the dark periods that the average posture of the leaves becomes higher as the leaves mature (Table 1-1). The amplitude of the nyctinastic movement decreases and is barely measurable after these three days, though younger leaves of course still show it.

During the 14 h light period there is no clear "trough" or day minimum, although there is a significant fall in the position of the leaves during the first four hours of the light period. After that, the leaves stay more or less steady till the final two hours of the light period, when a small rise is usually detectable (Fig. 1-1b).

Fig. 1 - 1.

- a. Course of leaf movement through three successive dark periods (curves A,B,C), on  $14:\overline{10}$  light:dark cycles. Values are means for 20 replicates, with 95% confidence limits represented by vertical bars. The horizontal bars below the graph shows the light dark periods diagrammatically.
- b. Course of leaf movement during the 14 h light period only. Table of the data is not given.
- ↑ time at which measurements commenced.



Time (h) from beginning of established light period

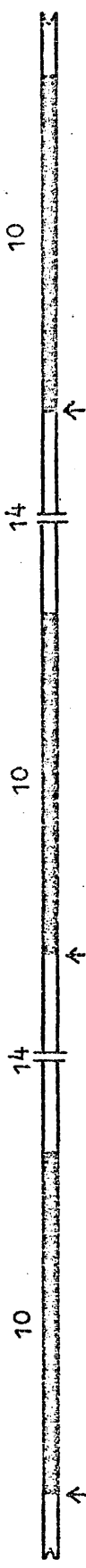


Table 1 - 1

Course of leaf movement through three successive dark periods, on 14:10 light:dark cycles. The plants aged 12 days at the commencement. Values are means for 20 replicates, with 95% confidence limits.

(Data of Fig. 1 - 10).

Time (h)	Mean leaf angle in degrees	Time (h)	Mean leaf angle in degrees
12th day			
14*	102.00 ± 4.76	20	137.25 ± 3.91
16	123.75 ± 5.87	22	132.75 ± 3.44
18	136.75 ± 3.70	24	114.25 ± 2.66
13th day			
14*	104.00 ± 3.45	19	133.50 ± 2.03
15	121.00 ± 3.36	20	131.50 ± 3.40
16	129.25 ± 2.55	22	123.75 ± 3.47
17	132.50 ± 3.98	24	121.00 ± 2.81
18	134.75 ± 3.76		
14th day			
14*	113.75 ± 1.48	20	134.25 ± 3.82
16	132.50 ± 2.79	22	130.00 ± 2.28
18	136.00 ± 3.53	24	125.00 ± 4.80

± 95% confidence limits

\* start of dark period after previous established light period of 14 h.

1 - 2. When plants were raised on a 12:12 light:dark cycle, the nyctinastic movement followed a slightly different course. After the initial steep rise within the first two hours of the dark period, the leaves remained in this raised position for two hours or so before a further small movement took them to the night maximum position (Fig. 1-2). The movement was then soon reversed. The difference between the 18th and the 20th hour in the dark is not statistically significant (Table 1-2); the movement is certainly reversed at this point and becomes significant at the 22nd hour. Although there are differences in the course of the nyctinastic movement following the 14 h and 12 h light periods, the night maximum leaf position or, rather, point of reversal of movement, is in each case usually 18 hours from the commencement of the light period. With the 14 h light period this phase occurs often at the 20th hour as in the first cycle in Fig. 1-1 and in some subsequent experiments.

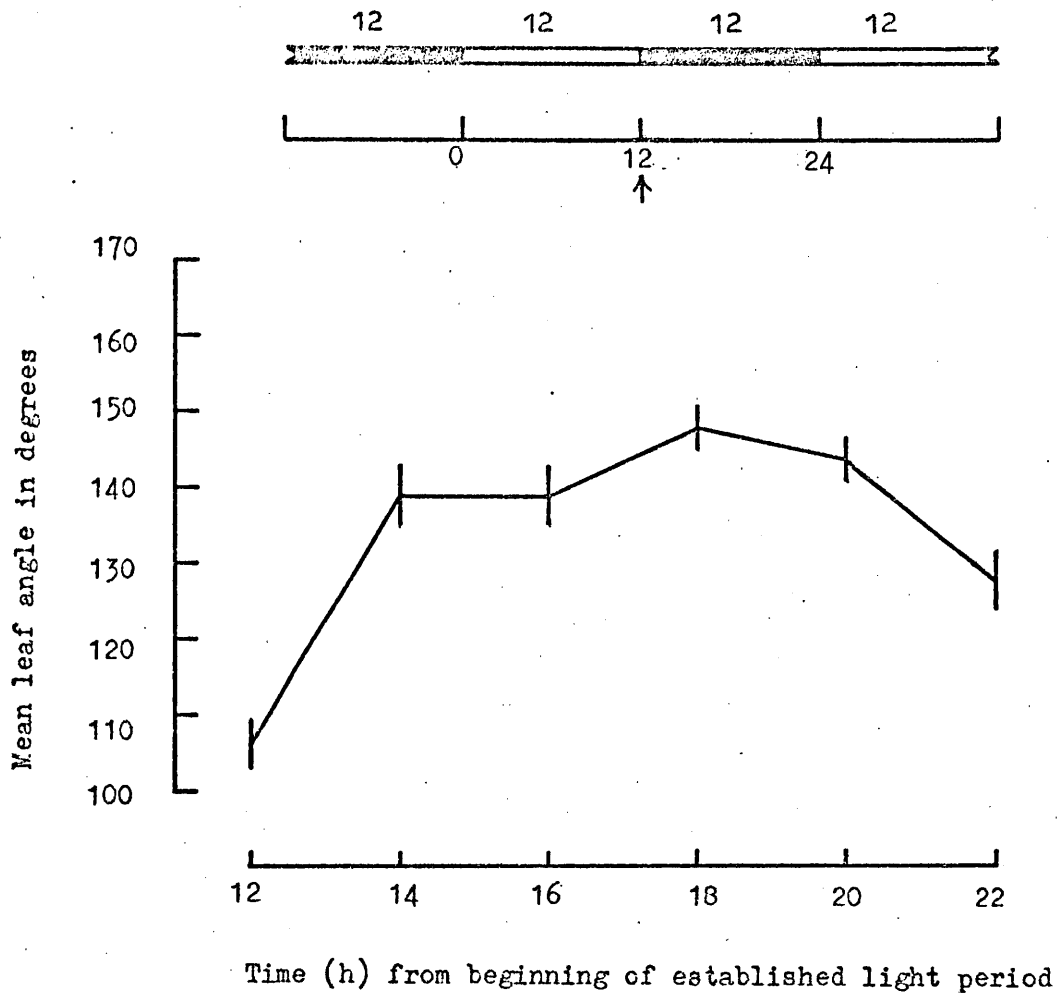


Fig. 1 - 2. Course of leaf movement during dark period, on  $12:12$  light:dark cycles.

The values are means of 20 replicates, with 95% confidence limit represented by vertical bars. The horizontal bar above the graph shows the dark period diagrammatically.

↑ time at which measurements commenced.

Table 1 - 2

Course of leaf movement during the dark period, on 12:12 light:dark cycles.

Values are means of 20 replicates, with 95% confidence limits.

(Data of Fig. 1-2).

Time (h)	Mean leaf angle in degrees
12*	105.75 ± 3.49
14	139.25 ± 3.66
16	138.75 ± 3.71
18	147.75 ± 2.88
20	144.50 ± 3.29
22	128.50 ± 3.65

± 95% confidence limits

\* start of dark period after previous established light period of 12 h.



Nyctinastic movement following single short light periods.

A. Short light periods commencing at established "dawn".

1 - 3. Plants raised on 14-h light periods were given a final light period of 4 h, 7 h, or 10 h, and measurements of the leaf angle were taken over the following eight hours during which the plants remained in darkness.

The results (Fig. 1-3; Table 1-3) show that there is no significant movement of the leaves during the eight hours of darkness following the short light periods of 4 h and 7 h; following a 10 h light period, however, the leaves were significantly raised within two hours after which the movement slowed down. The leaves were in the raised position when the final measurement was taken at the conclusion of the eighth hour of darkness. Reference to Table 1-3 will show that following 4 h light there is no significant change in the leaf angle from the 2nd to the 8th hour in the dark. There appears to be a downward trend in the curve because leaf angle at 0 h dark is significantly higher. Following 7 h light, the only significant rise is at the 6th hour of dark.

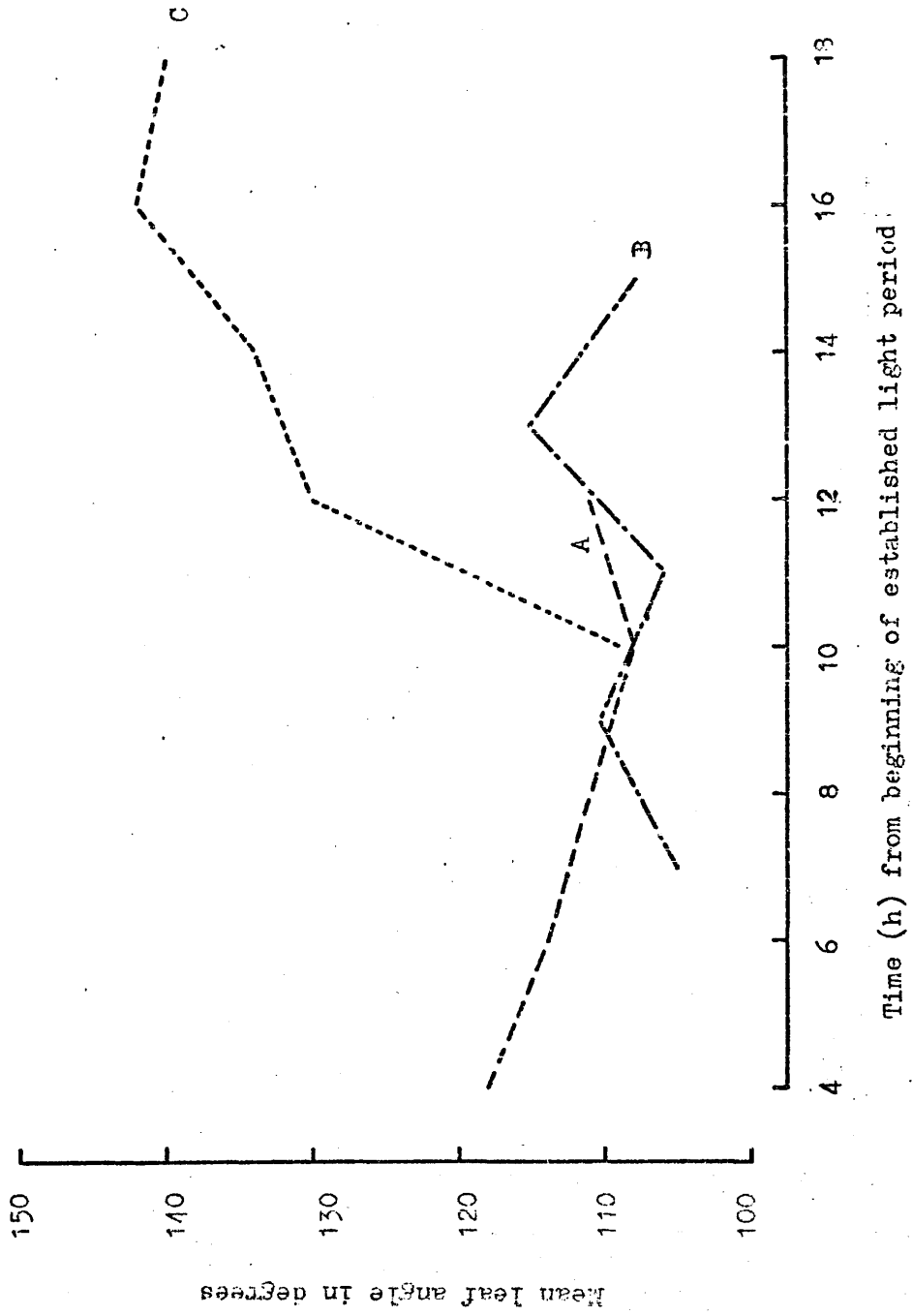
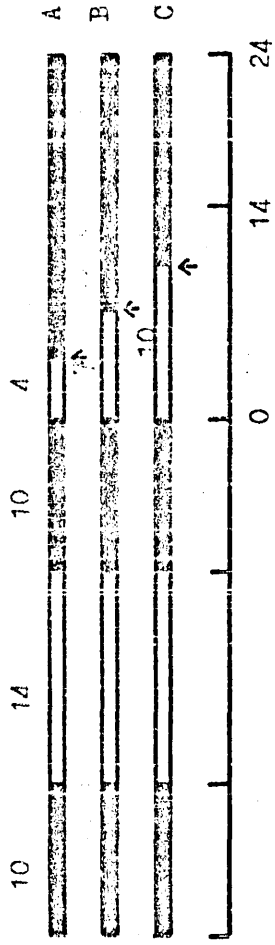
1 - 4. The treatments with short light period described above were repeated in another experiment (Fig. 1-4; Table 1-4) in which the measurement of leaf angle was extended so as to cover the time when the next dark period of the established 14:10 cycle was due. It was found that although there was no movement in the dark immediately following illumination for the first four hours of the light period, a significant nyctinastic movement occurred after a lapse of 10 h in the dark, that is, at a time corresponding to the commencement of the next regular dark period of the established 14:10 light:dark cycle

Fig. 1 - 3. Course of leaf movement after a final light period of variable length.  
 Measurements at 2 h intervals at the end of each treatment.  
 4 h light period (--- A); 7 h light period (--- B); 10 h light  
 period (----- C).

Values are means of 16 replicates.

Horizontal bars above the graph show the light:dark pattern.

↑ time at which measurements commenced.



Time (h) from beginning of established light period

Table 1 - 3

Course of leaf movement after a final light period of variable length. Plants raised on a 14:10 light:dark cycles were subjected to short light periods of 4 h, 7 h or 10 h following a regular dark period (see diagram on Fig. 1 - 3). Measurements were taken every 2 h at the end of each treatment.

Values are means of 16 replicates.

(Data of Fig. 1 - 3).

Light treatment	Time (h) in the dark				
	0*	2	4	6	8
4 h	118.90 d	114.50 def	111.70 efg	108.75 efg	111.85 efg
7 h	105.45 g	110.60 efg	105.75 g	115.75 de	108.40 fg
10 h	109.65 efg	130.60 c	134.65 bc	142.30 a	140.75 ab

N.B. When values share a common letter, they do not differ significantly at 5% level (Duncan's M.R.T.).

\* Commencement of the measurements in all the treatments.

Table of Analysis of Variance

Source	Sum of squares	Degrees of freedom	Mean squares	Variance ratio (F)
Light treatment	915.88	2	457.94	22.30***
Hours in dark	132.28	4	33.07	16.05***
Interaction	392.50	8	49.06	23.70***
Replication	16.60	14	1.19	0.58 <sup>ns</sup>
Error	434.34	225	2.06	

\*\*\* - Significant at 0.1% level  
ns - Non-significant.

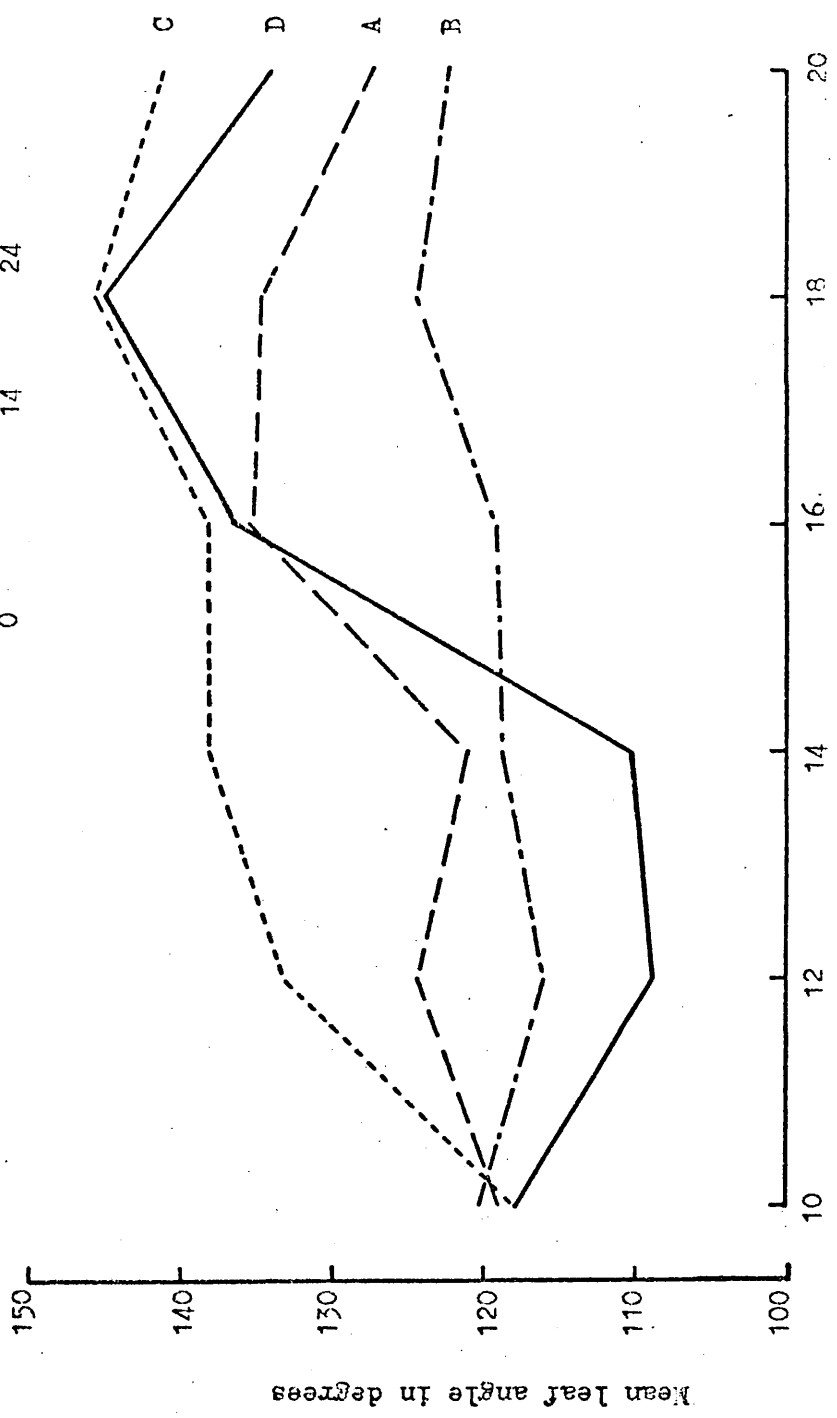
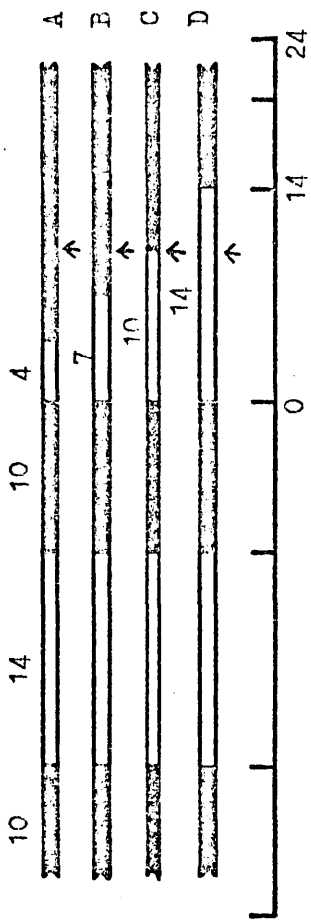
Fig. 1 - 4. Course of leaf movement after final light period of variable length. Measurements taken at 2 h intervals after 10 h from beginning of light period.

4 h light period (--- A); 7 h light period (- . - . - B); 10 h light period (----- C); 14 h light period (—— D).

Values are means of 16 replicates.

Horizontal bars above the graph show the light:dark pattern.

↑ time at which measurements commenced.



Time (h) from beginning of established light period

Table 1-4

Course of leaf movement after final light periods of variable length. Measurements taken at 2 h intervals after 10 h from beginning of light period. A final light period of 4 h, 7 h, 10 h or 14 h was given following regular 14:10 light:dark cycles (see diagram on Fig. 1 - 4).

Values are means of 16 replicates.

(Data of Fig. 1 - 4).

Light treatment	Time (h) from beginning of light period					
	10	12	14	16	18	20
4 h	119.65 efg	124.65 de	121.85 fde	135.00 c	134.45 c	127.50 d
7 h	120.00 efg	116.25 gh	113.75 ghk	119.05 efg	124.05 fde	122.80 fde
10 h	118.00 fg	133.75 c	138.10 abc	138.75 abc	145.10 a	141.90 ab
14 h	118.00 fg	109.05 k	110.00 hk	136.55 bc	145.10 a	134.05 c

N.B. When values share a common letter, they do not differ significantly at 5% level (Duncan's M.R.T).

Table of Analysis of Variance

Source	Sum of squares	Degrees of freedom	Mean squares	Variance ratio (F)
Light treatment	532.59	5	106.52	38.18***
Time	709.33	3	236.44	84.74***
Interaction	441.32	15	29.42	10.54***
Error	1076.25	360		

\*\*\* - Significant at 0.01% level.

on which the plants were raised. A movement also occurred following the 7 h light period at this time, but the movement was much slower and the maximum less than that following only 4 h light (Fig. 1-4). The nyctinastic response following a 10 h light period was peculiar in that movement commences as soon as the light is terminated, but after about 6 hours there was a definite significant rise to the maximum position. In this respect, the course of nyctinasty following a 10 h light period resembles that following a 12 h light period as described above (Fig. 1-2). Further experiments employing 10 h light periods will be described in a later section..

It is clear from the above results that although nyctinastic response does follow short light treatments of 4 h and 7 h given at the beginning of a subjective light period, the movement does not begin till a certain subsequent time in the dark has elapsed. We also know that 10 h light treatment results in an immediate nyctinastic response.

#### B. Minimum light period for immediate nyctinastic response.

1 - 5. The following experiment establishes the shortest light treatment (starting from the end of regular dark) required to produce an immediate response on darkening. Treatments of 8 h, 9 h and 10 h of light were given and measurements of the leaf angles were made every two hours as soon as the lights were switched off. The results are shown in Fig. 1-5. In each case the significant leaf movement commenced soon after the plants were subjected to darkness.



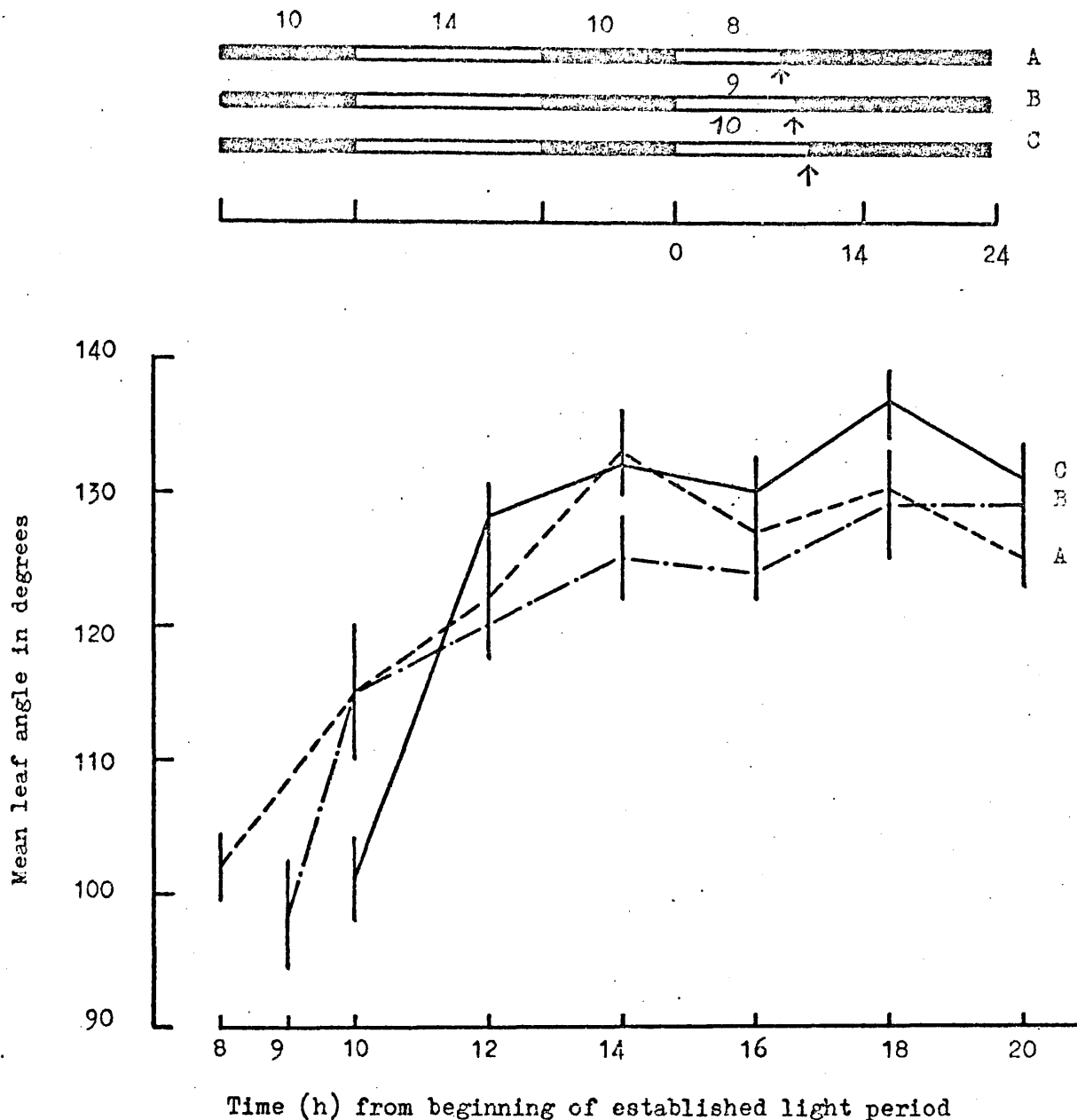


Fig. 1 - 5. Course of leaf movement after a final light period of 8 h, 9 h and 10 hours. Measurements taken during the dark at the end of each treatment.

8 h light period (--- A); 9 h light period (- · - · - B)  
10 h light period (— C).

Values are means of 16 replicates with 95% confidence limits.

Treatments are shown diagrammatically above the graph.

↑ time at which measurements commenced.

Table 1 - 5

Course of leaf movement after a final light period of 8 h, 9 h and 10 hours. Measurements were taken during the dark at the end of each treatment (see diagram on Fig. 1 - 5).

(Data of Fig. 1 - 5)

Time (h)	Light treatment		
	8 h	9 h	10 h
8*	102.15 ± 2.74	-	-
9	-	98.75 ± 4.07	-
10	115.00 ± 5.43	115.60 ± 4.95	101.85 ± 2.89
12	122.80 ± 4.23	120.00 ± 2.74	128.40 ± 2.72
14	133.40 ± 3.32	125.90 ± 3.11	132.80 ± 2.17
16	127.50 ± 3.07	124.05 ± 2.23	130.60 ± 2.55
18	130.30 ± 2.84	129.65 ± 3.82	137.50 ± 2.38
20	125.90 ± 2.28	129.35 ± 2.35	131.55 ± 3.03

± 95% confidence limit

\* hours from beginning of established light period.

C. Short light periods ending at established "dusk".

1 - 6. Short light periods were given confined to the final 4 h or final 7 h of the established 14 h light period (the preceding dark period being correspondingly lengthened). Leaf angles were measured from the termination of the light treatment (Fig. 1-6). In each case a good nyctinastic response was observed. As the interaction is non significant, all three treatments differ from each other at all levels. Very little difference of the curves is observed, except that the leaves were at a lower angle at the start of the dark period following the shorter light periods as compared to the control, which received the full 14 hours light.

D. Four hours light at various times of the subjective light period.

From the results of the experiments described above, it is clear that a good nyctinastic response with a maximum (in the dark) about 18 h from the commencement of the last established light period may be induced by four hours light given over either the first or the last 4 h of that light period (the rest of which may be exchanged for darkness). In order to investigate these responses further, it was decided to include a four hours light treatment which does not provide either a light-on or a light-off signal at the established times.

1 - 7. Thus the following experiment was done: four hours light was given at the beginning (hours 0-4), the middle (5-9), or the end (10-14) of an established light period and leaf angles were measured from the time of the commencement of the established dark period. The results (Fig. 1-7, Table 1-7) indicate that compared to the previously reported course of nyctinasty following light given

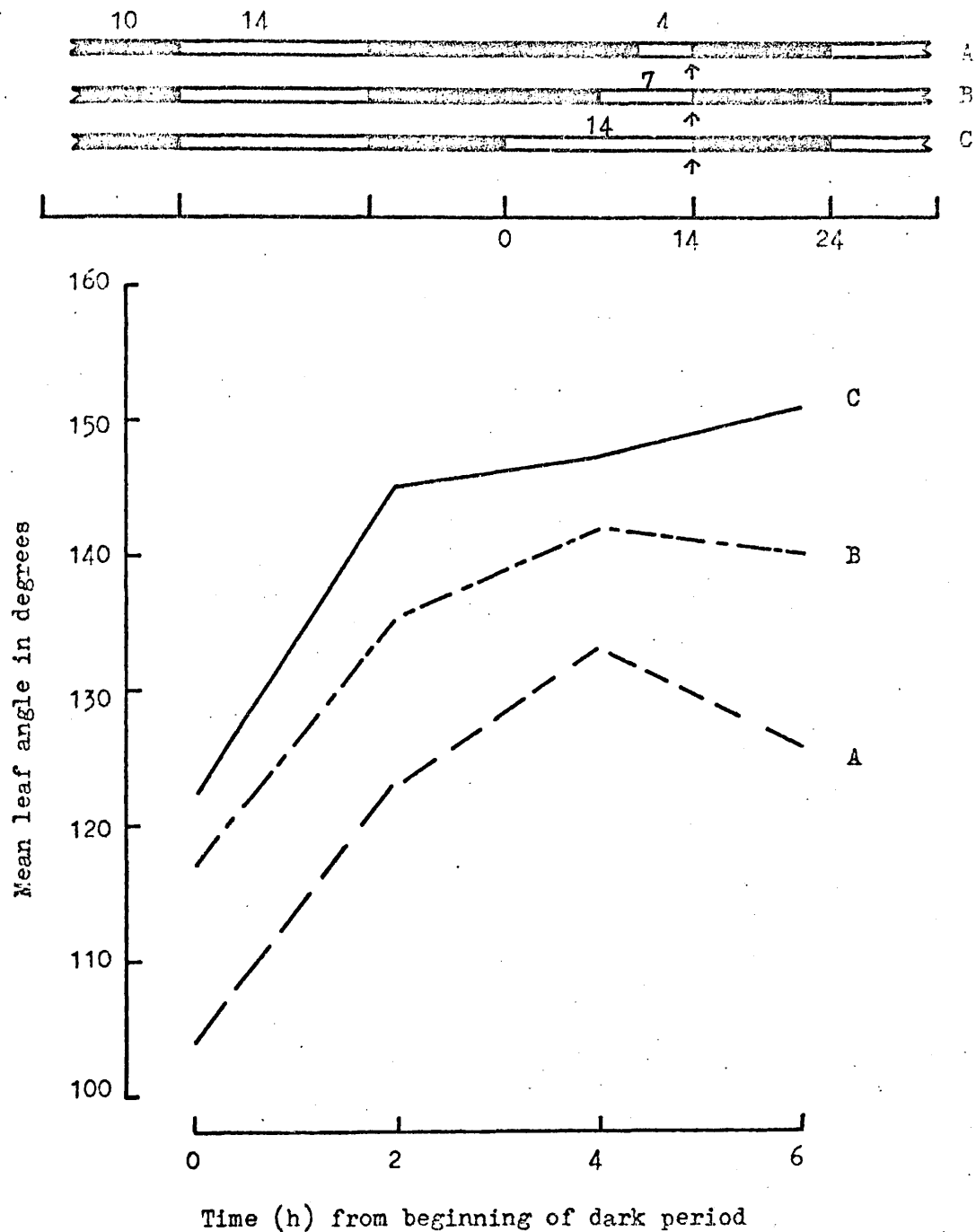


Fig. 1 - 6. Nyctinastic leaf movement following shorter light periods of 4 h or 7 h terminating at the end of an established light period. 4 h light A; 7 h light B; 14 h light C. Values are means of 16 replicates.

The light conditions of the treatments are shown diagrammatically above the graph.

↑ time at which measurements commenced.

Table 1 - 6

Nyctinastic leaf movement following shorter light periods of 4 h or 7 h terminating at the end of established light period (see diagram on Fig. 1 - 6). Control set of plants received normal 14 h light.

Values are means of 16 replicates.

(Data of Fig. 1 - 6).

Light treatment	Time (h) from beginning of dark period			
	0	2	4	6
4 h	104.50	123.55	133.40	126.40
7 h	117.15	136.25	142.65	140.60
14 h	122.65	145.45	147.50	151.40

Table of Analysis of Variance

Source	Sum of Squares	Degrees of freedom	Mean Squares	Variance ratio (F)
Light treatment	508.24	2	254.12	126.42 <sup>***</sup>
Time	852.28	3	284.09	141.33 <sup>***</sup>
Interaction	22.78	6	3.79	1.53 <sup>ns</sup>
Replication	20.92	15	1.39	0.69 <sup>ns</sup>
Error	332.20	165	2.01	--

\*\*\* - Significant at 0.01% level

ns - Non-significant at 0.5% level

Note: As the interaction is non-significant, the treatments differ significantly from each other at all levels.

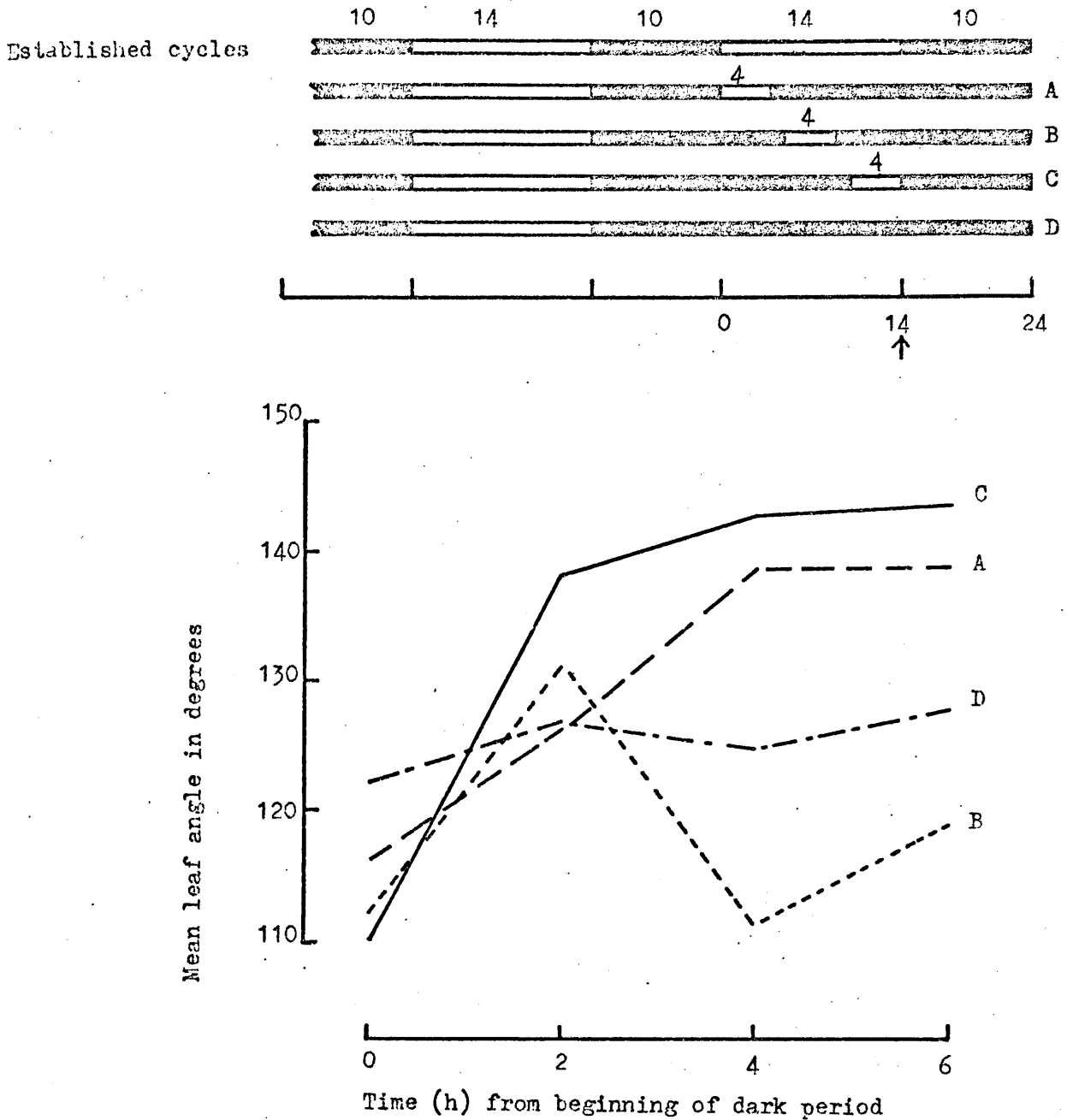


Fig. 1 - 7. Nyctinastic leaf movement following 4 h light at different Times during the established light period. (A) 4 h light in the beginning; (B) 4 h light in the middle; (C) 4 h light at the end and (D) uninterrupted darkness. Values are means of 10 replicates. Light and dark conditions are shown diagrammatically above the graph.

↑ time at which measurements commenced.

Table 1 - 7

Nyctinastic leaf movement following 4 h light at different times during the established light period (see diagram on Fig. 1 - 7).

Values are means of 10 replicates.

(Data of Fig. 1 - 7).

Light treatment	Time (h) from beginning of dark period			
	0	2	4	6
0 - 4 h	116.00 ef	126.00 cd	139.50 ab	139.00 ab
5 - 9 h	112.00 f	131.50 bc	110.50 f	119.50 def
10 - 14 h	110.50 f	138.50 ab	143.00 a	144.00 a
Whole period dark	122.00 cde	127.00 ed	125.00 cde	128.50 c

N.B. When the values share a common letter they do not differ significantly at 5% level (Duncan's M.R.T.).

Table of Analysis of Variance

Source	Sum of squares	Degrees of freedom	Mean squares	Variance ratio (F)
Light treatment	209.22	3	69.74	29.179***
Time	313.07	3	104.35	43.661***
Interaction	255.56	9	28.39	11.878***
Error	345.10	144	2.39	

\*\*\* - Significant at 0.01% level.

for the first or last four hours of the established light period, the movement following light given in the middle of the light period is peculiar in that the initial steep rise over the first two hours of darkness is immediately followed by an equally sharp significant fall during the next two hours, and there is a further slight rise after this. In the case of the continuous dark treatment added to ascertain the influence of a possible "memory" of the established rhythm, there is no significant change in the leaf angle during the whole six hours.

It is clear that the sensitivity to light during the light period is not constant. Four hours of light, which is very effective in producing the nyctinastic response when given in the beginning and at the end of the light period, is not so effective when applied in the middle of the main light period.



E. Effect of short light period following 12:12 photoperiodic cycles.

In the above experiments it was observed that 4 h light in the beginning only of the established light period (14 h light) produced a nyctinastic response during the following established 10 h dark period. The response corresponds closely with that of plants which receive light for the full light period of 14 h, although with smaller amplitude. One possible interpretation of the results could be that plants having been grown on a 14 h light period, the response with the 4 h treatment is more of an after-effect or "memory" of the previous light:dark cycles, than an effect of the light treatment itself. This point has been touched on already in connection with the "continuous dark" treatment included in the previous experiment. It is further investigated in the experiment which follows.

1 - 8. Plants were grown on a 12:12 light:dark cycle (instead of the routine 14:10). As we know from figure 1-2, the nyctinastic response follows a rather different pattern from that with a 14:10 cycle (Fig. 1-1). A 4 h light treatment was given in the beginning of the established light period (12 hours) and measurements were taken in the subsequent darkness.

Figure 1-8 shows that significant leaf movement begins immediately after the end of the established light period in the plants that received the full 12 h light, but movement following 4 h only of light does not begin until two hours later, and is comparable to the movement following a final 4 h light period on the 14:10 cycle. The results suggest that the timing and course of the movement following the 4 h light period is controlled by the underlying endogenous rhythm.

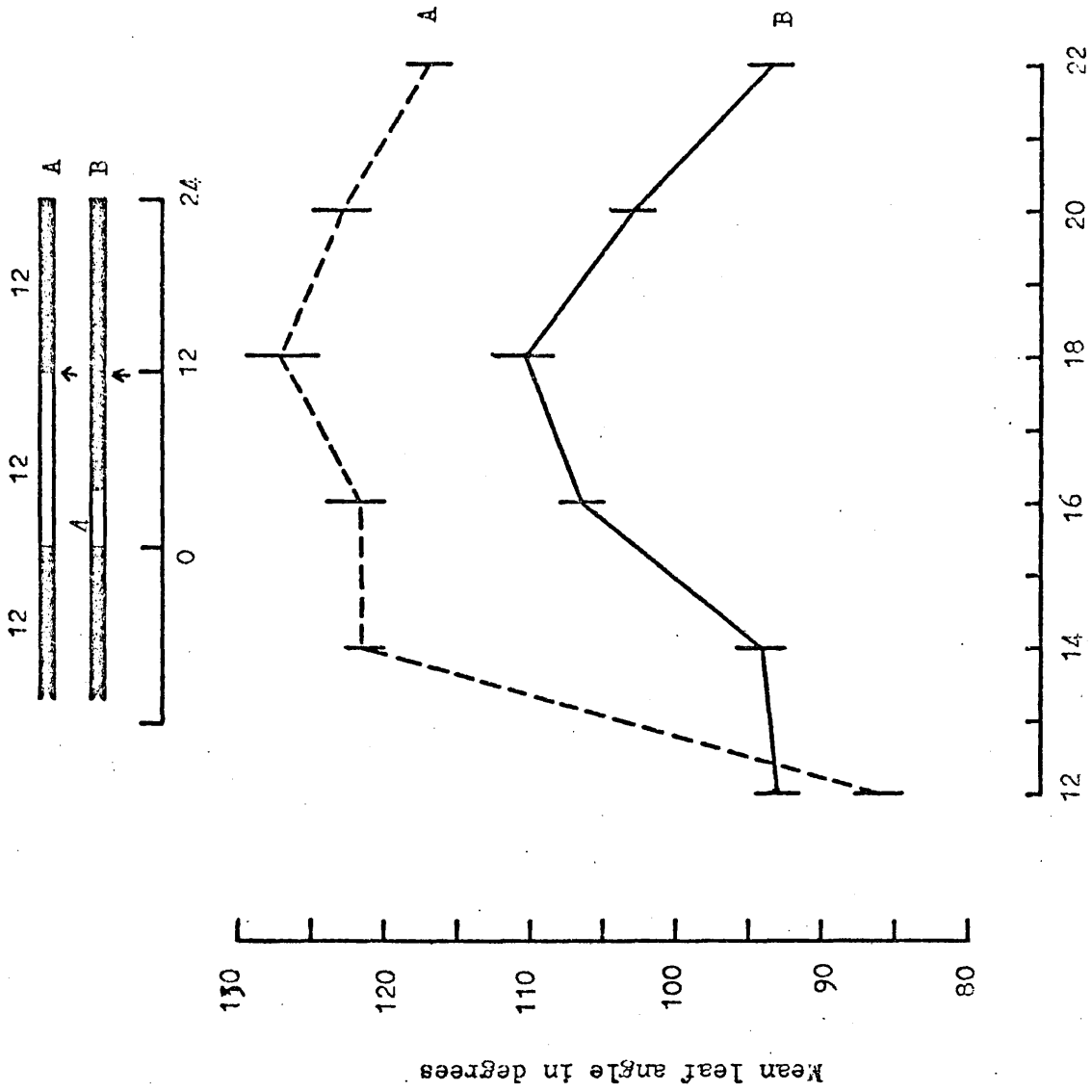
The course of leaf movement on passing from light to darkness is interpreted then as due to the superposition of two components: one, an

Fig. 1 - 8. Course of leaf movement following 4 h light in the beginning of established light period on 12:12 light:dark cycles. Control plants received the full 12 h light.

Values are means of 16 replicates with 95% confidence limits.

The horizontal bars above the graph show the treatments diagrammatically.

↑ time at which measurements commenced.



Time (h) from beginning of light period

Table 1 - 8

Course of leaf movement following 4 h light in the beginning of established light period, on 12:12 light:dark cycles. Control plants received full 12 h light. Measurements were taken during the dark at 2 h intervals.

Values are means of 16 replicates.

(Data of Fig. 1 - 8).

Time (h)	Light treatment	
	12 hours	4 hours
12*	86.25 $\pm$ 2.66	93.10 $\pm$ 2.90
14	121.85 $\pm$ 1.91	94.35 $\pm$ 3.35
16	121.75 $\pm$ 4.00	106.85 $\pm$ 2.15
18	127.80 $\pm$ 4.76	110.60 $\pm$ 3.62
20	123.10 $\pm$ 4.34	103.10 $\pm$ 2.90
22	117.80 $\pm$ 2.92	93.75 $\pm$ 3.37

$\pm$  95% confidence limit

\* Hours from beginning of established light period of 12 hours.

immediate response due to the transition from light to darkness; and two, a rhythmic component, regulated by the endogenous oscillation, which determines the timing of the maximum leaf position or the point of reversal of movement. It is the superposition of these two which results in the changes of curvature of Fig. 1-2 and 1-8. When the photoperiodic cycle is 14:10, the two effects superimpose so closely that they cannot be separated.

Nyctinastic movement following an extended light period.

1 - 9. It remains now to examine the effect of extending the light period into the phase of the rhythm normally characterized by rhythmic nyctinasty. Plants raised on the 14:10 light:dark cycle were given a final light period of 18 h or 20 h and the leaf angle was measured at the end of this extended light period and through the following dark period. The leaf angle was also recorded at the time corresponding to the end of the established 14 h light period. There was only a small change in angle whilst the plants remained in the light (see B, C in Fig. 1-9); they were about  $10^{\circ}$  higher at the end of the 20-h light period than they were at the end of the 14 h light period. When the light treatment was 18 h, the nyctinastic movement raised the leaves almost to the level attained by the leaves of the control which had been in darkness for an additional 4 h, and the reverse movement commenced soon after the maximum was reached, so that the 'recovery' almost duplicated that of the control (Fig. 1-9 A, B). At the 22nd hour the values do not differ significantly for any of the three light treatments (Fig. 1-9, Table 1-9). The movement following the 20 h light period was of lower amplitude and the maximum almost exactly coincided with the position of the control leaves at the 22nd hour, although the recovery after this was significantly slower than that of the control.

The result of this experiment shows that the response to transition from light to darkness is influenced by a changing 'sensitivity' throughout the 24 h cycle. We have already seen in the previous experiments how the immediate responsiveness of the leaves does not develop until about the 8th-9th hour of the light period. We see now that the responsiveness decreases through the "dark" period (established

night) as the movement is postponed due to the extension of the light period.

Fig. 1 - 9. Nyctinastic movement following light periods longer than the established light period.

Light periods are 14h, 18h, 20h. Measurements were taken every 2 h during the dark at the end of each treatment.

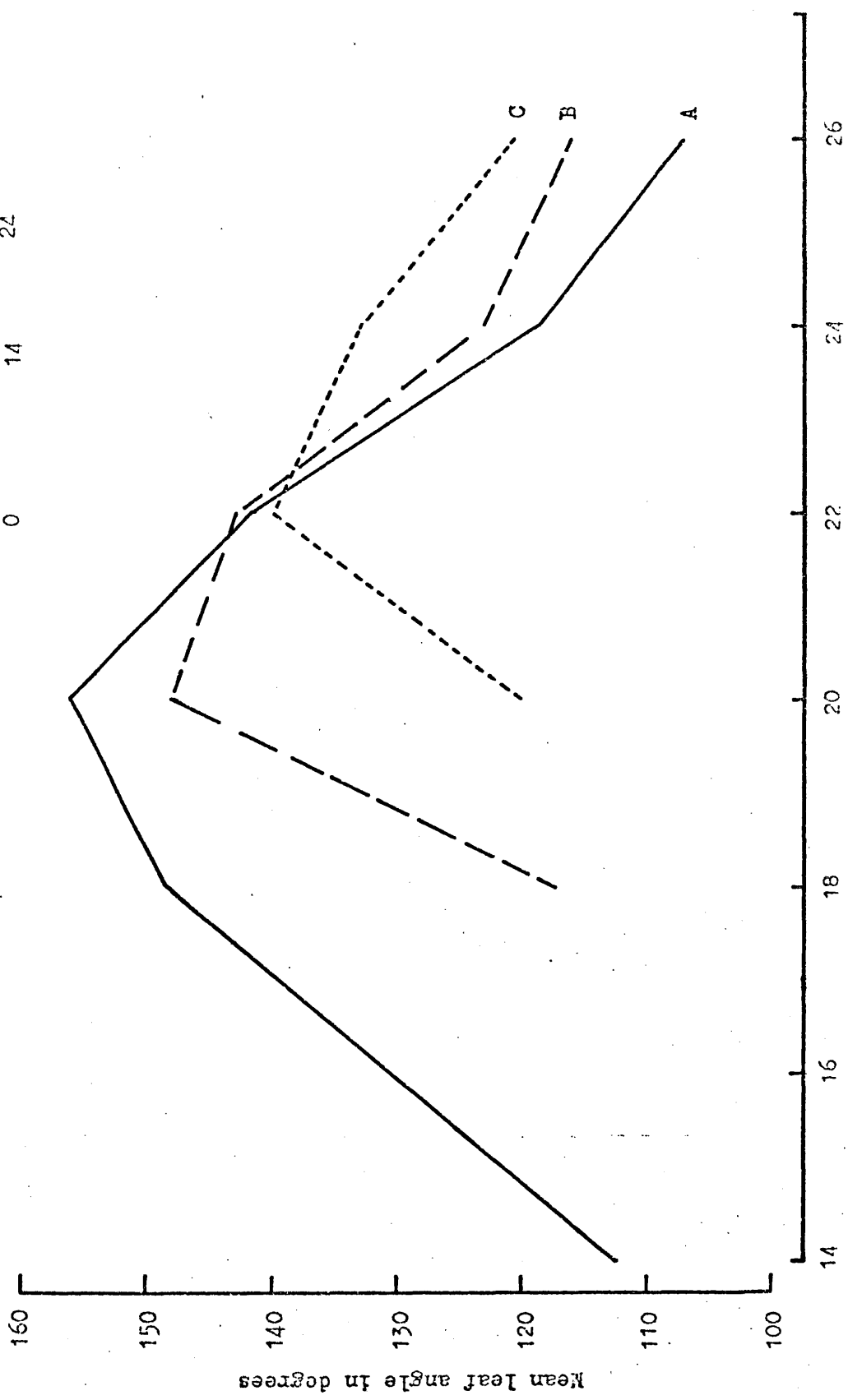
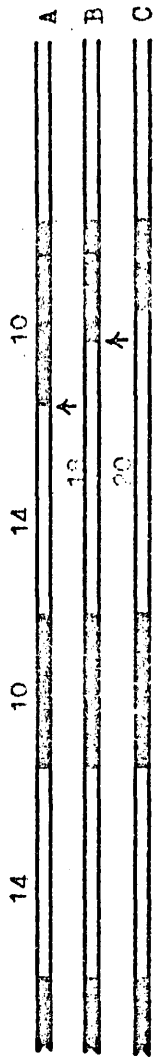
Light treatments are shown diagrammatically above the graph.

Values are means of 14 replicates.

Note: Curve A is comparable to Fig. 1-1 A and Fig. 1-4 D. The whole series is a continuation of the set in Fig. 1-4.

↑ time at which measurements commenced.





Time (h) from beginning of the established light period

Table 1 - 9

Nyctinastic movement following light periods longer than established/0 light period. Light treatment of 10 h or 20 h were given (see diagram on Fig. 1 - 9).

Measurements were taken during the dark at the end of each treatment.

Values are means of 14 replicates.

(Data of Fig. 1 - 9).

Light treatment	Time (h) from beginning of light period						
	14	18	20	22	24	26	28
14 h	113.55 <sup>+</sup>	149.65 a	156.45	142.15 bc	119.30 de	107.30 g	--
18 h	--	117.80 de	148.75 ab	143.75 abc	123.40 d	116.05 ef	--
20 h	--	--	120.35 de	140.35 c	133.75 h	121.95 de	110.35 <sup>*</sup> fg

+ - This figure was not included in further analysis.

N.B. When the values share a common letter, they do not differ significantly at 5% level (Duncan's M.R.T.).

\* value is not shown on the graph.

Table of Analysis of Variance

Source	Sum of squares	Degrees of freedom	Mean squares	Variance ratio (F)
Light treatment	129.53	2	64.76	35.78 <sup>***</sup>
Time	1452.32	4	363.08	200.59 <sup>***</sup>
Interaction	366.11	8	45.76	25.28 <sup>***</sup>
Error	354.54	195	1.81	

\*\*\* - Significant at 0.01% level.

Nyctinastic movement following light given after 24 hours darkness.

From the results of the foregoing experiments it can be seen that no movement occurred unless light is given during the 14 hour following the previous movement (see Fig. 1-7) and that when the light was extended beyond the established day the movement was delayed and reduced in amplitude (Fig. 1-9).

In the last experiment the long light periods commenced at the usual light-on time; and since light periods of 4 h, 10 h, 12 h and 14 h all result in a night maximum position about the 18th hour after this instant (Fig. 1-4) and the present ones are not far different, the rhythmic responsiveness might in fact be determined by this light-on signal. In the experiment now to be described light signals were given following 24 h uninterrupted darkness to see whether the rhythmic response to light persists through the prolonged dark period when no actual movement would have taken place.

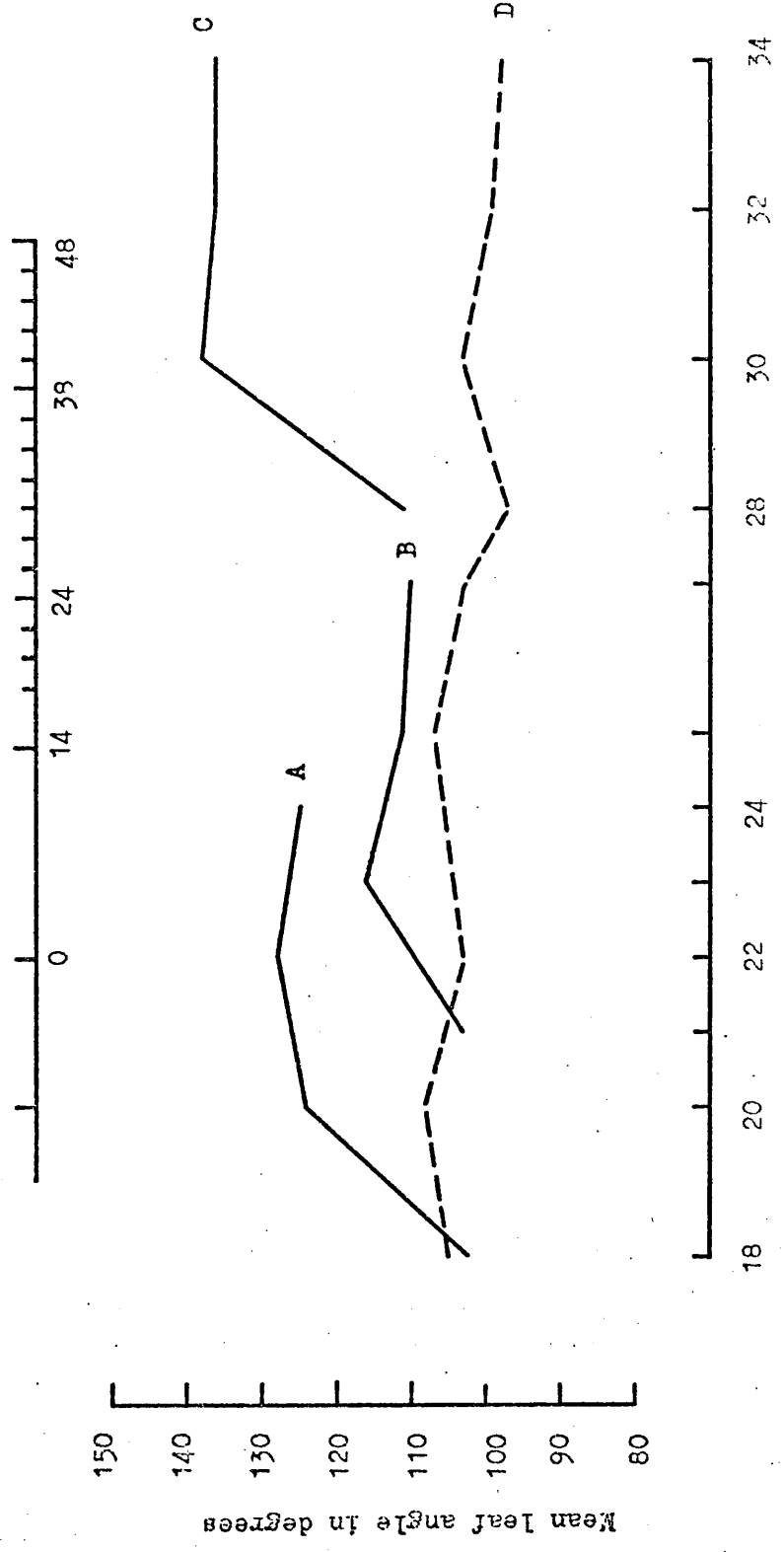
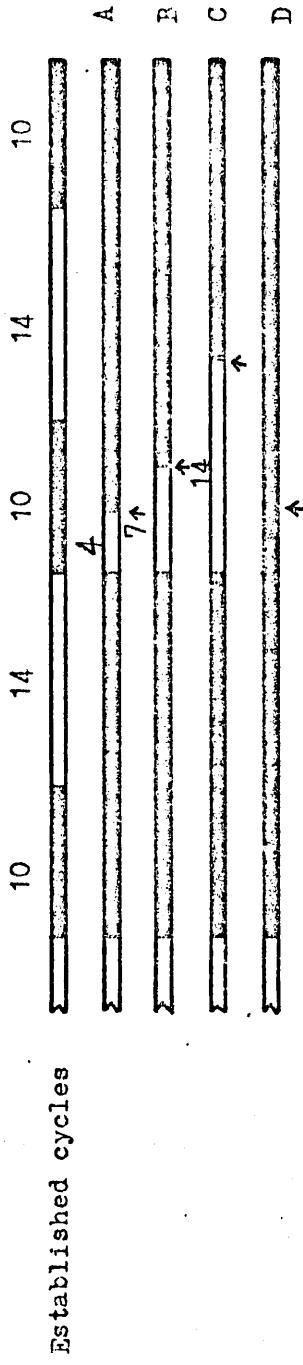
1 - 10. Plants were grown on a 14:10 light:dark regime, but kept in darkness for 24 h before the treatments. All three treatments of 4 h, 7 h and 14 h light began at the same time (i.e., at the beginning of the next established night).

Fig. 1-10 shows that 4 h light produced an immediate significant nyctinastic response in the following dark (Fig. 1-10 A). It seems that failure of expression of leaf movement in continuous dark is due to the lack of a light stimulus, although it is still under the control of the underlying endogenous clock. Four hours given at this particular time delays the phase of the leaf movement (predicted on a 14:10 light:dark regime) by 4 h, the maximum now occurring at 22 h. Following 7 h light the peak is of lower but still significant amplitude and is delayed by a further hour, with the reversal of leaf movement at the 23rd hour.

Fig. 1 - 10. Course of leaf movement following 4 h, 7 h or 14 h light treatment given after an initial 24 h dark. An uninterrupted dark control (curve D) is also given. Measurements were taken every 2 h at the end of each treatment. Values are means of 16 replicates.

Treatments are shown diagrammatically above the graph.

↑ time at which measurements commenced.



Time (h) from beginning of established light period

Table 1 - 10

Course of leaf movement following 4 h, 7 h and 14 h light treatments given after an initial 24 h dark. Measurements were taken every 2 h at the end of each treatment. Values are means of 16 replicates (see diagram on Fig. 1 - 10).

(Data of Fig. 1 - 10).

Light treatment	Time (h) in the dark			
	0	2	4	6
4 h	20.43 d	24.84 b	25.62 b	25.15 b
7 h	20.68 d	23.31 c	22.18 cd	22.03 c
14 h	22.25 cd	27.87 a	27.28 a	27.28 a

N.B. When the values share a common letter they do not differ significantly at the 5% level (Duncan's M.R.T.).

Table of Analysis of Variance

Source	Sum of squares	Degrees of freedom	Mean squares	Variance ratio (F)
Light treatment	542.84	2	271.42	121.71***
Time	565.72	3	188.57	84.56***
Interaction	74.95	6	12.49	5.60**
Error	402.49	180	2.23	

\*\*\* - Significant at 0.01% level.

The response following 14 h light is again of large amplitude and although measurements were not continued beyond the maximum, the reversal of movement may reasonably be expected to have taken place within an hour or two of the last reading. This full movement took place at a time that would correspond to the early hours of the next established day on the original rhythm. No other treatment evoked a movement at such a time and it must be assumed that a completely new rhythm has been established.

Table 1-10 shows that the initial rise within the first two hours of the dark in all the treatments is statistically significant, after which the leaves show no significant rise or fall in the next few hours.

Nyctinastic movement following longer periods of continuous light.

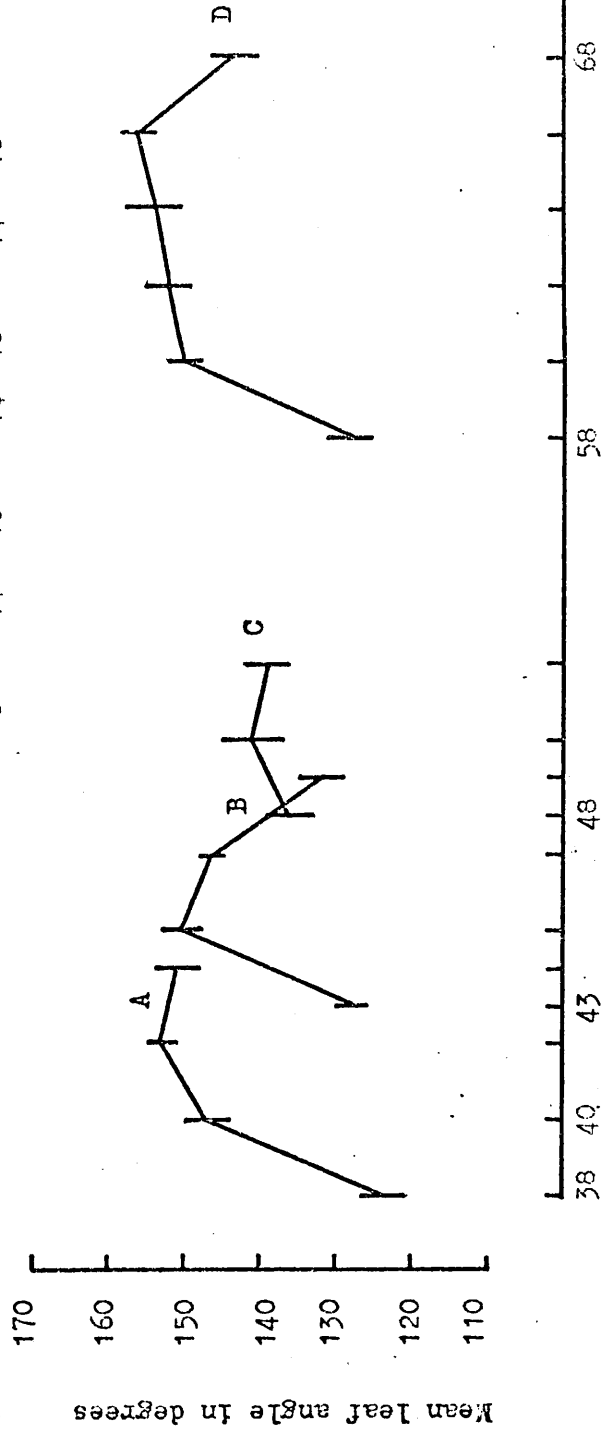
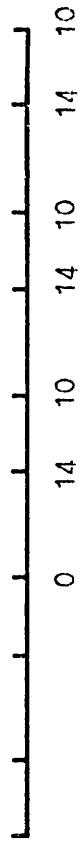
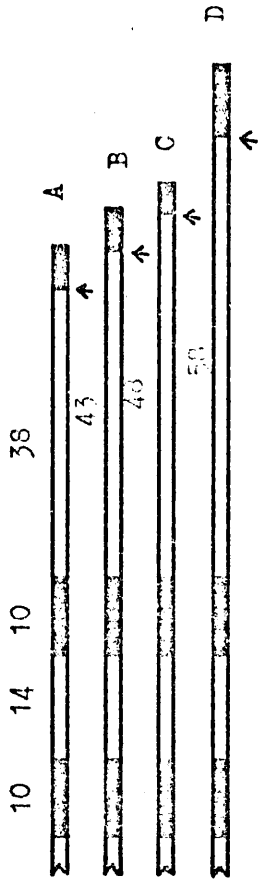
1 - 11. Plants grown on the 14:10 regime were left in light after an established 14 h light period. Some pots were put into the dark 24 h after the end of this period, that is, at a time corresponding to the end of the following light period and after they had received 38 h continuous light. Samples were measured at the time of transfer to dark and at intervals of 2 h thereafter. Further transfers to darkness were made after 43 h light and after 48 h light (this last corresponding to an established light-on instant). Lastly, the remaining plants were transferred to darkness after a total of 58 h continuous light.

The nyctinastic response on darkening at a time corresponding to the beginning of a dark period (an established night) was comparable to the response normally occurring at that time (Fig. 1-11 A; compare Fig. 1-1 A) and also 4 h later; Fig 1-11 B resembles in its course that of Fig. 1-9 B, while no significant response was evoked by transfer to darkness at the time of commencement of an established day (Fig. 1-11 C). The response evoked after 58 h light was typical for darkening at the 10th hour of an established day (Fig. 1-11 D; compare Fig. 1-4 C).

The movements evoked on transfer to darkness are seen to be strongly influenced by an underlying circadian rhythm (Fig. 1-10).



Fig. 1 - 11. Nyctinastic response of continuously illuminated plants.  
After the final dark period plants were transferred to continuous light terminated after 38, 43, 48 or 58 hours.  
Measurements were taken every 2 h at the end of each treatment.  
Values are means of 18 replicates with 95% confidence limits.  
The light treatments are shown diagrammatically above the graph.  
↑ time at which measurements commenced.



Time (h) from beginning of established light period

Table 1 - 11

Nyctinastic response of continuously illuminated plants.

After a final dark period, plants are given 38, 43, 48 or 58 hours light. Measurements are taken every 2 h in the dark at the end of each treatment (see diagram on Fig. 1-11). Values are means of 18 replicates.

(Data of Fig. 1 - 11).

Time (h)	Light treatment			
	38 h	43 h	48 h	58 h
0	123.05 ± 3.21	128.05 ± 2.44	136.40 ± 3.17	128.60 ± 3.06
2	146.95 ± 2.85	151.95 ± 3.36	141.65 ± 3.82	150.30 ± 2.49
4	153.30 ± 2.42	146.65 ± 2.42	138.45 ± 2.69	152.20 ± 3.11
6	151.40 ± 3.06	131.40 ± 2.94	—	153.60 ± 3.61
8	—	—	—	155.55 ± 2.24
10	—	—	—	143.05 ± 3.20

± 95% confidence limit.

Comments.

The main changes of the leaf movement cycle occur during the dark period. A rising phase commences as soon as the light period is terminated and culminates at the night maximum position, at which point the movement is reversed and the lowering phase follows. With light periods of 10, 12 and 14 hours, the point of reversal of movement or the night maximum position is in each case about 18 h after the beginning of the light period. This corresponds to the situation in Phaseolus, in which the night maximum position on all 24 h cycles occurs about 18 h after the light is switched on (Bünning 1944, Flügel, 1949).

Transition from light to darkness does not result in an immediate nyctinastic response unless the light period is at least 8 or 9 h long. The behaviour in Linum differs here from the nyctinastic response of plants with pulvinate leaves, eg., Albizzia, Mimosa and Samanea, where movement may be evoked at any time following illumination although the response does vary with the subjective time of the day (Jaffe and Galston 1967; Fondeville, Borthwick and Hendricks 1966; Palmer and Asprey 1958). On the other hand, as little as 4 h of light given over the final four hours of the established light period will produce full movement (Fig. 1-6 A, Fig. 1-7 C), and 4 h given at the beginning of the light period will do the same (Fig. 1-7 A). In both these cases movement takes place with the night maximum position 18 h from the commencement of the established light period, i.e., at the time normal to the established regime.

When seven hours of light were given in the beginning of the established light period, the response was much less than that after 4 h (Fig. 1-4 B). Four hours in the middle of the established 14 h light period produced a movement (Fig. 1-7 B) different from that

following 4 h at either the beginning or the end. There is then a suggestion that light given in the middle of the established light period is not only less effective in producing nyctinasty, but actually inhibits the response when it is extended beyond the first 4 h and terminates about the middle of the light period.

Since 4 h light is sufficient to potentiate a movement, then clearly the fact that no immediate nyctinastic movement occurs before the 8th hour of light period (see Fig. 1-5) cannot simply be due to minimum requirement for a light period of this length. Also, even after an extended light period movement varies according to the subjective time the light is terminated. Following longer periods of uninterrupted light, there was a clear indication of a changing responsiveness to the light/dark transition. There is no significant movement when this transition is made at the beginning of the established day even after exposure to light for 48 h; yet when made before that, and also 10 h later, a response can be elicited (Fig. 1-11). The shape of the curve following 58 h light (Fig. 1-11 D) and 10 h light (Fig. 1-4 C) are remarkably alike. The inference is that since both these treatments end at the tenth hour of the established day, they are both under the influence of an endogenous rhythm which has maintained circadian time despite the lack of light:dark cycles.

An established regime of regular light and dark cycles is important in producing a nyctinastic response, but the results show that the response cannot be explained as due to this alone. Changing responsiveness to the light/dark transition throughout the 24 h period strongly suggests that an endogenous rhythm is involved which influences leaf movement. This will be further examined in Section 2.

SECTION 2

ENDOGENOUS RHYTHM AND NYCTINASTY

Endogenous rhythm and nyctinasty.

Introduction.

It is current practice to define a biological rhythm as endogenous, i.e., controlled by an internal oscillating system, if it complies with certain criteria first proposed by Pittendrigh (1954), and recently stated in more general terms by Wilkins (1969). These have been discussed in relation to leaf movements in the general introduction to this thesis.

For technical reasons, or due to the nature of the specific rhythmic system under observation, it may not be possible to apply all the criteria. Very few of the many circadian phenomena listed by Cumming and Wagner (1968) have in fact been so tested. Since light is required to produce leaf movement in Linum, it is not possible to study the free-running rhythm of movement in uninterrupted darkness because the movement ceases altogether. This limits the sort of experiments routinely done to bring about vigorous phase shifts with single light signals during constant dark.

The results reported in the previous section, while not providing conclusive proof, nevertheless strongly indicated the influence of an endogenous rhythm in regulating the nyctinastic leaf movement. The following experiments are more directly concerned with such a rhythm.

The leaf movement is observed under constant light and temperature for three cycles. A shift in the phase of rhythmic nyctinasty is demonstrated.

The rhythm of movement is entrained to a non-diurnal 20 h light: dark cycle, which aids in the interpretation of the phase responses of this rhythm.

## Results.

### Leaf movement under constant light.

It has been demonstrated that plants left in continuous dark beyond the established night on the 14:10 light:dark regime exhibit no further significant leaf movement (Fig. 1-7 D) and that light is essential to potentiate the response. In the experiment described below therefore, the leaf movement is followed in continuous light.

2 - 1. Plants grown on a 14:10 light:dark cycle were transferred to continuous light conditions on the 12th day which is the age when the measurement of the leaf angle becomes useful. When illumination was continued beyond the 14 h light period, an upward movement of the leaf commenced at the 16th hour. The leaf movement continued over a period of 6 h to reach a maximum position at the 22nd hour (Fig. 2-1 A; Table 2-1). Thus the maximum was delayed by 3 to 4 h compared to the normal response in darkness (see Fig. 1-1 A). Leaf movement continued for one further cycle after which it ceased. During the course of the second cycle in constant light, the movement closely followed the normal curve (the curve obtained in the 14:10 light:dark regime), but the period length between the two maxima was only 20 h. The delay noted above was therefore transitory and the last maximum occurring at the established time.

The cessation of the leaf movement cannot be ascribed solely to the damping effect of continuous light, since, as described in the previous section, the movement diminishes even in the light:dark cycles as the leaves mature.

The results clearly demonstrate that a rhythmic movement persists in the absence of a light/dark transition which normally evokes it. Little can be said about the free-running rhythm in light on the basis of only two complete cycles, but since the second maximum occurs in



Fig. 2 - 1, Course of leaf movement in continuous light for three successive days.

Measurements were taken during the "subjective" dark periods.

Each value is the mean of 16 replicates with 95% confidence limits represented by vertical bars, The horizontal bar indicates the treatment.

↑ time at which measurements commenced,

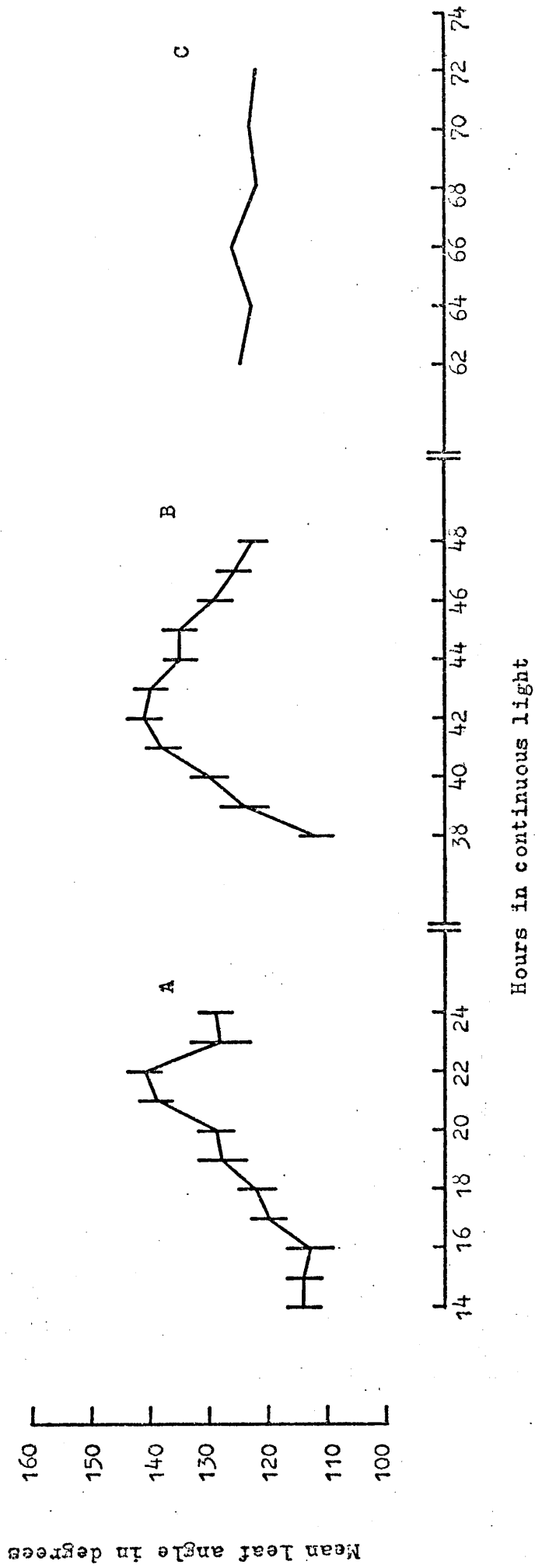


Table 2 - 1

Course of leaf movement in continuous light for three successive days. Measurements were taken during the subjective dark periods (see diagram on Fig. 2 - 1).

Each value is the mean of 16 replicates.

(Data of Fig. 2 - 1).

Time (h)	Mean leaf angle	Time (h)	Mean leaf angle
12th day			
14*	114.05 ± 3.249	20	129.70 ± 3.145
15	114.70 ± 3.689	21	139.70 ± 2.991
16	113.15 ± 4.547	22	141.55 ± 3.332
17	120.65 ± 3.486	23	128.45 ± 5.729
18	122.20 ± 3.886	24	129.70 ± 2.828
19	128.15 ± 4.547		
13th day			
14*	112.80 ± 2.911	20	135.65 ± 3.350
15	124.05 ± 4.373	21	135.00 ± 3.891
16	130.95 ± 3.793	22	129.35 ± 3.750
17	138.45 ± 3.468	23	125.95 ± 3.257
18	141.90 ± 2.919	24	123.55 ± 1.750
19	140.00 ± 2.908		
14th day			
14*	125.00	20	122.20
16	123.85	22	122.80
18	126.25	24	122.20

± 95% confidence limit

\* Start of subjective dark period after the light period of 14 h.

phase with the rhythm previously established on the  $14:\overline{10}$  cycle one may tentatively conclude that there is no permanent shift of phase despite the delay in the first cycle under continuous light.

It was shown previously that the nyctinastic system is potentiated by the 8th hour following a 'dawn' (light-on) signal so that a response will occur if the plants are then transferred to darkness (Fig. 1-5 A). From this time onwards light may be regarded as 'holding down' the leaves since they will begin to rise as soon as light is withdrawn. However the results just described suggest that light is unable to prevent a movement beyond the 16th hour, when rhythmic control overrides its direct action on the system.

Shift in the night maximum.

It has been shown in Section 1 that the timing of the night maximum leaf position remains constant (18th hour of the 24 h cycle) irrespective of the length of the light period up to 14 h and that a minimum of 4 h of light is sufficient to produce the rhythmic nyctinastic response.

From the published phase response curves in other organisms (Pittendrigh 1965; Halaban 1968) it would be expected that a light signal given during the established night, if sufficient to produce rhythmic nyctinasty would also shift the phase of the rhythm. The following experiment was designed with this possibility in mind.

2 - 2. A period of 4 h light was given over the final 4 h of the established night on the 14:10 regime, after which the plants were transferred to continuous dark. Measurements were taken every hour commencing 12 h from beginning of light treatment. Fig. 2 - 2 shows that the response occurred with a night maximum position 18 h after the beginning of the light treatment, which is 4 h advanced compared with the established rhythm or to the phase of the rhythmic movement produced by 4 h light given from the beginning of the established day. This is a characteristic response of a circadian rhythm to light given in the late subjective night. Only the first cycle is, of course, shown in this experiment and it is not a demonstration of a steady state phase shift (see previous experiment). However, the reality of this as a true phase response of the circadian rhythm seems very likely.

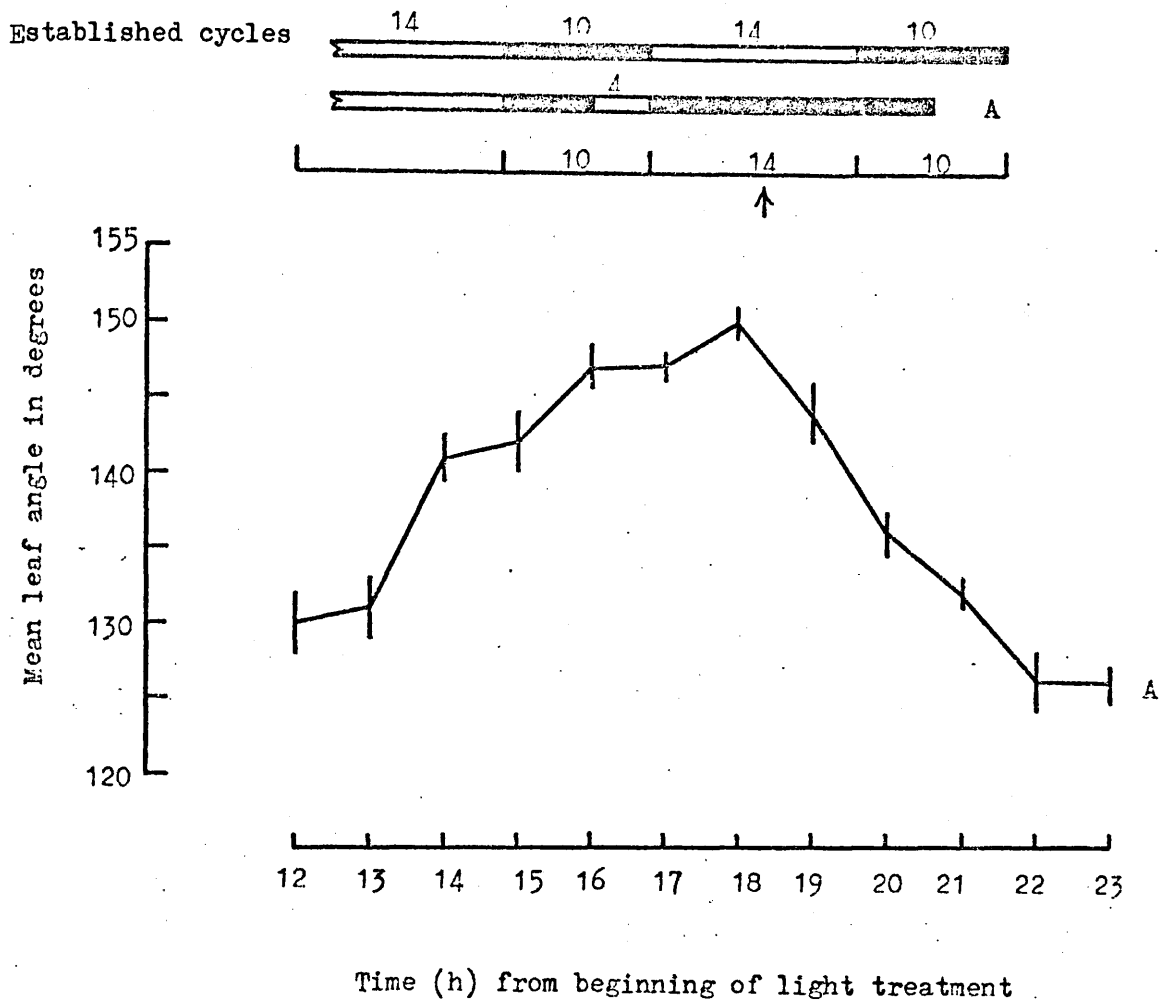


Fig. 2 - 2. Course of leaf movement following 4 h light treatment given during the late subjective dark period. Measurements commenced 12 hours from beginning of the light treatment. Each value is the mean of 20 replicates with 95% confidence limits.

↑ time at which measurements commenced.

Table 2 - 2

Course of leaf movement following 4 h light treatment given during the late established dark period. Measurements commenced 12 h from beginning of light treatment (see diagram on Fig. 2 - 2).

Each value is the mean of 16 replicates.

(Data of Fig. 2 - 2).

Time (h)	Mean leaf angle	Time (h)	Mean leaf angle
12*	130.95 ± 4.48	18	150.00 ± 2.57
13	131.55 ± 3.98	19	144.05 ± 4.58
14	141.55 ± 3.60	20	136.90 ± 3.49
15	142.50 ± 4.13	21	132.80 ± 2.17
16	147.20 ± 2.91	22	126.25 ± 1.69
17	147.20 ± 2.57	23	126.90 ± 2.55

± Confidence limit at 95% level.

\* - hours from beginning of light treatment.

Entrainment to non-diurnal (20 h) cycle.

The occurrence of the night maximum position at a constant time after the established "dawn" in the 24 h light:dark regime suggests that the phase of the circadian rhythm may be set by the light-on signal. It was seen, however, in other results that when the light period commenced later in the day this relationship did not hold and again the night maximum position occurred at the time expected in the established regime. It remains therefore to test the relationship between "dawn" and night maximum position in a regime other than the 24 h cycle of alternating light and darkness. Entrainment of a circadian rhythm to light:dark cycles of less than 24 h is usually possible and a cycle of 20 h was chosen for the experiment since the 10 h light period had already been shown to produce a characteristic pattern of movement (Fig. 1 - 4 C).

2 - 3. Plants 11 days old grown on a 14: $\overline{10}$  light:dark cycle were transferred to 10: $\overline{10}$  light:dark regime, the first light period starting at the established time. On this new 20 h cycle, the third subjective night comes symmetrically in the middle of a subjective day of the original 24 h cycle.

14: $\overline{10}$



10: $\overline{10}$





The nyctinastic leaf movement was recorded during the second and the third subjective night. Response was typical of a 10 h light period. The time interval between the two maxima was 20 h, thus movement had become entrained to the new light:dark cycle. It is seen that the maxima occur 18 h after the commencement of the light period, as in all previously described examples of 24 h cycles. Although the difference between the leaf position at the 16th h and the 18th h is not significant (Table 2 - 3), there is a clear reversal of movement from the 18th h in both cycles.

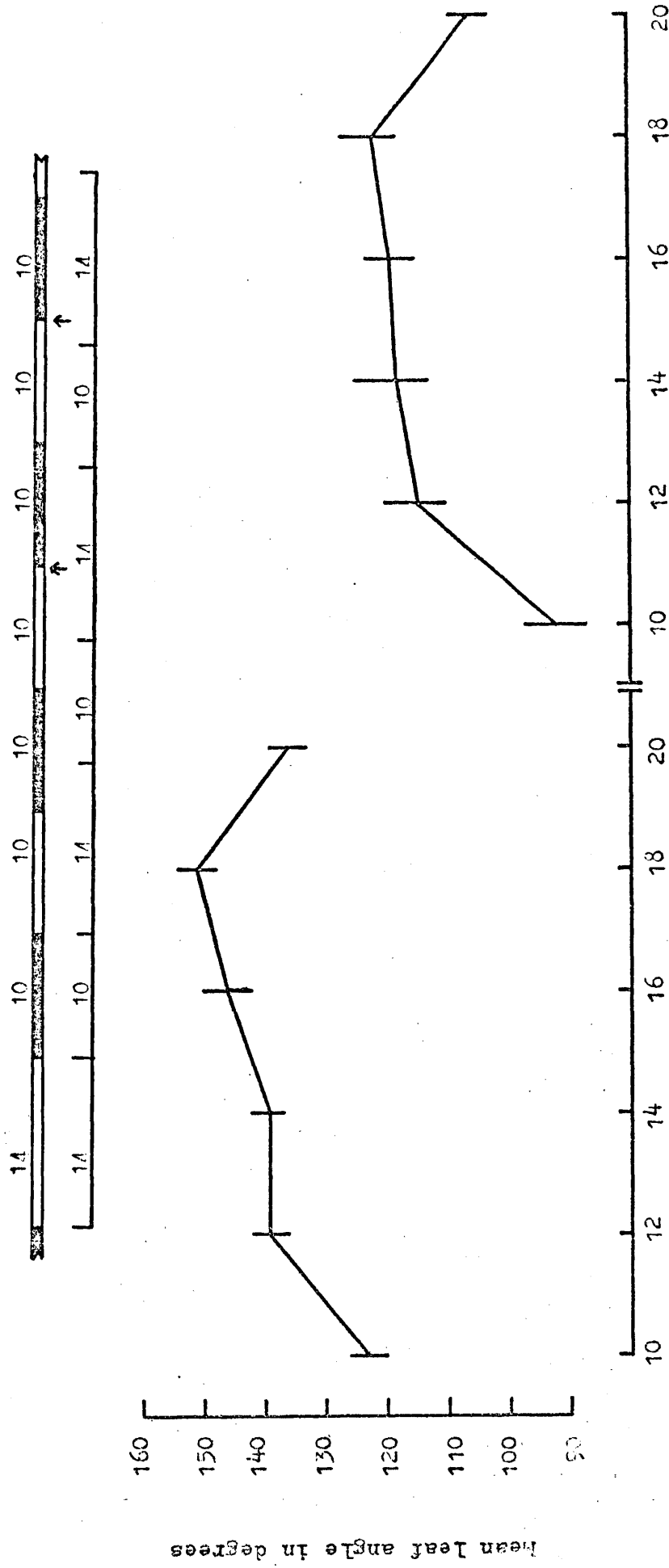
Fig. 2 - 3. Course of leaf movement through two successive dark periods on 10:10

light:dark cycles for three days.

Each value is the mean of 20 replicates with 95% confidence limits

represented by vertical bars. The light:dark pattern in shown diagrammatically above the graph.

↑ time at which measurements commenced.



Time (h) from beginning of established light period

Table 2 - 3

Course of leaf movement through two successive dark periods on 10:10 light:dark cycles for three days (see diagram on Fig. 2 - 3).

Each value is the mean of 20 replicates.

(Data of Fig. 2 - 3).

Time (h)	Mean leaf angle	Time (h)	Mean leaf angle
12th day			
10*	112.75 ± 3.43	16	135.50 ± 4.22
12	129.00 ± 3.36	18	140.75 ± 2.87
14	129.00 ± 2.22	20	126.50 ± 3.04
13th day			
10*	82.00 ± 4.78	16	108.75 ± 3.86
12	105.25 ± 5.39	18	112.00 ± 4.64
14	107.75 ± 4.58	20	96.00 ± 3.45

± Confidence limit at 95% level.

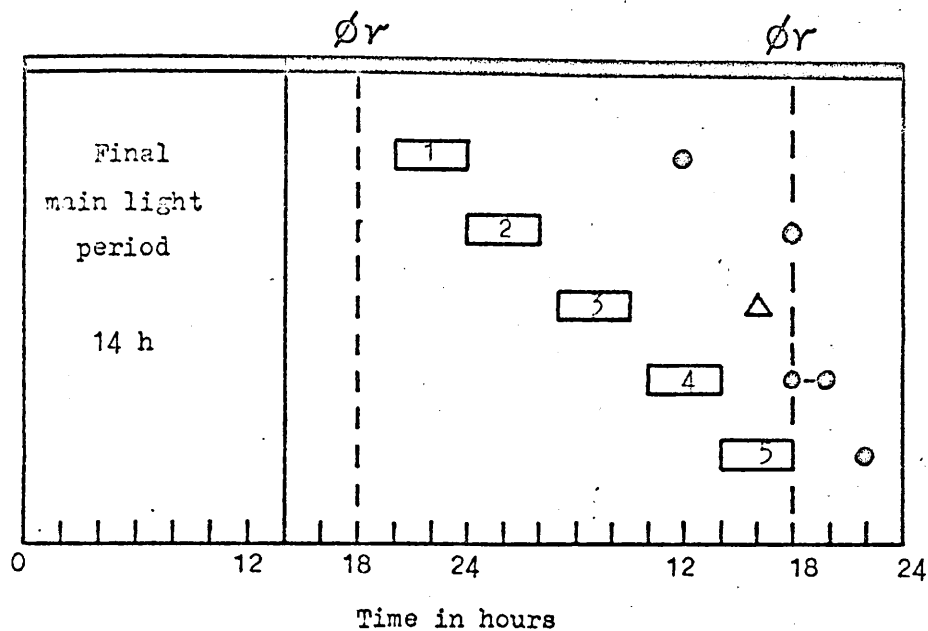
\* - Hours from beginning of light period.

Section 2Comments.

The experiments described in this Section provide additional evidence that the leaf movement is regulated by an endogenous clock. Although movement ceases when plants are exposed to abnormally long periods of darkness, it continues for at least two established cycles in continuous light with a circadian periodicity (Fig. 2 - 1). The movement hardly lasts long enough to provide an adequate characterisation of the "free-running" rhythm. The diminishing movement in continuous light cannot be ascribed to an actual running down of the endogenous clock due to the conditions imposed, for as discussed in the previous Section, the movement of the leaves used in this investigation decreases in amplitude and then ceases as the leaves mature even in unchanged and favourable light:dark cycles. It is quite possible therefore, that although the movement of these leaves soon stops under constant illumination this is to be attributed to the process of maturation and loss of susceptibility, and that the endogenous clock continues to function and controls other processes including the movement of younger leaves.

The results of the experiments described in this and the previous Section in which rhythmic nyctinastic movement was induced by a 4 h light break given during the first cycle of continuous darkness are summarised below: (see diagram on next page)

- (1) A 4 h light break at the end of the established dark period (subjective night) advances the following maximum by 4 h (Fig. 2 - 2).
- (2) A 4 h light break at the beginning of the established light period (subjective day) induces movement with the maximum at the predicted



$\emptyset r$  - phase reference point, i.e., time of night maximum leaf position on established 24 h light:dark regime.

○ - maximum leaf position of full rhythmic nyctinastic movement.

△ - peak of small abnormal leaf movement.

time, i.e., one complete cycle (24 h) after the previous phase reference point (Fig. 1 - 7).

- (3) A 4 h light break in the middle of the established day does not induce a full rhythmic movement and resulted in a small rise at the 16th h. The movement was soon reversed.
- (4) A 4 h light break at the end of the established day induces movement with the maximum at the predicted time or delayed by up to 2 h (Fig. 1 - 7).
- (5) A 4 h light break at the beginning of the subjective night induces movement with a maximum 4 h later than the predicted time (Fig. 1 - 10).

It will be seen that in (1) and (2) the maximum occurs 18 h after the beginning of the light treatment. As mentioned earlier, this suggests that the phase of the rhythm is set by the light-on signal since this constant relationship between "dawn" and the night maximum also exists for light periods of 10 h, 12 h and 14 h in the 24 h light:dark cycles. It is again apparent in the entrainment of the rhythm to the non-diurnal (20 h) 10:10 cycle (Fig. 2 - 3). Here it is as if "dawn" repeatedly resets the phase for each cycle since in order to maintain the constant phase relationship between "dawn" and nyctinastic movement a 4 h advance of phase is necessary in each cycle. When the short light break is given at the end of the subjective day (4) or in the early subjective night (5) a full normal movement occurs as soon as the light is switched off, the maximum position being attained within 4-6 h and this is also the case when longer light treatments are terminated at these times (Figs. 1-1, 1 - 6, 1 - 9, 1 - 10). There does not appear to be a constant phase-relation between the light-on signal and the leaf movement in this instance.

When light is given in the early subjective night (5), however, the phase of the movement is delayed. It is pertinent to compare the delayed movement achieved by prolongation of a light period which provided a "dawn" at the normal time (Fig. 1 - 9) and the movement induced by a shorter light period given over the early hours of the subjective night (Fig. 1 - 10) as in both cases light terminates at the same time of the subjective night. In the former instance (Fig. 1-9) the movement appeared to be regulated so as to coincide almost exactly with the cycle of movement associated with light periods of normal lengths. Movement was prevented by the extra hours of light, but when light was terminated a normal movement did not take place, only a smaller movement occurred confined within the "allotted time" on the

established rhythm. The actual rhythm that regulates movement was apparently not affected by this single extra-long light period and an immediate phase-delay had not taken place.

The second case is rather different. After a 24 h dark period a 4 h light break is followed immediately by a full normal nyctinastic movement ( (5) above and Fig. 1 - 10). This result appears to represent a real phase-delay of 4 h. Extending this light break to 7 h, however, only achieved a 1 h further delay and the movement was less, perhaps once again indicating a limitation set by the allotted time during which movement may take place. Although it may seem that a much greater delay was achieved in the experiment quoted (Fig. 1 - 10) when 14 h light was given, this is possibly better regarded as an advance relative to the following cycle. The maximum delay achievable in this first cycle then would be not more than 4 - 5 h. No further information was obtained to indicate period length of subsequent cycles.

Finally, the question of the damping of the endogenous rhythm in prolonged darkness must be considered. It has been shown that leaf movement ceases when darkness is prolonged through the established day, and that a period of light is necessary to induce leaf movement. Despite the observation that there is no rhythmic movement in prolonged darkness there is no evidence to suggest that the endogenous clock has itself ceased to function. It must surely be presumed to be still functioning to explain the foregoing facts. If the clock had ceased to function then the effect of a 4 h light break would be the same whenever in the 24 h cycle it was given. This is clearly not the case and the results suggest that the light is acting on different phases of an existing rhythm. In fact the results would have been predictable from the known phase responses of other circadian systems (Pittendrigh 1965; Halaban 1968; Moser 1962; Wilkins 1968).



SECTION 3

LEAF MOVEMENT AND THE LIGHT SPECTRUM

LEAF MOVEMENT AND THE LIGHT SPECTRUM.Introduction.

The results described so far have demonstrated that rhythmic leaf movements and the nyctinastic response both require light for induction but are both regulated by the endogenous clock. In addition to the inductive effect of light, it has been shown that short light breaks given at the appropriate phase in the circadian cycle will advance or delay the rhythmic nyctinastic movement, a phase-response similar to that found in other light-sensitive circadian systems. Further study of the light action is needed, in particular to determine which region of the spectrum is most effective for the induction of nyctinastic movement and in the phase responses.

As noted in the General Introduction, the phytochrome system exerts some control over leaf closure in several species of Leguminosae which exhibit a circadian rhythm of leaf movement. At the close of a period of polychromatic (white) light, phytochrome exists predominantly in the far-red light absorbing state-  $P_{fr}$ . Exposure to far-red light immediately after a main light period results in rapid transformation of  $P_{fr}$  to  $P_r$ , the non-active red light absorbing state (Borthwick et al., 1952 b; Borthwick 1964). Effects of irradiation with far-red light prior to transferring plants to darkness are therefore generally attributable to the low ratio of  $P_{fr}:P_r$  at the time of transfer.

The effect on the leaf movements of Linum of far-red radiation at the close of the main white light period has been studied and tested for reversibility by subsequent exposure to red radiation. The results are described in the following experiments, 3 - 1 to 3 - 6.

The red, blue and far-red regions of the spectrum have been tested for effectiveness in inducing rhythmic nyctinasty and shifting the phase (experiments 3 - 7 to 3 - 14).

Results.Investigation of involvement of phytochrome.Effect on nyctinastic movement of prior exposure to far-red radiation.

3 - 1. Plants were raised on 14:10 light:dark cycle for 12 days. At the close of the final 14 h light period, 15 min far-red radiation was given to some of the plants at the beginning of the dark period. The rest of the plants constituted a control set without the far-red treatment. Measurements were taken every 2 h through 12 h of darkness. The results shown in Fig. 3 - 1 demonstrated that the initial movement in the dark is not altered by the prior far-red treatment. There is no significant difference between the maximum position in the treatment and control. There is, however, a more rapid reversal of the movement following far-red treatment. The point of reversal of the movement is sharply defined at the 18th hour.

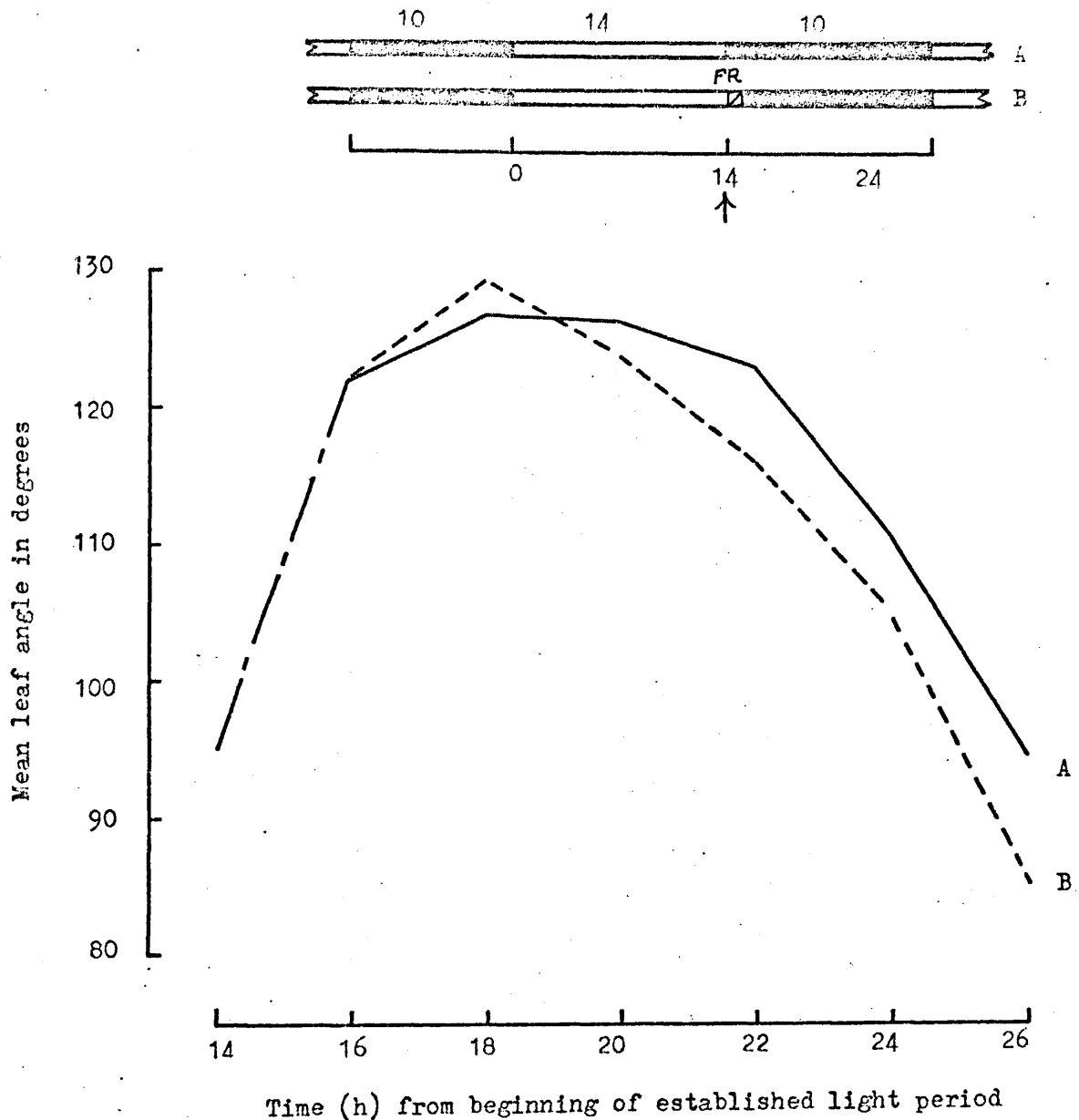


Fig. 3 - 1. Effect of 15 min far-red radiation on the nyctinastic leaf movement after 14 h light period. The control set of plants received 14 h white light only. Measurements were taken during the dark at 2 hourly intervals 14 h from the beginning of established light period. Each value is the mean of 16 replicates. The horizontal bars above the graph show the treatments diagrammatically.

↑ time at which measurements commenced.

Table 3 - 1

Effect of 15 min far-red light on the nyctinastic leaf movement after 14 h light period. The control set of plants received 14 h white light only. Measurements were taken during the dark at 2 hourly intervals, 14 h from beginning of established light period.

Each value is the mean of 16 replicates.

(Data of Fig. 3 - 1)..

Light treatments	Time (h) from beginning of established light period						
	14	16	18	20	22	24	26
Control	94.60+	122.65 c	128.10 ab	127.15 abc	123.75 bc	110.75 e	94.80 g
Far-red	—	122.95 c	129.80 a	127.65 ab	116.70 d	105.00 f	85.75 h

+ was not included in further analysis.

N.B. When values share a common letter they do not differ significantly at 5% level (Duncan's M.R.T.).

Table of Analysis of Variance

Source	Sum of squares	Degrees of freedom	Mean squares	Variance ratio (F)
Light treatment	40.04	1	40.04	19.92***
Time	2786.67	5	557.33	285.22***
Interaction	67.65	5	13.53	6.73**
Error	750.63	372	2.01	

\*\*\* Significant at 0.01% level

\*\* " " 0.1 % "

Far-red effect following 10 h light period.

Following a 10 h light period, the immediate rise is separated by a longer interval from the reversal of movement since the maximum position is apparently determined by the endogenous rhythm (see Fig. 2 - 3). The far-red effect was therefore investigated for this case too.

3 - 2. Plants were raised on a 14:10 light:dark cycle for 12 days. The final light period was reduced to 10 h. At the end of 10 h light, 15 min of far-red radiation was given to one set of plants before transferring them to complete darkness, the control plants being transferred directly to darkness. Measurements were taken every 2 h starting at the end of the light period.

The graph (Fig. 3 - 2) shows that the result is a little different from the previous case. The 15 min far-red after only 10 h white light does slow up the initial rising nyctinastic movement; it also led to a more precise night maximum position since the reverse movement, which occurs after 8 h in the dark, was faster when far-red light was given.

It appears, therefore, from this and the previous experiments, that the presence of  $P_{fr}$  at the time of transition from light to darkness leads to retardation of the reverse downward movement of the leaves after the maximum position has been reached. The upward movement of the leaves following a 14 h light period is not affected by the state of phytochrome, but following a 10 h light period the upward movement is more rapid when  $P_{fr}$  is present.

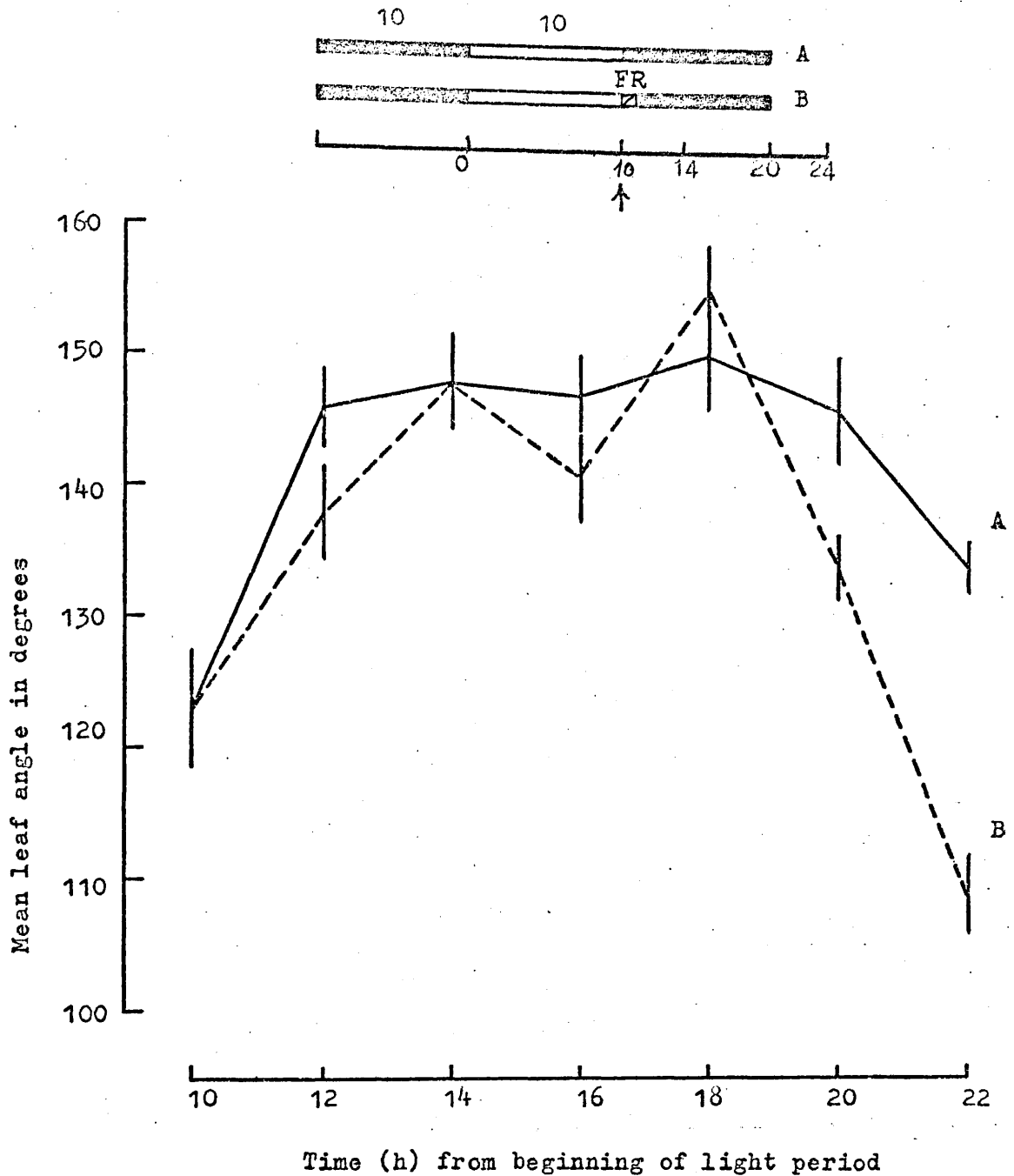


Fig. 3 - 2. Effect of 15 min far-red radiation on the nyctinastic leaf movement after 10 h light period. The control set of plants received 10 h white light only. Measurements were taken during the dark at 2 hourly intervals 10 h from beginning of established light period. Each value is the mean of 20 replicates. The diagram above the graph shows the treatments diagrammatically. ( Vertical lines on the curves indicate 95% confidence limits.)  
 ↑ Time at which measurements commenced.



Table 3 - 2

Effect of far-red radiation on the nyctinastic leaf movement after 10 h light period. The control set of plants received 10 h white light only. Measurements were taken during the dark at 2 hourly intervals 10 h from the beginning of established light period.

Each value is the mean of 20 replicates with 95% confidence limits.

(Data of Fig. 3 - 2).

Time (h) from beginning of established light period	Light Treatments	
	Control	Far-red
10	113.93 $\pm$ 4.79	—
12	135.85 $\pm$ 2.98	128.05 $\pm$ 3.73
14	137.50 $\pm$ 3.64	137.50 $\pm$ 2.40
16	137.20 $\pm$ 3.21	131.40 $\pm$ 3.49
18	139.70 $\pm$ 4.04	144.70 $\pm$ 3.57
20	135.55 $\pm$ 3.80	124.80 $\pm$ 2.53
22	124.40 $\pm$ 2.24	98.60 $\pm$ 2.92

$\pm$  95% confidence limits.

Reversibility of the far-red effect (A).

3 - 3. With experimental procedure similar to that in the above experiment 3 - 1, reversal of the far-red effect was attempted by giving to an additional set of plants 15 min of red radiation immediately after the far-red treatment. Only the reverse downward movement was followed from the 6th to the 12th hour in darkness.

As shown in Fig. 3 - 3a a true reversal of the far-red effect was not achieved by subsequent exposure to red radiation. The slopes of the regressions (Fig. 3 - 3b) are identical, indicating that the rate of the downwards movement was the same with both treatments. However, the red radiation led to a vertical displacement of this slope: the reason for this is not apparent from the present results but is made clear in the following experiment.

Reversibility of the far-red effect (B).

3 - 4. After the final 14 h light period some plants were given 15 min far-red and others were given 15 min far-red followed immediately by 15 min red radiation, before transferring them to darkness. To a further set of plants an interval of 1 h of darkness was interposed between the far-red and red treatments.

It is seen in Fig. 3 - 4a, and 3 - 4b that a complete reversal of the effects of far-red radiation was not achieved with red light and consequently the introduction of a 1 h dark interval did not greatly alter the effect of the red light treatment although it resulted in a more rapid fall between the 6th and 8th hour. However, in both cases where red light followed far-red, the rapid reversal of movement characteristic of far-red treated plants appeared to have been normalized. This would account for the vertical displacement of the falling part of the curve seen in the result of experiment 3 - 3.

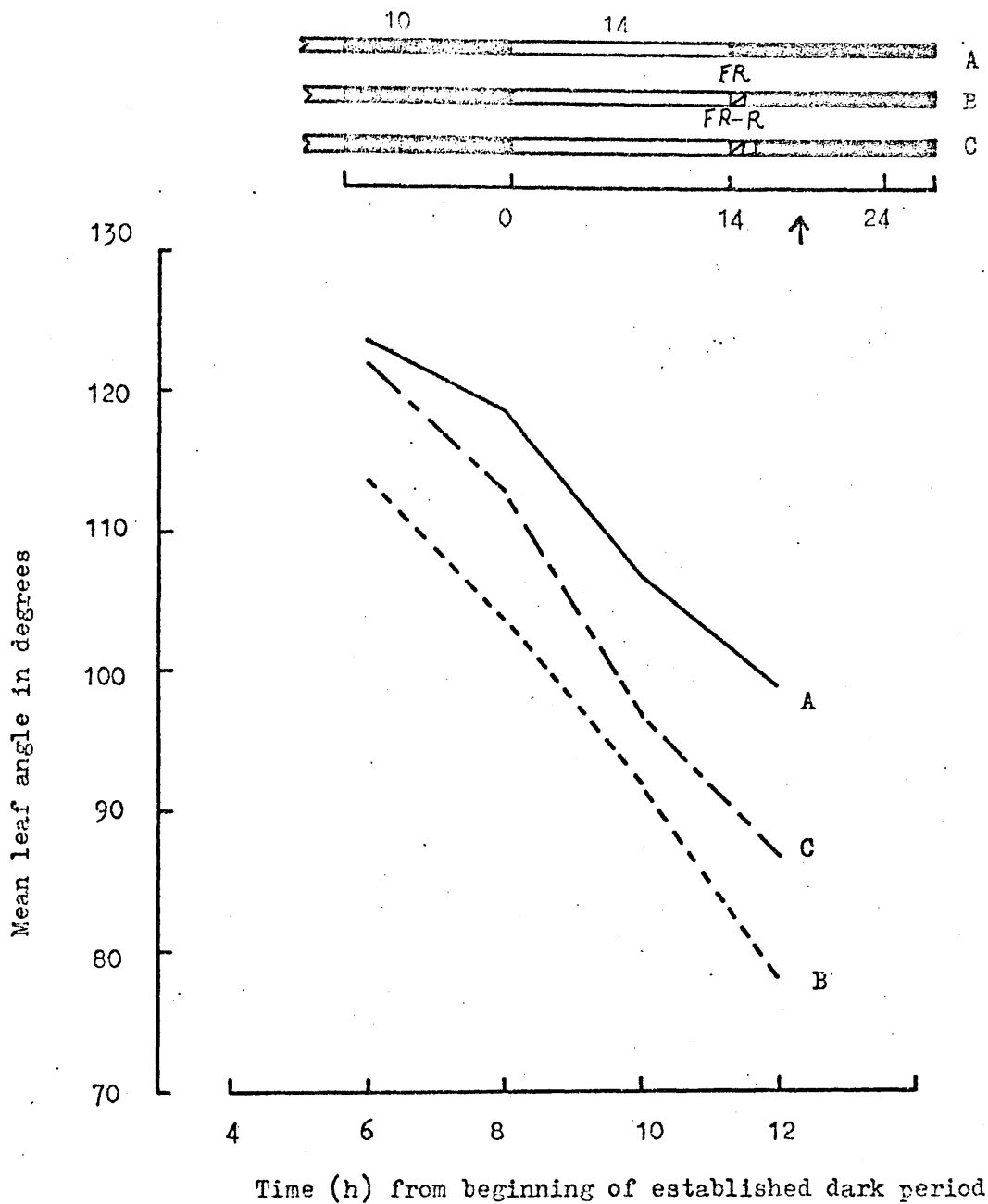


Fig. 3 - 3 a. Effect of far-red radiation and of subsequent exposure to red radiation after the 14 h light period. Light period followed (A) uninterrupted darkness; (C) 15 min far-red, 15 min red, then darkness; (B) 15 min far-red, then darkness. Measurements were taken at 2 hourly intervals 6 h from beginning of established dark period. Each value is the mean of 14 replicates. Treatments are shown diagrammatically above the graph.

↑ time at which measurements commenced.

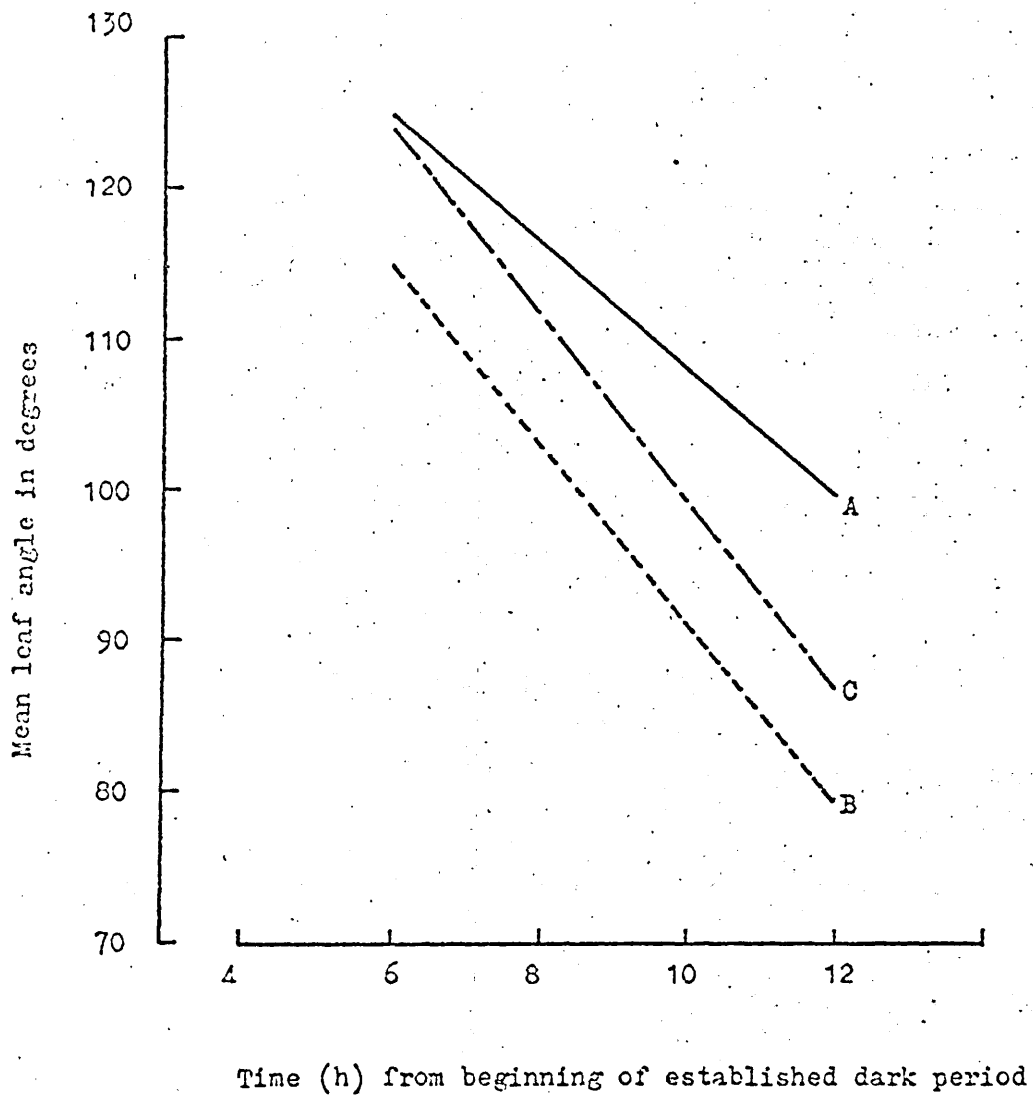


Fig. 3 - 3 b. Regression lines for curves presented in Fig. 3 - 3 a.

$$Y = 125.35 - 8.55 X \quad (\text{curve A})$$

$$Y = 115.30 - 12.00 X \quad (\text{curve B})$$

$$Y = 123.85 - 12.20 X \quad (\text{curve C})$$

Table 3 - 3

Effect of far-red radiation and subsequent exposure to red radiation after the 14 h light period. Measurements were taken at 2 hourly intervals 6 h from beginning of established dark period.

Each value is the mean of 14 replicates.

(Data of Fig. 3 - 3).

Light treatments	Time (h) from beginning of established dark period			
	6	8	10	12
Control	124.10	119.10	107.50	99.45
FR	114.25	104.10	92.85	78.00
FR-R	122.85	113.90	97.65	87.50

Table of Analysis of Variance

Source	Sum of squares	Degrees of freedom	Mean squares	Variance ratio (F)
Light treatments	260.02	2	130.01	46.432 <sup>***</sup>
Time	1011.11	3	337.00	120.357 <sup>***</sup>
Interaction	34.45	6	5.75	2.05 <sup>ns</sup>
Error	400.75	143		

\*\*\* - Significant at 0.01% level  
 ns - non significant.

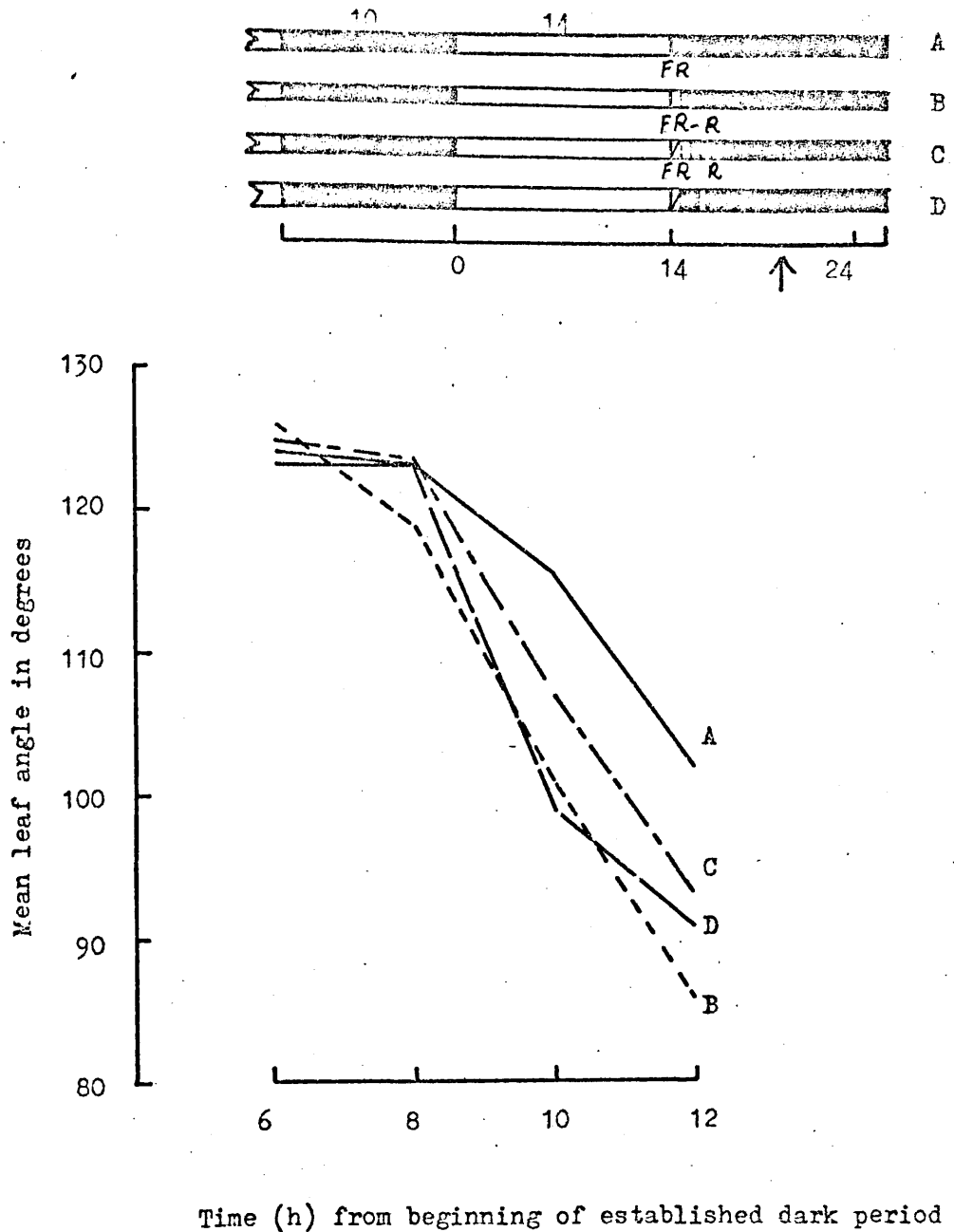


Fig. 3 - 4 a. Reversibility of far-red effect by red radiation and effect of the dark interval between the far-red and red treatments after 14 h light period. The control set of plants received 14 h white light only. Measurements were taken during the dark at 2 hourly intervals 4 h from beginning of established dark period. Each value is the mean of 14 replicates. Treatments are shown diagrammatically above the graph.

↑ time at which measurements commenced.

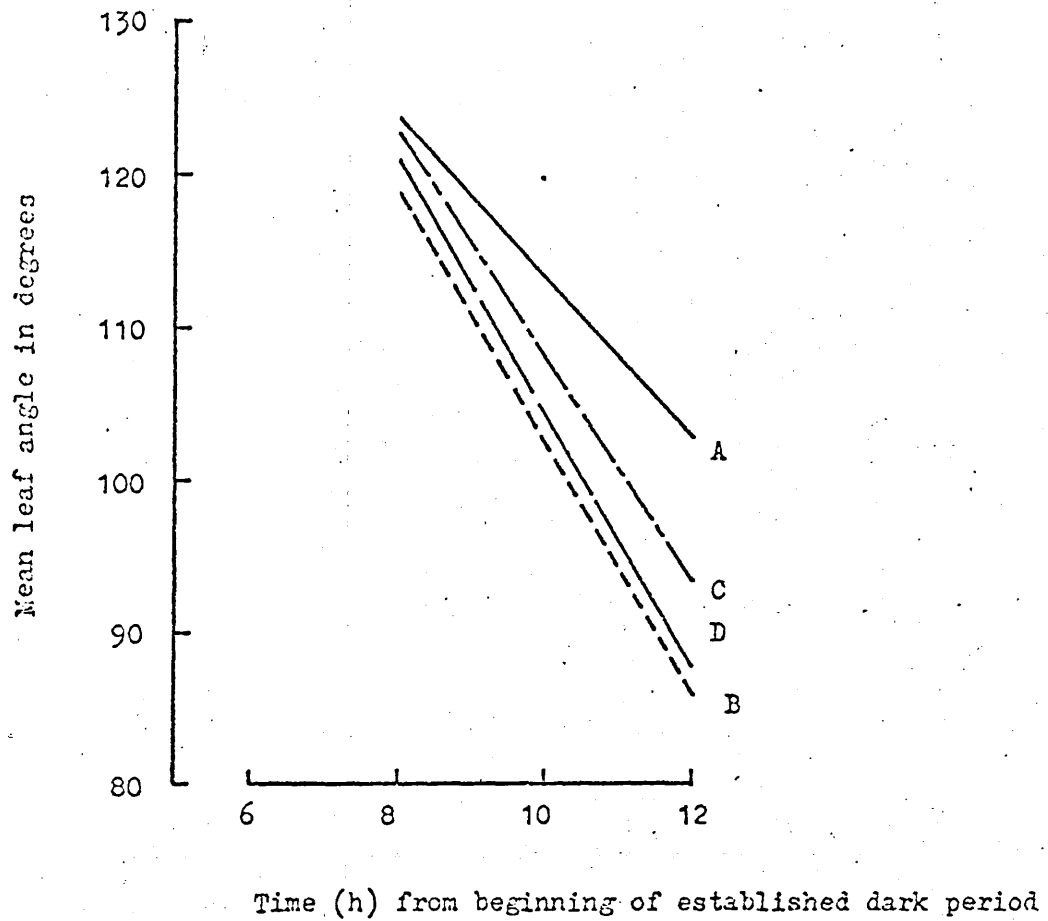


Fig. 3 - 4 b. Regression lines for curves presented in Fig. 3 - 4 a.

$$Y = 124.05 - 10.25 X \quad (\text{curve A})$$

$$Y = 119.15 - 16.70 X \quad (\text{curve B})$$

$$Y = 122.85 - 14.65 X \quad (\text{curve C})$$

$$Y = 120.95 - 16.35 X \quad (\text{curve D})$$

Table 3 - 4

Reversibility of far-red effect by red radiation and effect of 1 h dark interval between the far-red and red irradiation after the 14 h light period. The control set of plants received 14 h white light only. Measurements were taken during the dark at 2 hourly intervals 4 h from beginning of established dark period.

Each value is the mean of 14 replicates.

(Data of Fig. 3 - 4).

Light treatments	Time (h) from beginning of established dark period			
	4	6	8	10
Control	124.45 a	123.20 ab	115.50 c	102.65 d
FR	126.40 a	119.45 bc	101.95 d	86.05
FR-R	124.60 a	123.20 ab	107.50	93.90 f
FR-D-R	125.00 a	123.75 a	99.10 d	91.05 f

N.B. When values share a common letter they do not differ significantly at 5% level (Duncan's M.R.T.).

Table of Analysis of Variance

Source	Sum of squares	Degrees of freedom	Mean squares	Variance ratio (F)
Light treatment	83.34	3	27.91	38.60***
Time	1480.37	3	493.45	682.50***
Interaction	93.57	9	10.39	14.37***
Error	150.56	208	0.72	

\*\*\* - Significant at 0.01% level.



Far-red radiation following short light period.

It is clear from experiment 3 - 1 that the rising of the leaves in darkness following a 14 h light period is not regulated by phytochrome, although the reverse at the peak of the movement is made more precise when far-red is given at the beginning of dark. A small increase in the rate of leaf rising movement did occur, however, when far-red followed a 10 h light period (Fig. 3 - 2 B). This last result also showed the long lasting effect of the far-red irradiation since the effect on the reverse movement came some 8 h after the far-red treatment.

The following experiments were designed to explore the possibilities of the phytochrome regulation of the nyctinastic movement in the early hours of the light period. Although nyctinastic movement does not occur on transfer to darkness in the early hours of the light period, the rhythmic nyctinastic movement is induced by the first 4 h light of the daily light period (see Fig. 1 - 4 A).

3 - 5. Plants were grown on 14:10 light:dark cycles, and light was terminated after 4 h of the final light period. This was followed immediately by 15 min far-red radiation, and the plants were then transferred to complete darkness. Another set of plants received their far-red treatment 10 h after the end of 4 h light treatment, i.e., at the time corresponding to the end of the established light period. The control plants received the 4 h white light only with no far-red treatment. Measurements were taken every 4 h at the beginning of the last established dark period, when leaf movement begins. In the preliminary experiment, it had been ascertained that no movement occurred until the 10th hour in the dark.

Fig. 3 - 5 shows that far-red given at the end of the short light

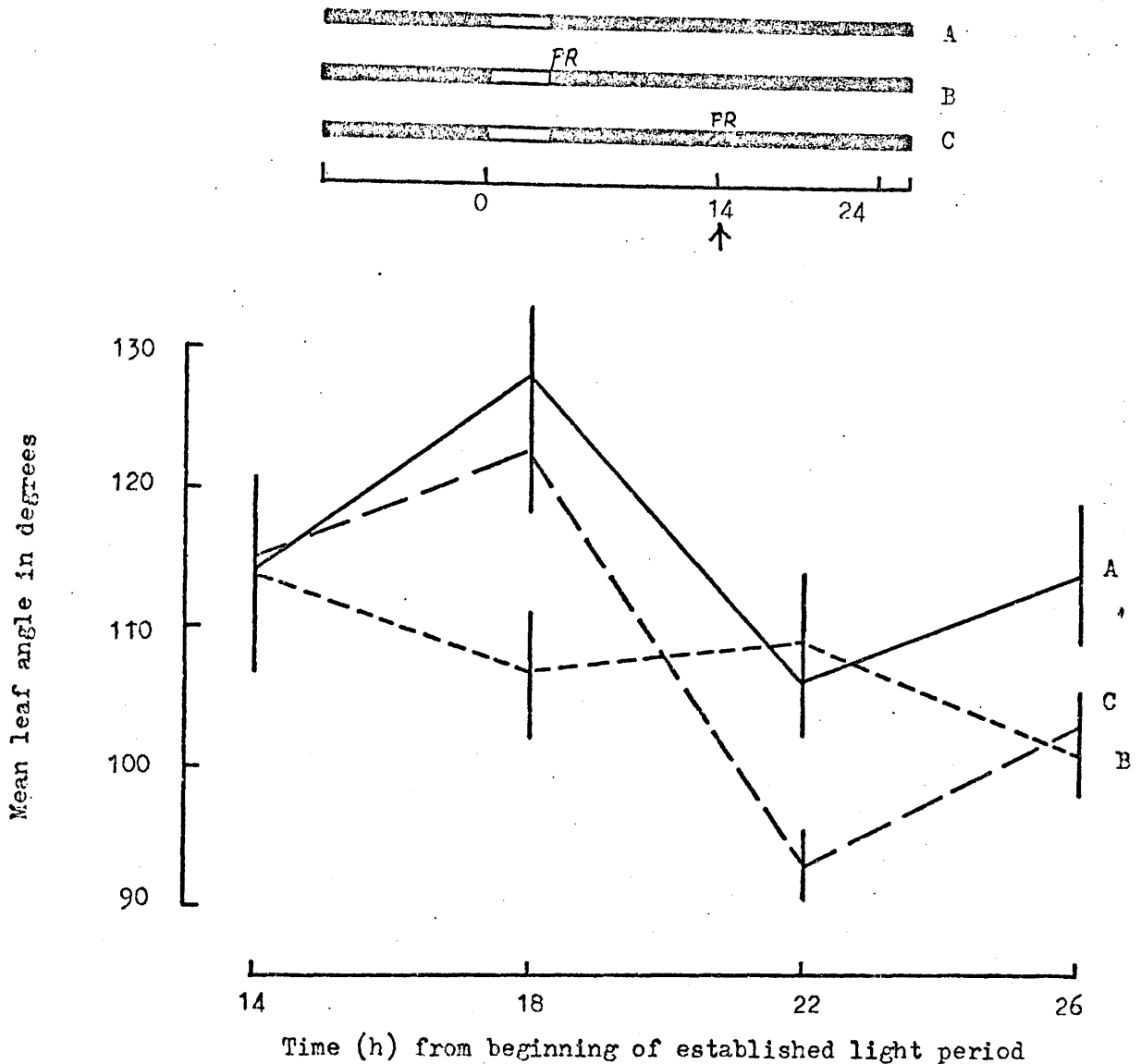


Fig. 3-5. Effect of far-red radiation on nyctinastic leaf movement after 4 h light treatment.

(A) uninterrupted dark; light followed by (B) immediately 15 min. far-red; (C) 10 h dark then 15 min. far-red.

Measurements were taken during the dark every 4 h, commencing 14 h from beginning of established light period. Each value is the mean of 16 replicates with 95% confidence limits. Treatments are shown diagrammatically above the graph.

↑ time at which measurements commenced.

Table 3 - 5

Effect of far-red radiation on nyctinastic leaf movement after 4 h light period. Light followed (A) immediately by 15 min far-red; (B) 10 h dark, then 15 min far-red; (C) uninterrupted dark. Measurements were taken during the dark every 4 h, commencing 4 h from beginning of established light period.

Each value is the mean of 16 replicates with 95% confidence limits.

(Data of Fig. 3 - 5).

Light treatments	Time (h) from beginning of established light period			
	14	18	22	26
Control	114.05 $\pm$ 3.91	128.45 $\pm$ 4.43	106.25 $\pm$ 2.82	114.70 $\pm$ 2.65
Far-red	114.05 $\pm$ 4.08	107.80 $\pm$ 4.91	109.05 $\pm$ 4.59	101.55 $\pm$ 5.47
Dark-Far-red	115.30 $\pm$ 7.71	123.45 $\pm$ 4.43	93.40 $\pm$ 5.39	103.10 $\pm$ 3.35

$\pm$  95% confidence limits.

period completely nullifies the effect of light treatment, i.e., leaves are not raised.

On the other hand, when far-red is given after 10 h dark, the rising movement is slower than that of the control plants, and the reverse movement quicker and takes the leaves to a lower position, a response which is similar to that described at the end of a 14 h light period.

Reversibility of far-red effect following short light periods.

3 - 6. In the following experiment the reversibility of the far-red effect is investigated by giving red light immediately after the far-red and also by giving a short dark period between the end of light period and the far-red treatment to see if there is a rapid "escape" from the inhibitory action of far-red.

After the short light period of 4 h, in one series 15 min of far-red was given at once. Half the plants were then transferred to darkness; the other half were given 15 min red before similar transfer. In the second series 1 h dark was interposed before the far-red treatment which was otherwise as before.

Fig. 3 - 6 shows that 1 h in darkness before the far-red treatment makes that treatment ineffective, although the falling movement still shows the enhancement noted in all plants that had received 15 min far-red. Comparing Fig. 3-5 and Fig. 3 - 6 there is close similarity between movement with 1 h delay and movement with 10 h delay of the far-red treatment. Red following far-red soon after a short light period completely reverses the inhibitory effect of far-red.

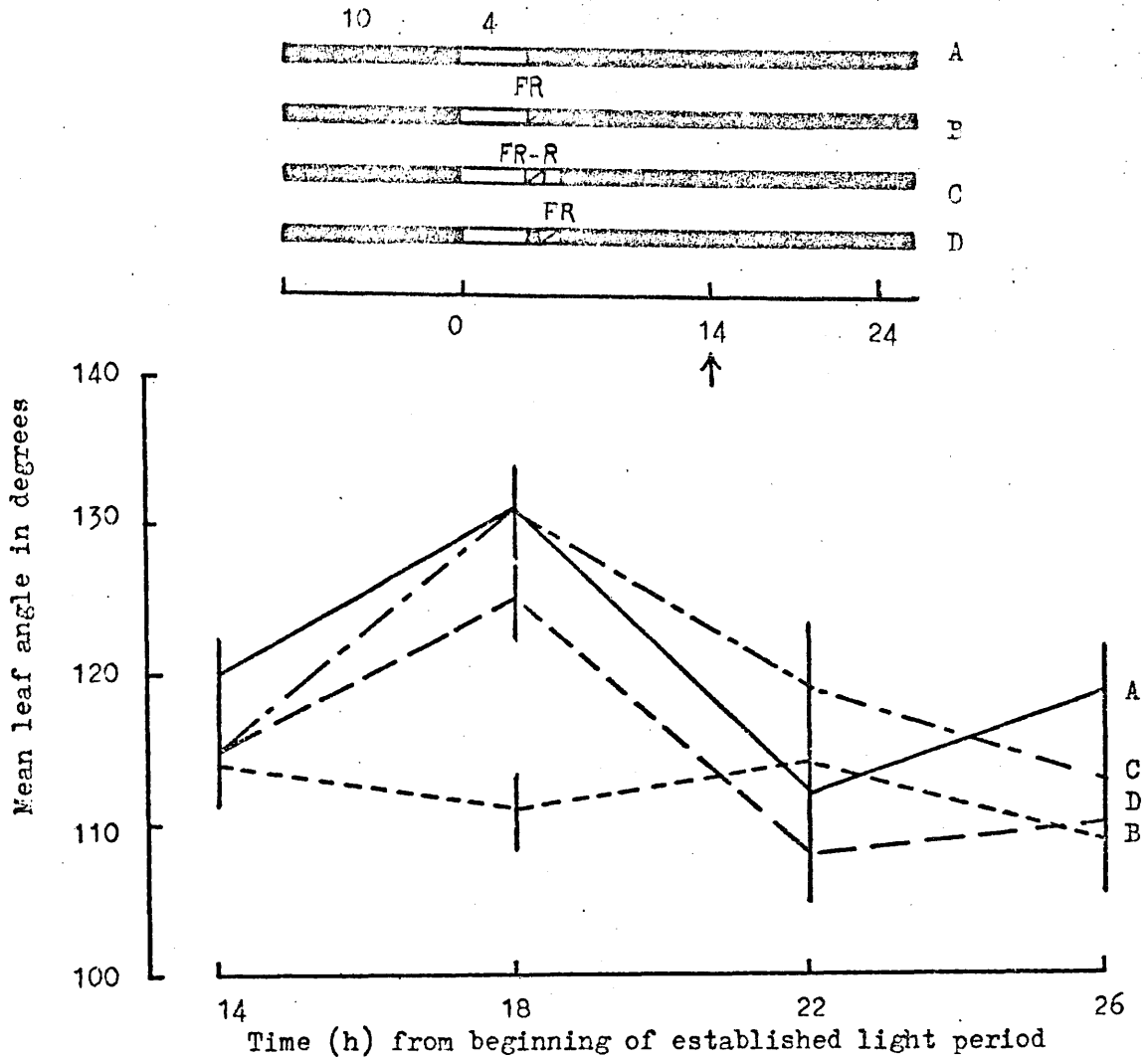


Fig. 3-6. Effect of far-red radiation on nyctinastic leaf movement after 4 h light treatment. Light period followed by (A) control treatment with uninterrupted darkness following 4 h light; light period followed by (B) 15 min. far-red and (C) 15 min. far-red then 15 min. red; (D) 1 h dark, then 15 min. far-red. Measurements were taken during the dark every 4 h, commencing 14 h from beginning of established light period. Each value is the mean of 20 replicates with 95% confidence limits. Treatments are shown diagrammatically above the graph.

↑ time at which measurements commenced.

Table 3 - 6

Effect of far-red radiation on nyctinastic leaf movement after 4 h light treatment.

Light period followed by (A) 15 min far-red; (B) 15 min far-red, then 15 min red; (C) 1 h dark then 15 min far-red; and (D) control treatment with uninterrupted darkness following 4 h light.

Measurements were taken during the dark every 4 h, commencing 14 h from beginning of established light period.

Each value is the mean of 20 replicates with 95% confidence limits.

(Data of Fig. 3 - 6).

Light treatments	Time (h) from beginning of established light period			
	14	18	22	26
Dark	120.50 $\pm$ 3.03	131.50 $\pm$ 2.02	109.75 $\pm$ 3.43	119.50 $\pm$ 3.55
Far-red-Red	115.00 $\pm$ 2.98	131.50 $\pm$ 2.53	119.75 $\pm$ 3.83	113.75 $\pm$ 3.47
Dark-Far-red	115.50 $\pm$ 3.42	125.25 $\pm$ 2.34	108.25 $\pm$ 4.18	110.00 $\pm$ 2.63
Far-red	114.50 $\pm$ 2.83	111.25 $\pm$ 3.12	114.00 $\pm$ 2.90	109.00 $\pm$ 2.90

$\pm$  95% confidence limits.

Light quality and induction of rhythmic nyctinastic movement.

It has been shown that 4 h of white light is required to potentiate the nyctinastic leaf movement (see Fig. 1 - 4 C). It is of interest to enquire what part of the spectrum is responsible for this. Various wavelengths were accordingly given in the early part of the light period to establish the most effective part of the spectrum inducing the nyctinastic response.

3 - 7. Plants were raised on a 14:10 light:dark cycle for 12 days. Blue, red and far-red were given for the first 4 h of the main light period respectively to three sets of plants. A control set of plants received 4 h unfiltered (white) light. After the various light treatments, plants were transferred to darkness. Measurements were taken every 4 h starting at the beginning of the established dark period (i.e., at the time at which rising movement normally starts).

Fig. 3 - 7 shows that red light induces the movement as effectively as white light. Blue and far-red have no effect at all (Table 3 - 7).

Light given in the middle of established day.

White light given in the middle of the established day results in a small rise during the first 2 h of the established dark period after which the movement is reversed (See Fig. 1 - 7 B). Blue and red light were given to see if these on their own have a different effect.

3 - 8. Plants were grown on the usual 14:10 light:dark regime. During the final established day, white light, blue light and red light were given during the middle 4 h only to three different sets of plants respectively. For the rest of the established day plants were kept in darkness. Measurements were started at the beginning of the established dark period and taken every 2 h for 10 h. The control treatment received the full light period of 14 h white light.

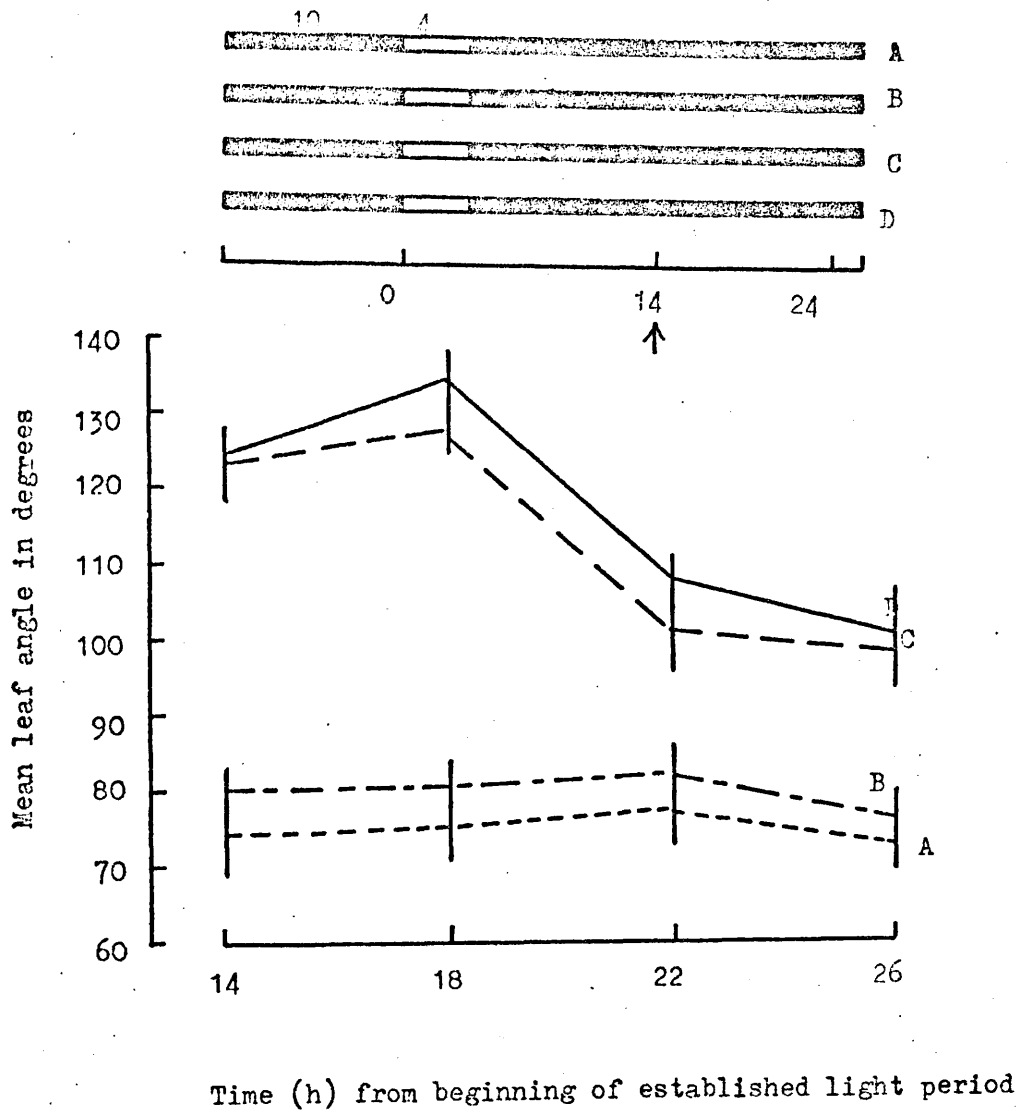


Fig. 3 - 7. Nyctinastic leaf movement following 4 h light of various wavelengths, given in the beginning of the established 14 h light period.

(A) far-red; (B) blue; (C) red light and (D) control set of plants received 4 h white light. Measurements were taken during the dark every 4 h, commencing 14 h from beginning of established light period. Each value is the mean of 20 replicates with 95% confidence limits.

Treatments are shown diagrammatically above the graph.

↑ time at which measurements commenced.



Table 3 - 7

Nyctinastic leaf movement following 4 h light of various wavelengths, given in the beginning of the established 14 h light period. (A) 4 h far-red; (B) 4 h blue; (C) 4 h red light; and (D) control set of plants received 4 h white light only.

Measurements were taken during the dark every 4 h, commencing 14 h from beginning of established light period.

Each value is the mean of 20 replicates with 95% confidence limits.

(Data of Fig. 3 - 7).

Light treatments	Time (h) from beginning of established light period			
	14	18	22	26
White	123.50 $\pm$ 4.43	127.00 $\pm$ 3.07	101.50 $\pm$ 3.04	98.25 $\pm$ 5.59
Blue	80.00 $\pm$ 3.13	80.25 $\pm$ 3.17	82.00 $\pm$ 4.19	76.50 $\pm$ 3.06
Red	124.00 $\pm$ 4.72	134.25 $\pm$ 3.33	108.25 $\pm$ 5.28	101.50 $\pm$ 4.59
Far-red	74.25 $\pm$ 5.23	72.25 $\pm$ 4.22	77.00 $\pm$ 4.25	75.00 $\pm$ 3.22

$\pm$  95% confidence limits.

Fig. 3 - 8 shows that none of these treatments produced the normal nyctinastic response when given in the middle of the subjective day. The small rise and fall observed in the set of plants that received blue light is similar to the small movement found with the white light treatment (see Fig. 1 - 7 B). It is also seen that the red light treatment resulted in the leaves being held at a slightly higher angle compared to the white light treatment.

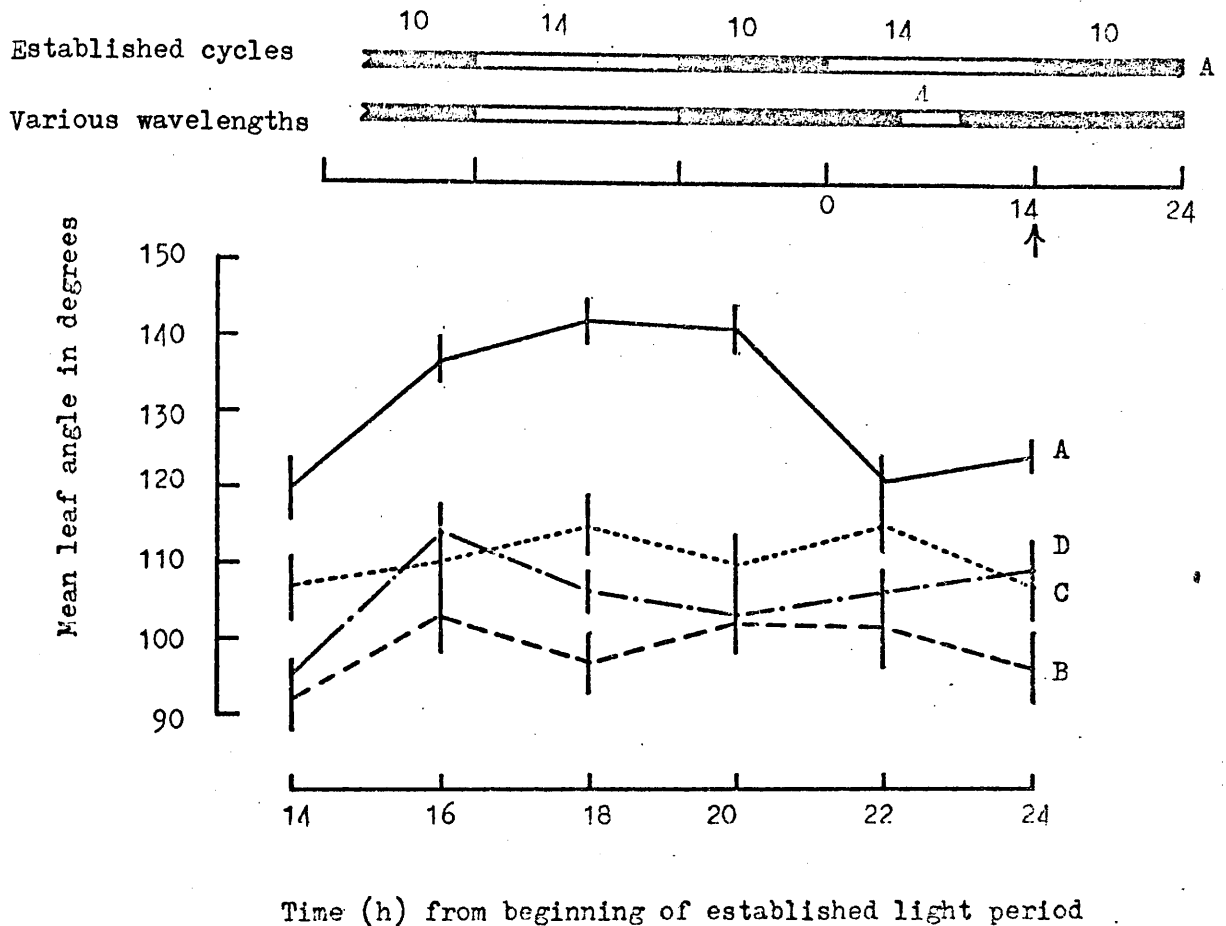


Fig. 3 - 8. Nyctinastic leaf movement following 4 h light of various wavelengths given in the middle of the established light period. (A) Control set of plants received 14 h white light; (B) 4 h white light; (C) 4 h red light and (D) 4 h blue light. Measurements were taken every 2 h, commencing 14 h from beginning of established light period. Each value is the mean of 20 replicates with 95% confidence limits. Treatments are shown diagrammatically above the graph.

↑ time at which measurements commenced.

Table 3 - 8

Nyctinastic leaf movement following 4 h light of various wavelengths given in the middle of the established light period. (A) 4 h white light; (B) 4 h blue light; (C) 4 h red light; (D) control set of plants received 14 h white light. Measurements were taken every 2 h, commencing 14 h from beginning of established light period.

Each value is the mean of 20 replicates with 95% confidence limits.

Time (h) from beginning of established light period	Light treatments			
	White	Blue	Red	Control
14	92.50 $\pm$ 1.94	95.75 $\pm$ 3.66	107.00 $\pm$ 4.25	120.50 $\pm$ 3.94
16	103.25 $\pm$ 3.96	114.75 $\pm$ 5.39	110.25 $\pm$ 4.05	137.00 $\pm$ 3.09
18	97.25 $\pm$ 2.99	106.00 $\pm$ 4.06	115.75 $\pm$ 4.38	142.25 $\pm$ 25.7
20	102.50 $\pm$ 3.27	103.75 $\pm$ 4.23	110.75 $\pm$ 3.50	141.25 $\pm$ 303
22	102.25 $\pm$ 3.44	105.75 $\pm$ 5.05	115.00 $\pm$ 3.71	121.75 $\pm$ 3.50
24	96.00 $\pm$ 3.68	109.00 $\pm$ 3.99	107.00 $\pm$ 4.65	124.00 $\pm$ 2.28

$\pm$  95% confidence limits.

Induction of rhythmic nyctinastic movement by red light breaks during darkness.

The following experiments were designed to explore the possibility of inducing the leaf movement by short red light breaks during darkness where longer periods of white light are required for such response. We have already seen that 4 h red light is about as effective as white light when given during the first 4 h of the established light period.

3 - 9. The plants were grown as usual on 14:10 light:dark cycles. On the 12th day, 15 min red light (600 nm) was given at "dawn", in the middle and at "dusk" of the subjective day. Otherwise plants were kept in the dark. Measurements were taken for 6 h (the time during which raising of leaf is normally completed) beginning at the commencement of the established dark period.

Fig. 3 - 9 shows that 15 min red light at the 7th h produced good nyctinastic movement (In another experiment not described here a similar result was achieved by 15 min red light given at the 9th hour). The other two treatments were not so effective. The light treatment at "dusk" initiated a weak response. Light in the beginning resulted in irregular "up" and "down" movements during these six hours.

Reversibility of rhythmic nyctinastic response induced by red light.

3 - 10. In the following experiment, the induction of the nyctinastic response by 15 min red light only when it is given in the middle of the subjective day was tested for reversibility by subsequent exposure to far-red light.

The experimental procedure was the same as in all of the above mentioned experiments. Fifteen minutes of red was immediately followed by 15 min far-red before transferring them to dark. 15 min of far-red

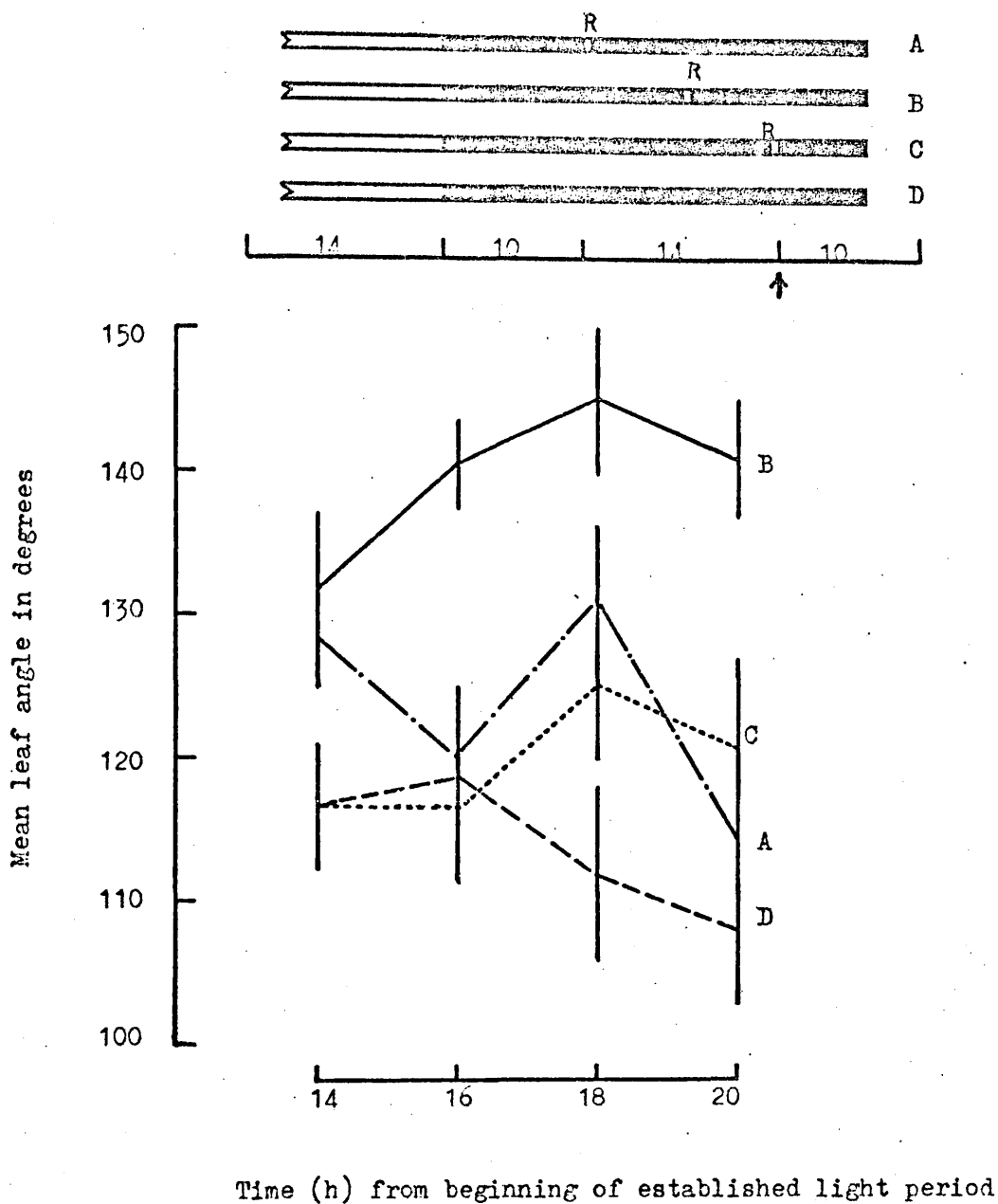


Fig. 3 - 9. Effect of 15 min red light irradiation given at different times during the subjective light period. (A) 15 min red at "dawn"; (B) in the middle; (C) at "dusk"; (D) control set of plants received uninterrupted darkness. Each value is the mean of 20 replicates with 95% confidence limits. Treatments are shown diagrammatically above the graph.

↑ time at which measurements commenced.

Table 3 - 9

Effect of 15 min red light irradiation given at different times during the subjective light period.

(A) 15 min red light at "dawn"; (B) in the middle; (C) at "dusk"; (D) control set of plants received uninterrupted darkness.

Each value is the mean of 20 replicates with 95% confidence limits.

(Data of Fig. 3 - 9).

Light treatments	Time (h) from beginning of established light period			
	14	16	18	20
A	129.50 $\pm$ 3.48	120.25 $\pm$ 4.89	131.50 $\pm$ 5.23	114.75 $\pm$ 4.12
B	132.50 $\pm$ 4.83	141.00 $\pm$ 2.74	145.75 $\pm$ 5.39	141.25 $\pm$ 3.29
C	* 117.50 $\pm$ 3.91	119.75 $\pm$ 4.96	125.25 $\pm$ 5.12	121.00 $\pm$ 5.74
D	—	119.25 $\pm$ 3.09	112.00 $\pm$ 5.90	108.25 $\pm$ 4.75

$\pm$  95% confidence limits.

\* - value for C and D is the same.

alone was given to a set of plants at the middle of the subjective day, a control set of plants likewise receiving 15 min red. A dark control was run.

Fig. 3 - 10 shows that the red light effect in inducing the nyctinastic response is significantly reversed by immediate far-red treatment. Referring to Table 3 - 10, the change of angle between the 14th and 16th hour was greatly reduced following subsequent treatment with far-red light as compared to red alone.

When far-red is given alone the leaves show a greater downward trend compared to the dark control.



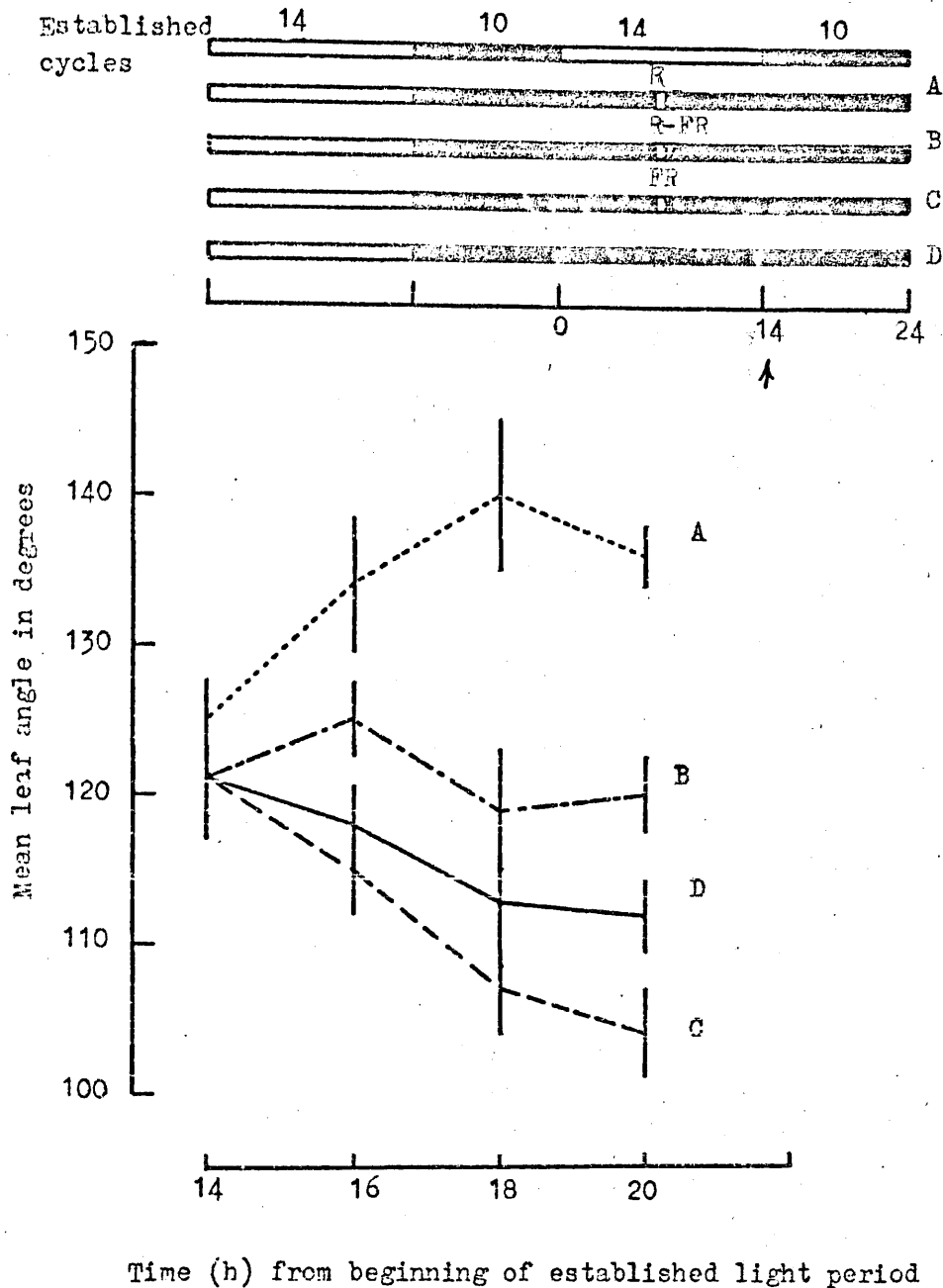


Fig. 3-10. Effect of red light irradiation given in the middle of the established light period and of subsequent far-red irradiation. 15 min red in the middle of established light period A; 15 min red followed by 15 min far-red before transferring them to darkness B; and 15 min far-red in the middle of established light period C. A dark control with no light treatment is also given D. Measurements are taken during the dark 14 h from beginning of established light period. Each value is the mean of 16 replicates. Vertical lines on the graph indicate 95% confidence limits. Treatments are shown diagrammatically above the graph. ↑ Time at which measurements commenced.

Table 3 - 10

Nyctinastic leaf movement following 15 min red, red and far-red and far-red alone in the middle of the established light period. Dark control with no light treatment is also given. Measurements were taken during the dark at 2 h intervals, 14 h from beginning of established light period.

Values are means of 16 replicates with 95% confidence limits.

(Data of Fig. 3 - 10).

Light treatments	Time (h) from beginning of established light period			
	14	16	18	20
Red	125.30 $\pm$ 2.83	134.00 $\pm$ 4.56	140.30 $\pm$ 4.91	136.25 $\pm$ 2.07
Red-Far-red	121.90 $\pm$ 2.73	125.30 $\pm$ 2.83	119.70 $\pm$ 3.95	120.00 $\pm$ 2.57
Far-red	122.20 $\pm$ 3.89	115.00 $\pm$ 3.22	107.50 $\pm$ 2.74	104.35 $\pm$ 3.21
Dark	121.25 $\pm$ 3.57	118.75 $\pm$ 2.48	113.15 $\pm$ 4.55	112.80 $\pm$ 2.91

$\pm$  95% confidence limits.

Induction of rhythmic nyctinastic response with 4 h white light and 15 min red in the middle of the established light period.

It has been shown in the previous experiments that phytochrome regulates the induction of leaf movement in the early hours of light period. Far-red irradiation following short light period of 4 h completely inhibits the rhythmic leaf movement in the following dark (Fig. 3 - 5). It is also now known that 4 h white light in the beginning or at the end of the established light period will induce the rhythmic nyctinastic response whereas 4 h light in the middle of the light period does not induce the normal rhythmic nyctinastic response (Fig. 1 - 7).

Observation from Fig. 3 - 9 shows that it is possible to potentiate a nyctinastic response with 15 min red light when given at an appropriate time. Fifteen minutes red light at the 7th h of the established light period resulted in a good rhythmic response, yet 4 h red light or any other wavelength spanning this time (5 - 9th h) did not (Fig. 3 - 8). It is also noted in Section 1 (Fig. 1 - 4) that white light continued beyond the first 4 h of the established light period could inhibit the response when terminated at the 7th h.

The following experiments are thus designed to clarify the peculiarities mentioned above of the light falling in the mid-subjective day.

3 - 11. Following the usual 14:10 light:dark regime, sets of plants were treated as follows:

Four hours white light was given in the middle of the established day alone and in combination with 15 min red light given at the end of 4 h treatment, or at the end of established day (i.e., after 5 h dark interval). The treatments with red light were also repeated without the 4 h white light. The plants remained otherwise in dark. These treatments are shown

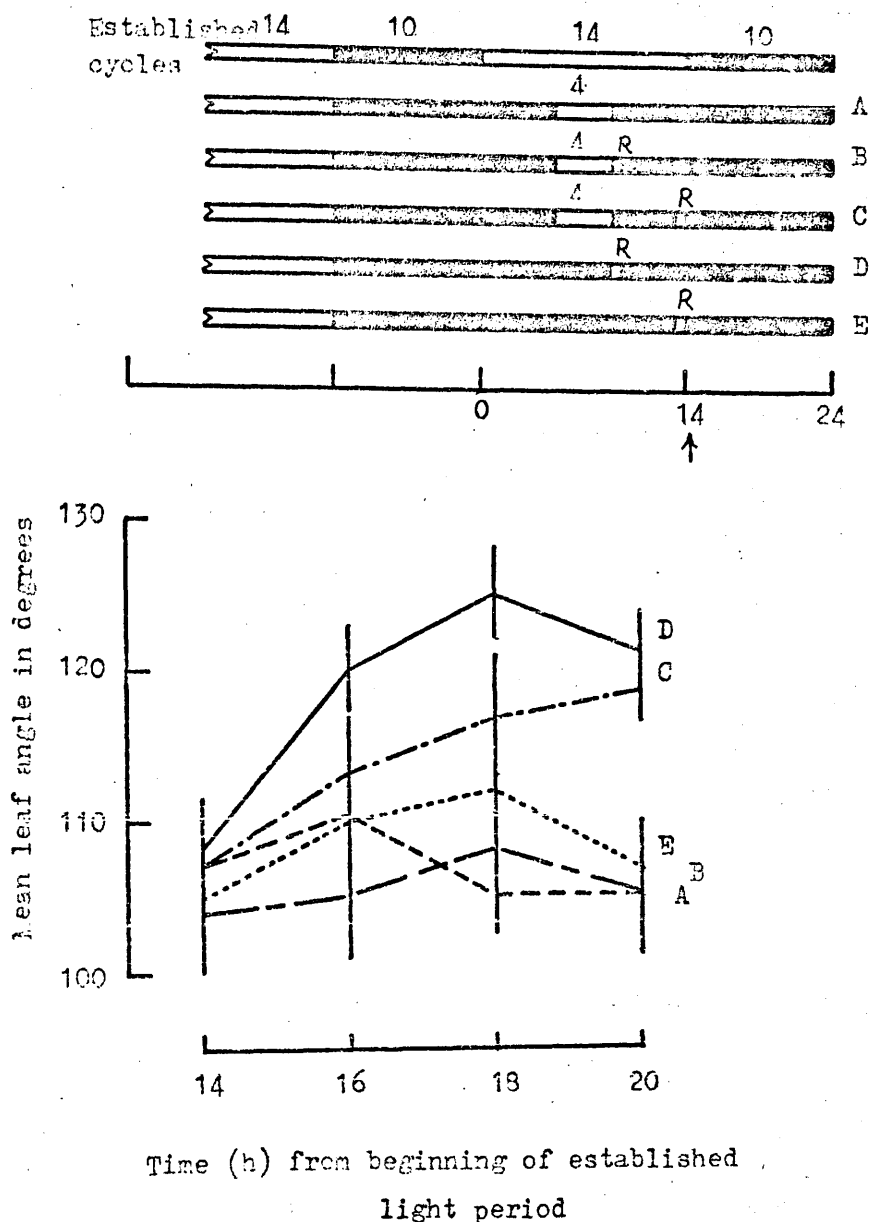


Fig. 3-11. Nyctinastic leaf movement following 4 h white light from 5-9th h of the established light period A; 4 h light followed by 15 min red B; 4 h followed 5 h dark and then 15 min red C; 15 min red at the 9th h D; and 15 min red at the 14th h of the established light period E. Measurements were taken during the dark 14 h from beginning of established light period. Values are means of 16 replicates. Vertical lines on the graph indicate 95% confidence limits. Treatments are shown diagrammatically above the graph. ↑ Time at which measurements commenced.

Table 3 - 11

Nyctinastic leaf movement following 4 h white light from 5 - 9th h of the established light period (A); 4 h white light followed by 5 min red (B); 4 h white light followed by 5 h dark and then 15 min red (C); 15 min red at the 9th h (D), and 15 min red at the 14th h of the established light period (E). Measurements were taken during the dark 14 h from beginning of established light period.

Values are means of 16 replicates with 95 % confidence limits.

(Data of Fig. 3 - 11).

Light treatment	Time (h) from beginning of established light period			
	14	16	18	20
A	107.50 $\pm$ 3.22	105.60 $\pm$ 4.54	110.65 $\pm$ 3.75	105.65 $\pm$ 3.75*
B	104.05 $\pm$ 4.66	105.30 $\pm$ 1.82	108.15 $\pm$ 2.35	105.65 $\pm$ 3.75
C	107.50 $\pm$ 3.22	110.95 $\pm$ 2.95	116.90 $\pm$ 3.75	118.75 $\pm$ 2.48
D	108.45 $\pm$ 3.18	120.30 $\pm$ 3.29	125.30 $\pm$ 2.83	121.90 $\pm$ 2.73
E	105.60 $\pm$ 4.54	113.75 $\pm$ 3.95	112.80 $\pm$ 4.45	107.50 $\pm$ 2.74

\* Values for A and B are the same.

$\pm$  confidence limits at 95% level.

diagrammatically above the graph (Fig. 3 - 11).

3 - 12. This experiment is in continuation of the above mentioned.

The treatments given were:

Four hours white light only in the middle of the established day to a control set of plants. Four hours light in the middle and 15 min red after a 5 h dark interval at the close of established day. Four hours white light in the middle of established day was followed immediately by 15 min red and a further 15 min red light given later at the close of the established day.

It is seen from Fig. 3 - 11 (curves B and D) that 15 min red following 4 h light is ineffective whereas red alone at this time (D) produces normal nyctinastic response. Comparing C and E, E produces the curve of a normal shape although of a lower amplitude whereas in C the rise is greater but the reversal of the movement is not seen. If the measurements had been continued beyond the 20th h it is possible that a delayed maximum may have been detected.

It is thus clear that it is the actual length of the period irradiated in the middle of the light period which is inhibitory to the rhythmic nyctinastic response (Fig. 3 - 8) which would occur if a very short period (15 min red) was given.

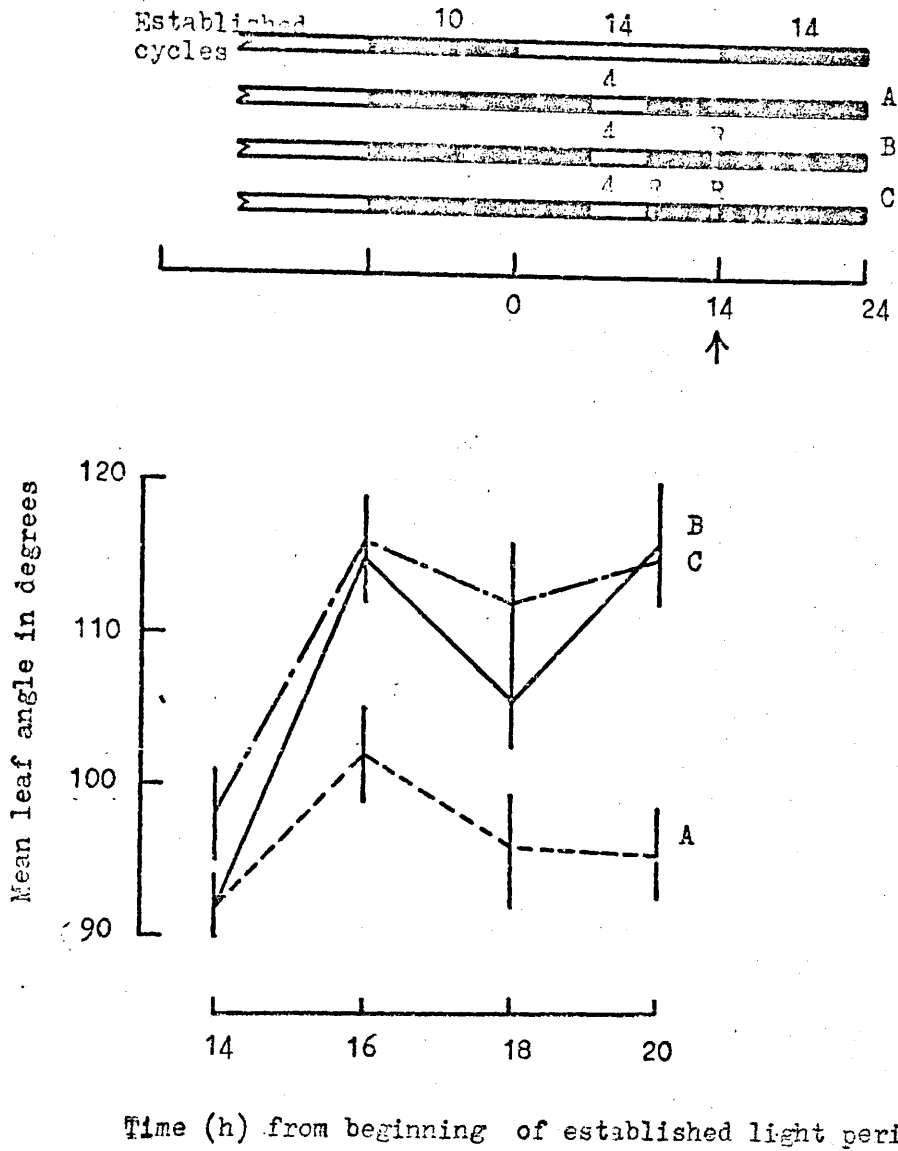


Fig. 3-12. Nyctinastic leaf movement following 4 h white light from 5-9th h of the established light period A; 4h white light followed 5 h dark and then 15 min red B; and 4 h white light followed 15 min red immediately and also after 5 h dark C. Measurements were taken during the dark 14 h from beginning of established light period. Values are means of 16 replicates. Vertical lines on the graph indicate 95% confidence limits. Treatments are shown diagrammatically above the graph.

↑ Time at which measurements commenced.

Table 3 - 12

Nyctinastic leaf movement following 4 h white light between 5 - 9th h (A); 4 h white light followed by 15 min red (B) and 4 h white light followed by 15 min red immediately and also after 5 h dark interval (C). Measurements were taken during the dark 14 h from beginning of established light period.

Each value is the mean of 16 replicates with 95% confidence limits.

(Data of Fig. 3 - 12).

Light treatment	Time (h) from beginning of established light period			
	14	16	18	20
A	92.75 $\pm$ 2.44	102.50 $\pm$ 3.11	96.15 $\pm$ 3.60	96.35 $\pm$ 2.99
B	98.40 $\pm$ 3.23	115.45 $\pm$ 2.97	112.95 $\pm$ 4.39	116.80 $\pm$ 3.31
C	92.75 $\pm$ 2.44	115.00 $\pm$ 3.06	106.60 $\pm$ 3.16	117.00 $\pm$ 4.61

$\pm$  confidence limits at 95% level.



Phase shift by different wavelengths of light.

Advancing phase.

It was shown in Section 2 (2 - 2) that 4 h light given during the late subjective night advances the phase of rhythmic movement so that the maximum position occurs 4 h earlier. The following experiment was therefore carried out in an attempt to determine the spectral region responsible for the phase advance. It was realized that since red light is necessary to induce the rhythmic leaf movement (3 - 7) any action of blue light alone would be unlikely, but a negative result with blue would not be conclusive proof that blue light could not affect phase.

3 - 13. Plants grown on a 14:10 light:dark regime for 12 days were given white, blue or red light for 4 h covering the end of the subjective night. After the light treatment the plants were transferred to darkness. Measurements were taken every hour during the dark, starting 8 h after the end of the light treatment.

Fig. 3 - 13 shows that the maximum position and the point of reversal of the movement occurs at the 18th h from beginning of light treatment for plants given white light and red light. Only small irregular movement follows the blue light treatment.

Comparing it with Fig. 3 - 7, where 4 h of blue and red lights were given in the early hours of established light period, the maximum leaf position is advanced by 4 h following red light given in the late established dark period.

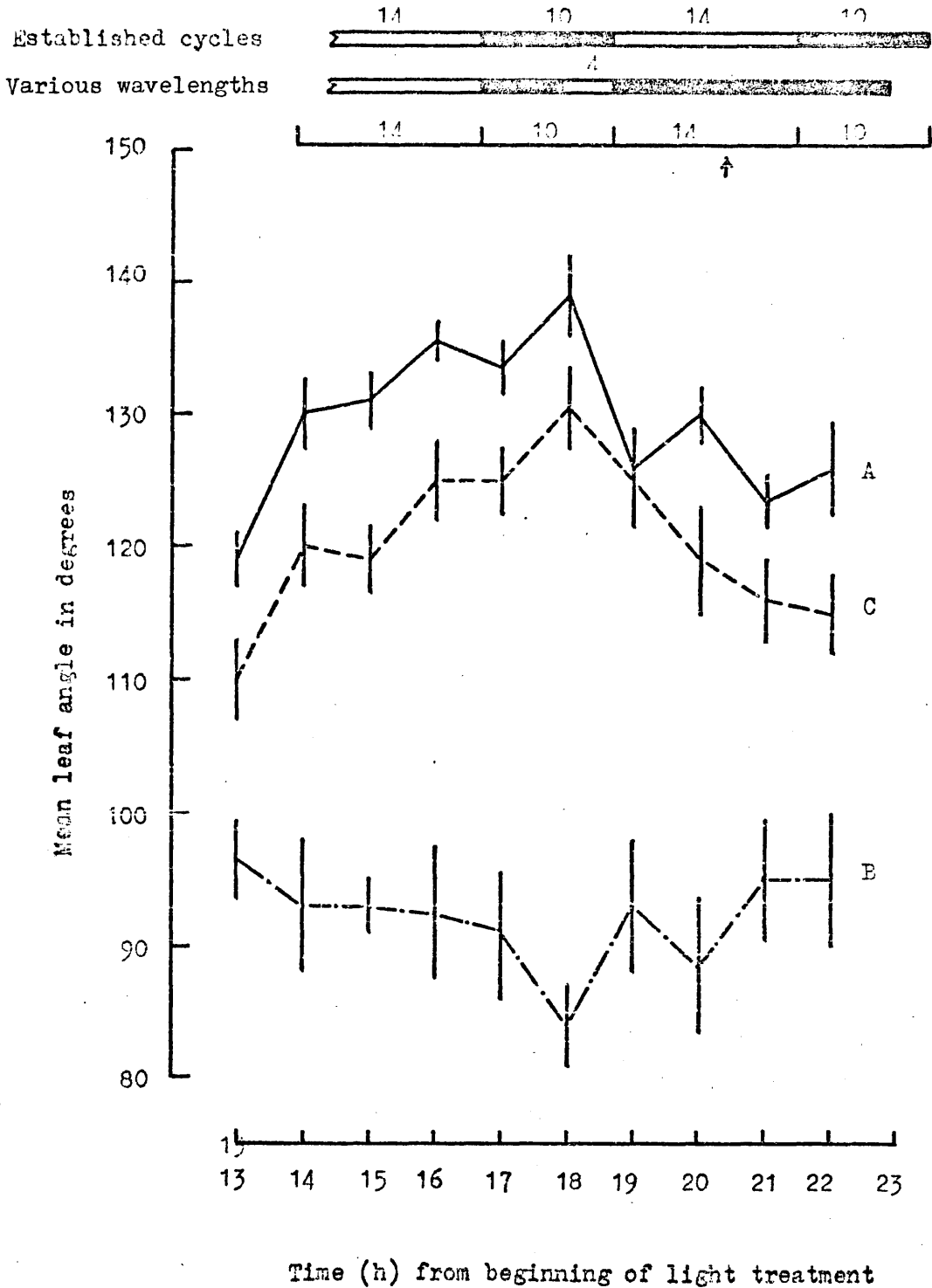


Fig. 3 - 13. Nyctinastic leaf movement following 4 h light of various wavelengths given during the later part of the established dark period. (A) 4 h white light; (B) 4 h blue light; (C) 4 h red light. Measurements were taken 13 h from beginning of light treatment. Each value is the mean of 16 replicates with 95% confidence limits. Treatments are shown diagrammatically above the graph.

↑ time at which measurements commenced.

Table 3 - 13

Nyctinastic leaf movement following 4 h light of various wavelengths given during the later part of the established light period.

Measurements were taken 13 h from beginning of light treatment.

Each value is the mean of 16 replicates with 95% confidence limits.

(Data of Fig. 3 - 13).

Time (h) from beginning of light treatments	Light treatments		
	White	Red	Blue
13	119.40 $\pm$ 2.31	109.70 $\pm$ 3.22	96.25 $\pm$ 3.19
14	130.00 $\pm$ 2.55	120.30 $\pm$ 3.03	92.80 $\pm$ 4.76
15	130.95 $\pm$ 2.34	119.40 $\pm$ 2.50	92.80 $\pm$ 2.05
16	135.65 $\pm$ 1.81	125.30 $\pm$ 2.90	92.50 $\pm$ 5.21
17	133.45 $\pm$ 2.42	125.30 $\pm$ 2.74	91.25 $\pm$ 4.84
18	139.40 $\pm$ 3.50	130.65 $\pm$ 3.08	84.05 $\pm$ 3.38
19	125.65 $\pm$ 2.79	125.00 $\pm$ 3.65	92.80 $\pm$ 5.05
20	129.70 $\pm$ 2.39	118.75 $\pm$ 3.94	88.75 $\pm$ 5.02
21	123.45 $\pm$ 2.44	116.25 $\pm$ 2.90	95.30 $\pm$ 4.55
22	125.95 $\pm$ 3.78	114.70 $\pm$ 2.90	100.00 $\pm$ 5.40

$\pm$  95% confidence limits.

Delaying phase.

When the light period is extended through the established dark period the maximum of the leaf movement in this cycle is delayed by about 4 h (see Fig. 2 - 1). It was concluded that a direct action of light holds the leaves down but eventually the rhythmic control overrides this. The following experiment was done to determine the spectral region responsible for this light action.

3 - 14. In this experiment the established light period (white light) was extended for 10 h (i.e., to cover the subjective night) with blue or red light in parallel treatments. Control plants received white light. Measurements were taken every 2 h starting from the 14th hour of the light period.

Fig. 3 - 14 shows that movement follows immediately during the treatment with red light, with a maximum at the 18th h, after which the movement is reversed. The response is similar, therefore, to the normal movement that occurs when plants are transferred to darkness after the established light period, although there is a more sharply defined peak in red light.

Following blue light the leaves show a slow but steady rise attaining a maximum position at the 22nd hour.

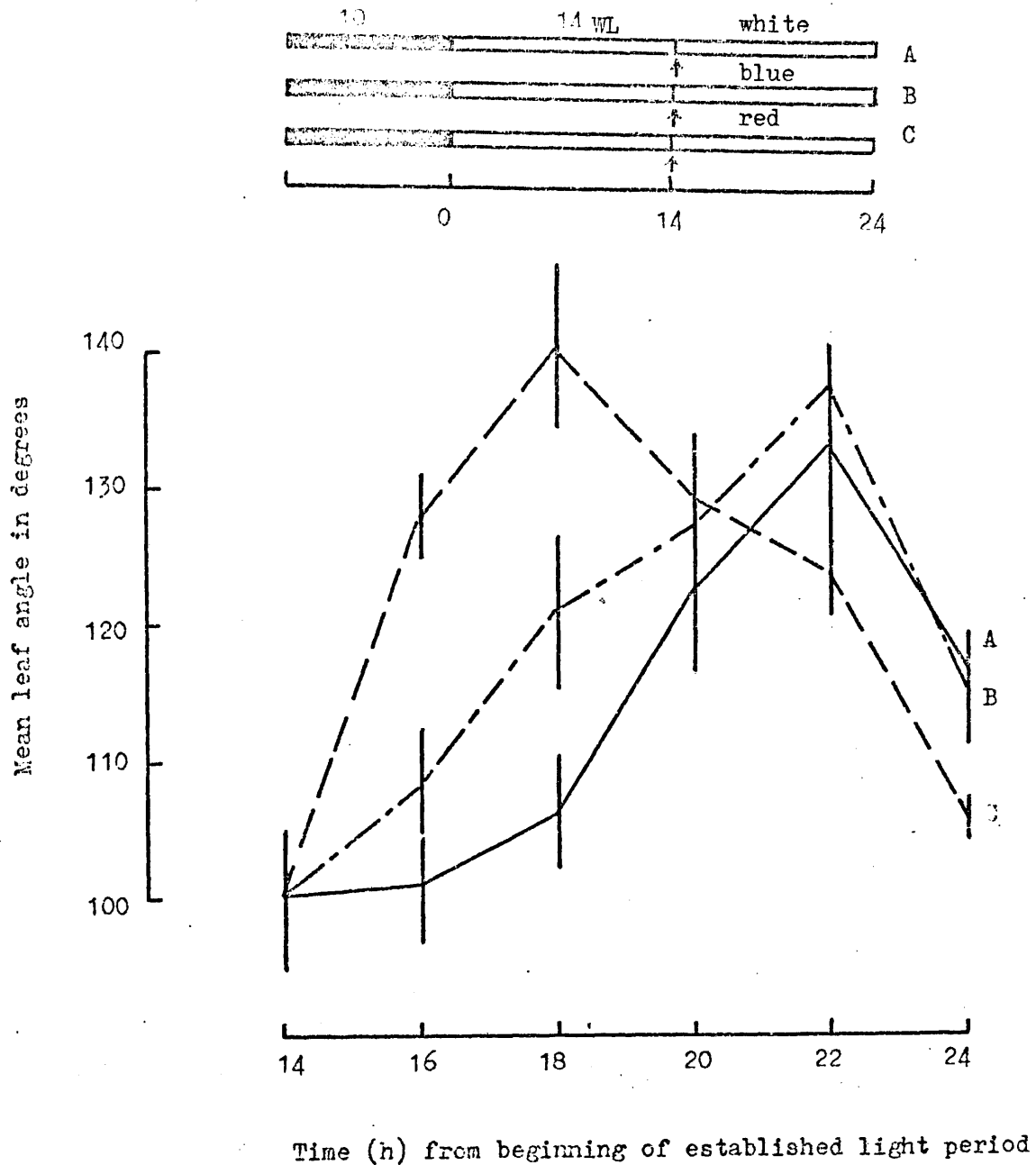


Fig. 3 - 14. Course of leaf movement during the extended light period with various wavelengths. Established 14 h light period followed (A) 10 h of white light; (B) 10 h of blue light; (C) 10 h of red light. Measurements were taken 14 h from beginning of established light period. Each value is the mean of 12 replicates with 95% confidence limits. Treatments are shown diagrammatically above the graph.

↑ time at which measurements commenced.

Table 3 - 14

Course of leaf movement during the extended light period with various wavelengths.

Measurements were taken 14 h from beginning of established light period.

Each value is the mean of 12 replicates with 95% confidence limits.

(Data of Fig. 3 - 14).

Time (h) from beginning of established light period	Light treatment		
	White	Blue	Red
14*	100.00 ± 4.97		
16	101.25 ± 4.09	108.75 ± 4.09	127.50 ± 3.18
18	116.25 ± 4.09	121.25 ± 5.50	140.40 ± 6.03
20	122.05 ± 6.03	127.05 ± 3.94	129.15 ± 4.03
22	133.30 ± 3.41	137.90 ± 3.44	129.55 ± 2.14
24	117.05 ± 2.14	115.40 ± 3.39	106.65 ± 1.56

± 95% confidence limits.

\* - value for white, blue and red light is the same.

Comments.

Three aspects of the involvement of the phytochrome system are shown in these results: (a) the induction of rhythmic nyctinasty; (b) partial control of the rising movement; and (c) partial control of the falling movement.

(a) Rhythmic nyctinasty (the cycle of movement in darkness whose timing is determined by the circadian rhythm) may be induced by 4 h white light and this is far-red reversible in the early part of the established day (Fig. 3 - 5 B). Thus, the removal of  $P_{fr}$  by far-red radiation at the close of the short light period prevents the leaf movement. It is confirmed by the fact that this far-red effect is reversible by red, which re-establishes  $P_{fr}$ . It was however found that  $P_{fr}$  action for the rising movement was completed by 1 h after the end of a short light period since a 1 h dark interval between the end of a light period and irradiation with far-red allowed the system to "escape" from far-red inhibition (Fig. 3 - 6 D). Such phytochrome control after a short light period has also been shown in the flowering response of Pharbitis nil (Fredericq 1964).

A brief (15 min) impulse of red light induced rhythmic nyctinasty when given about the middle (7th - 9th hour) of the established day, and also to a lesser extent when given at the close of the day (Fig. 3 - 9 B, C). A 15 min impulse was not alone sufficient to induce normal movement, when given at the commencement of the established day (although 4 h red induces normal movement) but following 4 h white light at this time, 15 min red light reversed the effect of prior treatment with far-red light and thus re-induced movement (Fig. 3 - 6 C). It is possible that in the early part of the day, a period in excess of 15 min is

necessary between the light-on signal and the onset of darkness for complete induction and phase-setting, and that merely providing  $P_{fr}$  by a single impulse of red light is not sufficient. This obviously needs further study.

Although 15 min red light is sufficient to induce the rhythm in the first cycle of darkness, it is not known if this would be the case in subsequent cycles. It is however clear that the failure of the leaves to move in darkness after the initial movement is not simply due to the cessation of photosynthesis.

(b) Although the absence of  $P_{fr}$  at the time of transfer to darkness (far-red treatment) slows down the rising movement at the end of a 10 h light period (Fig. 3 - 2), no such effect was found after the 14 h light period. The situation is possibly comparable to the action of phytochrome in legumes, e.g., Albizzia, where its control over leaf movement progressively diminishes through the light period (Hillman and Koukkari 1967).

(c) Exposure to far-red light prior to transferring the plants to darkness at both the 10th hour and the 14th hour led to a more sharply defined "peak" or maximum position since the reverse movement was made more rapid (Fig. 3 - 2). Several hours may elapse between treatment with far-red irradiation and the time when the effect is shown. Thus, when the far-red light was given 1 h after the 4 h white light period, it had no effect on the rising rhythmic movement, but the quickening of the falling movement was seen some 14 h after the treatment (Fig. 3 - 6 D). Full reversal of the far-red effect with subsequent red light was achieved following a short 4 h light period (Fig. 3 - 6 C). Following a 14 h light period, on the other hand, the reversal of far-red light was not complete (Fig. 3 - 3). Red light given after the far-red showed



an initial reversal of the movement, so that the peak was flattened again to its normal form. However, the overall effect of the falling curve was barely affected (Fig. 3 - 3 B).

The effect of 4 h white light in inducing the rhythmic movement and also advancing its phase when given in the late subjective night is seen to be brought about by the red region of the spectrum (Fig. 3 - 13). On the other hand, the action of light in delaying the rhythmic movement when the light period is extended through the established dark period is due to the blue end of the spectrum (Fig. 3 - 14), the response to red light being much the same as to darkness.

SECTION 4

ANATOMY AND FINE STRUCTURE OF THE BASE OF THE LEAF

Anatomy and fine structure of the base of the leaf.

Introduction.

Reports about anatomical and histological studies of leaves that display movement in response to external stimuli are mainly about pulvinate leaves, e.g., Mimosa and Albizzia. In these materials the specialized organ, the pulvinus, at the base of the leaf petiole, is considered to be responsible for initiating the movements. Anatomical investigations of the basal portion of the leaf were therefore undertaken in an attempt to determine whether there were any special features that might provide a structural basis for the mechanism of leaf movement in Linum. The leaf is without an apparent pulvinus but shows a well defined nyctinastic movement which is controlled by the endogenous rhythm, as well as phytochrome. The leaves are curved slightly towards the apical bud until epinastic growth straightens the leaf and takes it away from the bud. Thereafter the measurable periodic changes in angle occur. When the leaf angle changes the overall curvature of the leaf does not noticeably change (see Fig. 4) so regardless of whether it is due to differential growth or a reversible change in cell turgor, the differential change in length between the upper and lower sides of the leaf must be mainly confined to the region near the base of the leaf.

Methods.

Twelve days old plants grown in the usual 14:10 light:dark cycles were used. Five replicates were taken during the light period (at the 8th hour) and five during the dark period when the leaves were in the rising phase of movement (at the 16th hour). The basal part of the leaf along with a small portion of the stem was fixed in 6% gluteraldehyde at room temperature for 3 to 4 h. The replicates from the dark period

were fixed in the dark. The preparation methods for optical and electron microscopy are described in the Materials and Methods section of the thesis.

#### Observations.

There is obviously no pulvinus at the base of the Linum leaf, and serial sections taken in longitudinal and transverse planes and observed by optical microscopy (Figs. 4 - 1, 4 - 2) show no special features that could be immediately associated with a leaf movement mechanism and no difference between plants taken from light or dark. These sections show no difference between the cells on the adaxial and abaxial sides of the mid-vein except at the base where the inner periclinal walls of the epidermal cells and the outer (and to a lesser extent the inner) periclinal walls of the sub-epidermal cell layer are very thick, giving the sub-epidermal cells the appearance of collenchyma. This thickening extends below the leaf axil into the cortical tissue of the stem.

The electron micrographs (Figs. 4 - 3, 4 - 4, 4 - 5) show the differences between the adaxial and abaxial tissues of the leaf base more clearly. The most striking feature of the cells is the large number of vesicular invaginations of the plasmalemma (p in the figures). If these are to be identified as pinocytotic vesicles then there may be some indication of the cells which are involved in movement of water and ions through their plasmalemma. However, more material must be examined before any definite conclusion can be reached. These vesicles appear to be most numerous in the cells on the adaxial side of the leaf fixed during the dark period (Fig. 4 - 3 B) but they are also common in light fixed material (Fig. 4 - 6 A) and sometimes in the abaxial epidermis too (Fig. 4 - 5 B).

The epidermal cells near the leaf axil show a most unusual feature, particularly in the dark fixed material (Figs. 4 - <sup>5</sup>6, 4 - <sup>6</sup>7). The cytoplasm contains numerous small membrane-limited vesicles or vacuoles. In Fig. 4 - <sup>6</sup>7 B these are seen in one cell to completely fill the vacuolar space while in other cells here and in Fig. 4 - 7 A a larger vacuole is present and the numerous smaller vacuoles are confined within the peripheral cytoplasm. Fig. 4 - <sup>7</sup>8 shows the identifiable organelles in one of the epidermal cells.

#### Comments.

The cells described above with numerous small vacuoles are of great interest because they resemble to a remarkable degree the multivacuolate cells in the sub-epidermal tissue of the pulvinus of Albizzia (Satter et al., 1970). In the Albizzia pulvinus the cells of this contractile tissue are filled with vacuoles ranging in size from vesicles less than 0.2  $\mu$ m to others several  $\mu$ m across. They are smallest and most numerous in the outer layers, and there is a gradation in number and size of vacuoles in successive layers of cells between the epidermis and vascular bundle (loc. cit. p. 376).

Satter et al. comment on the unusual nature of this multivacuolate condition and note that small vacuoles are reported to appear in cells of the Mimosa pulvinule (Weintraub 1951), but the significance of these small vacuoles in the movement of water into and out of pulvinule cells requires further study.

The presence of these peculiar cells in the adaxial epidermis of the Linum leaf suggests a possible anatomical link between the mechanism of leaf movements in Linum and the specialized pulvinar movements of Albizzia.

As there is no pulvinus at the base of the leaf and there appear to be no obvious changes in the cells located at the base of the leaf that may on their own be responsible for the movement, a possibility that growth changes are involved should be considered. Further anatomical study with this in mind might provide more conclusive results.

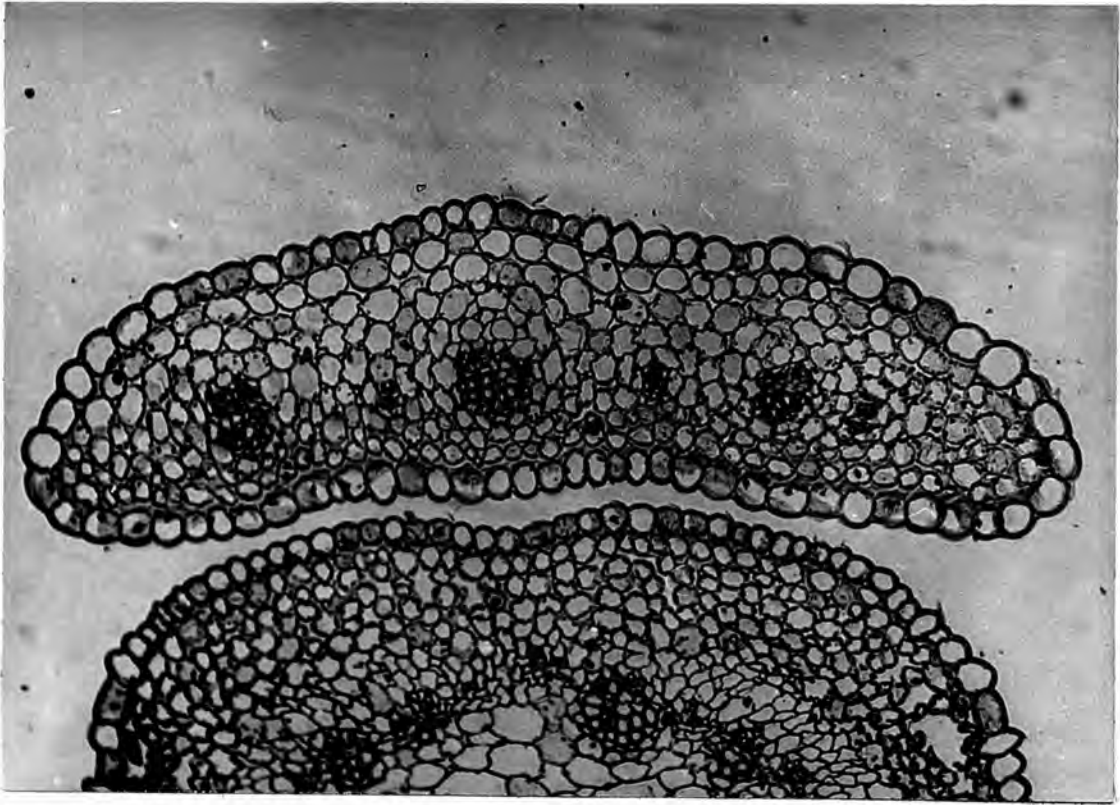
Fig. 4 - 1. Transverse section of the base of leaf of Linum.

The material was fixed in glutaraldehyde and embedded in wax. Twelve days old plants were used.

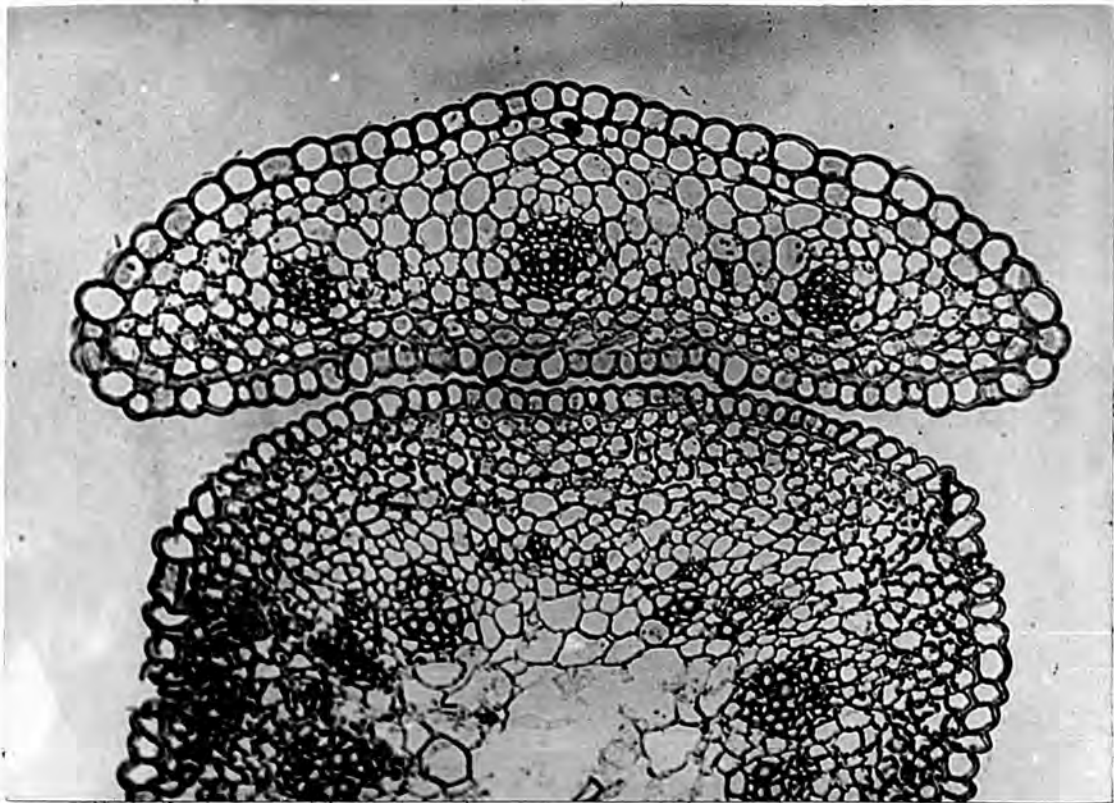
A. leaf from the plant in light (8th hour).

B. leaf from the plant in dark (16th hour).

Note little apparent difference between A and B; the sub-epidermal cells on the adaxial side are thick-walled.



A



B

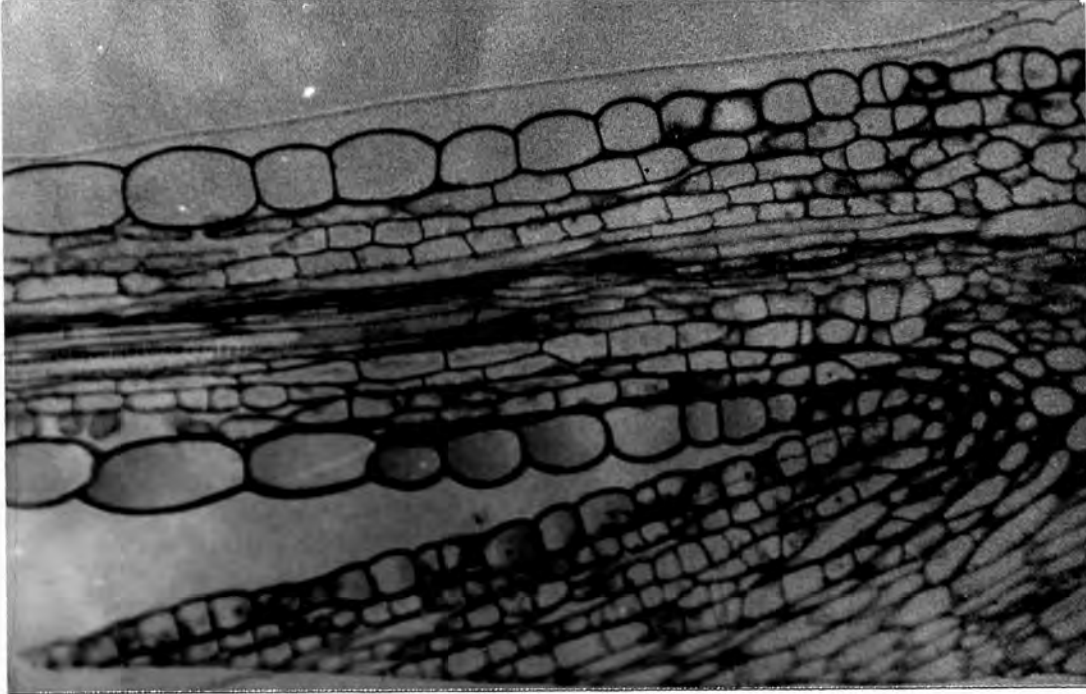


Fig. 4 - 2. Longitudinal section of the base of leaf of Linum.

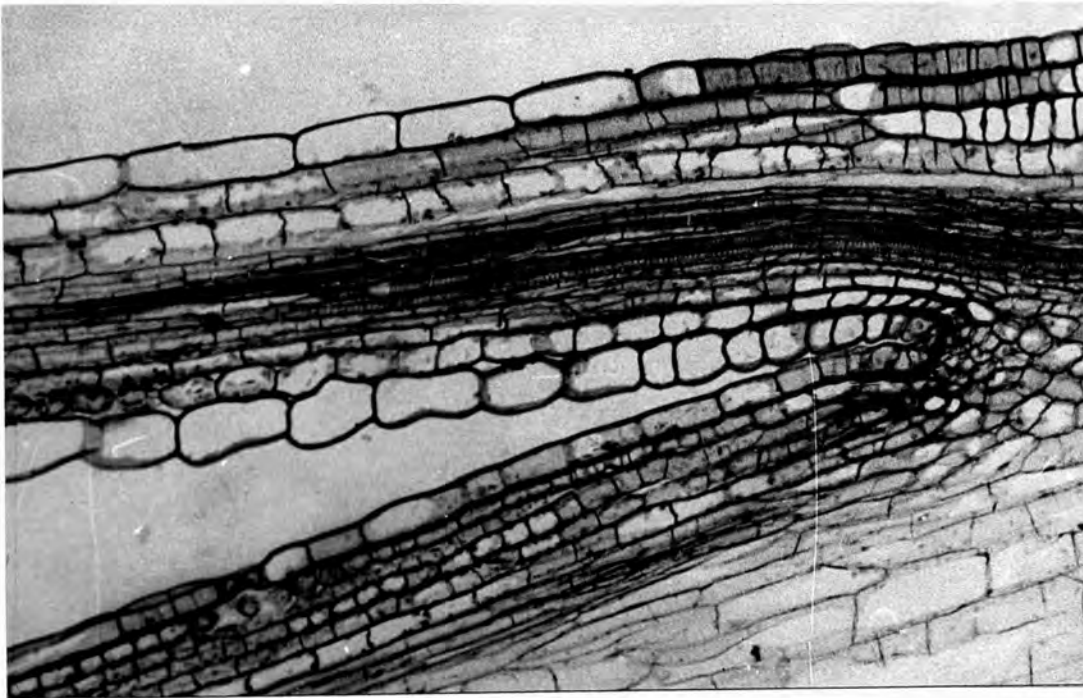
A. leaf from the plant in light (embedded in wax)

B. leaf from the plant in dark (embedded in resin)

Note no difference between A and B but periclinal walls of the sub-epidermal cells are obvious in the lower part of the leaf.



**B**



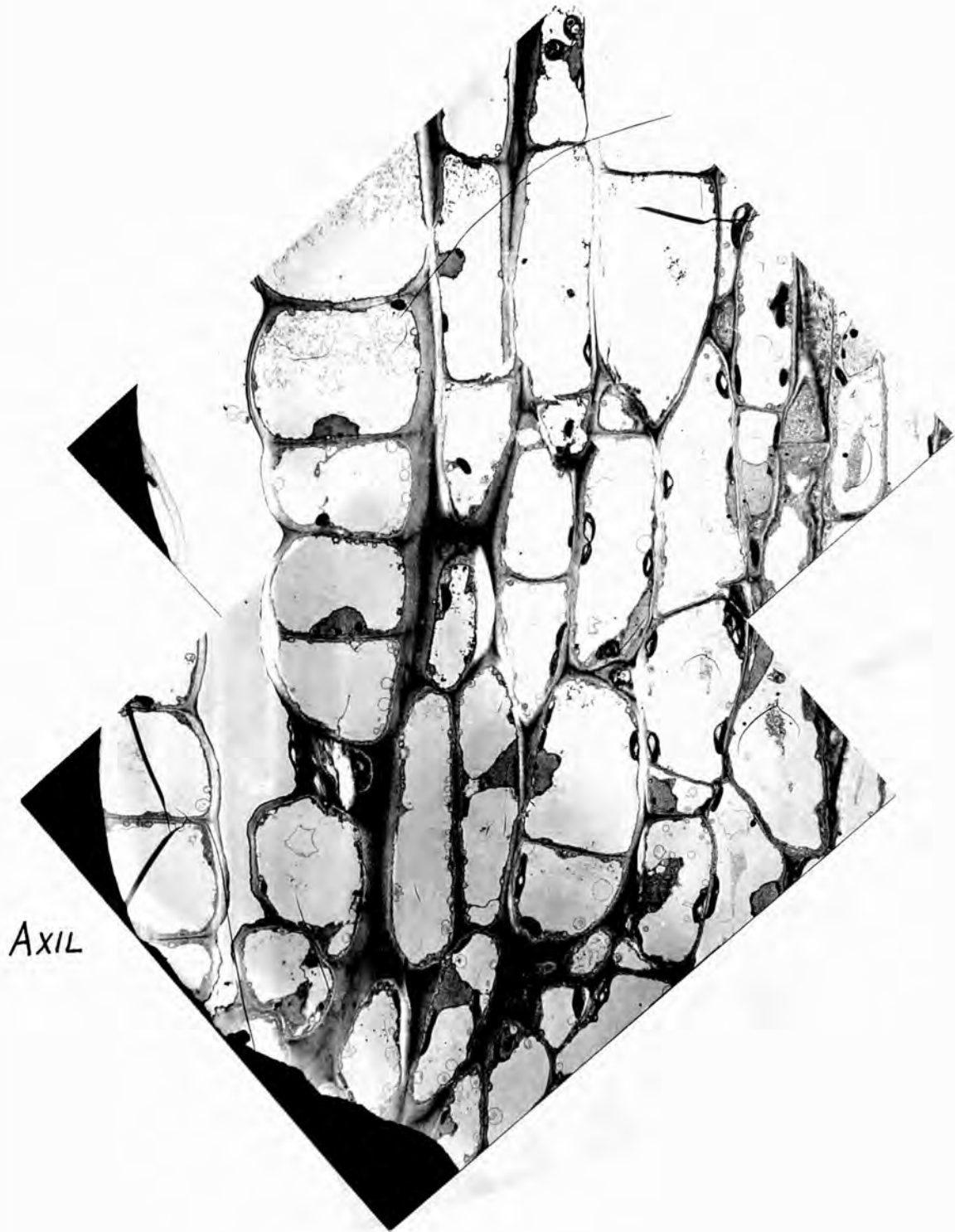
**A**

Fig. 4 - 3. Electron micrographs of the longitudinal sections of the base of leaf. The sections were 1  $\mu$  thick.

A. leaf from the plant in light.

B. leaf from the plant in dark.

Note a large number of vesicular invaginations of the plasmalemma (p). These are more numerous in B than in A.



**B**



AXIL

A

Fig. 4 - 4. Micrographs showing the epidermal and sub-epidermal tissue on the abaxial side of the leaf.

A. leaf from the plant in light.

B. leaf from the plant in dark.

There is no apparent difference in the cellular structure between A and B.

AXIL



A.

AXIL



B

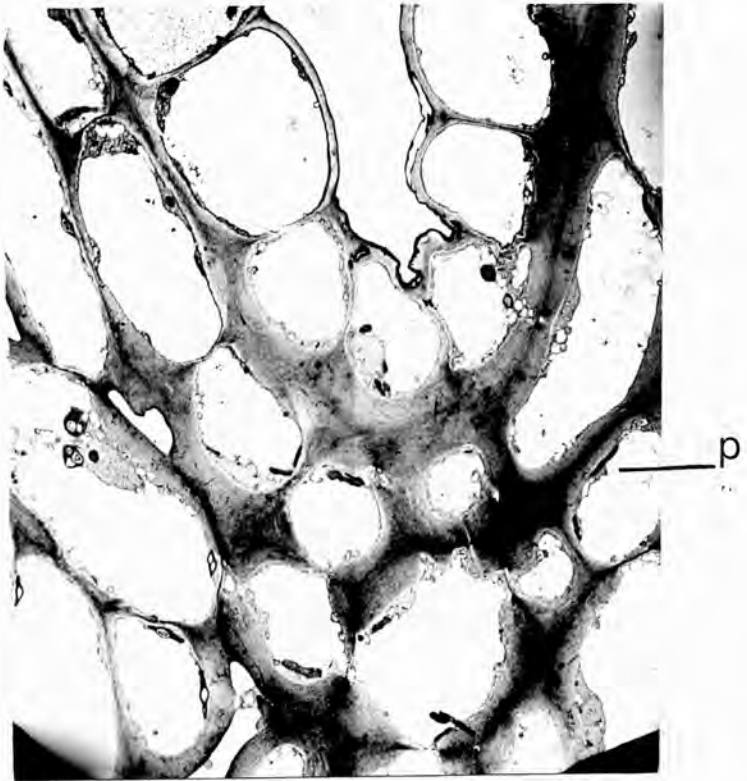
Fig. 4 - 5. Micrographs showing the tissue at the basal part of the leaf joint with stem.

A. leaf from the plant in light.

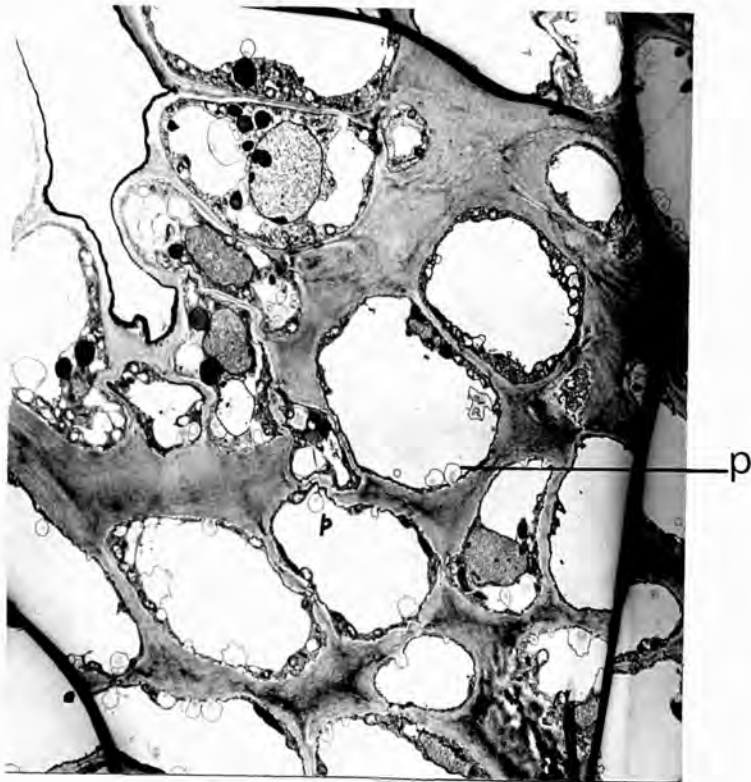
B. leaf from the plant in dark.

Note thick walls of the sub-epidermal cells in both A and B; numerous plasmalemma vesicles (p) and multivacuolate cells in B as compared to A.





**A**



**B**

Fig. 4 - 6. Epidermal cells in leaf angle at higher magnification.

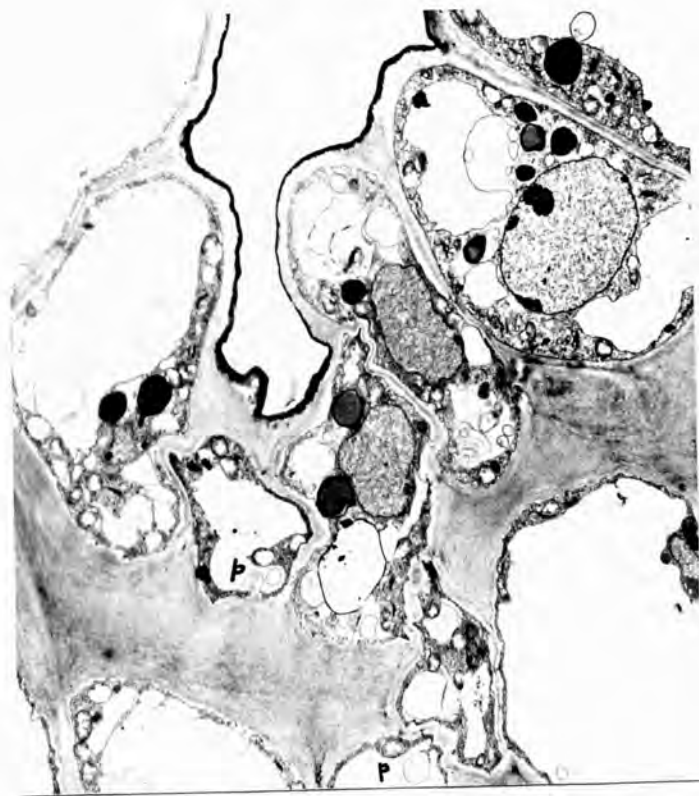
A. leaf from the plant in light.

B. leaf from the plant in dark.

Note much more multivacuolated epidermal cells in B as compared to A and plasmalemma vesicles are distinct.



A



B

Fig. 4 - 7. Micrographs of an epidermal cell in the leaf angle fixed during the dark.

Note the following identifiable sub-cellular structures:

o.w. outer wall of the cell.

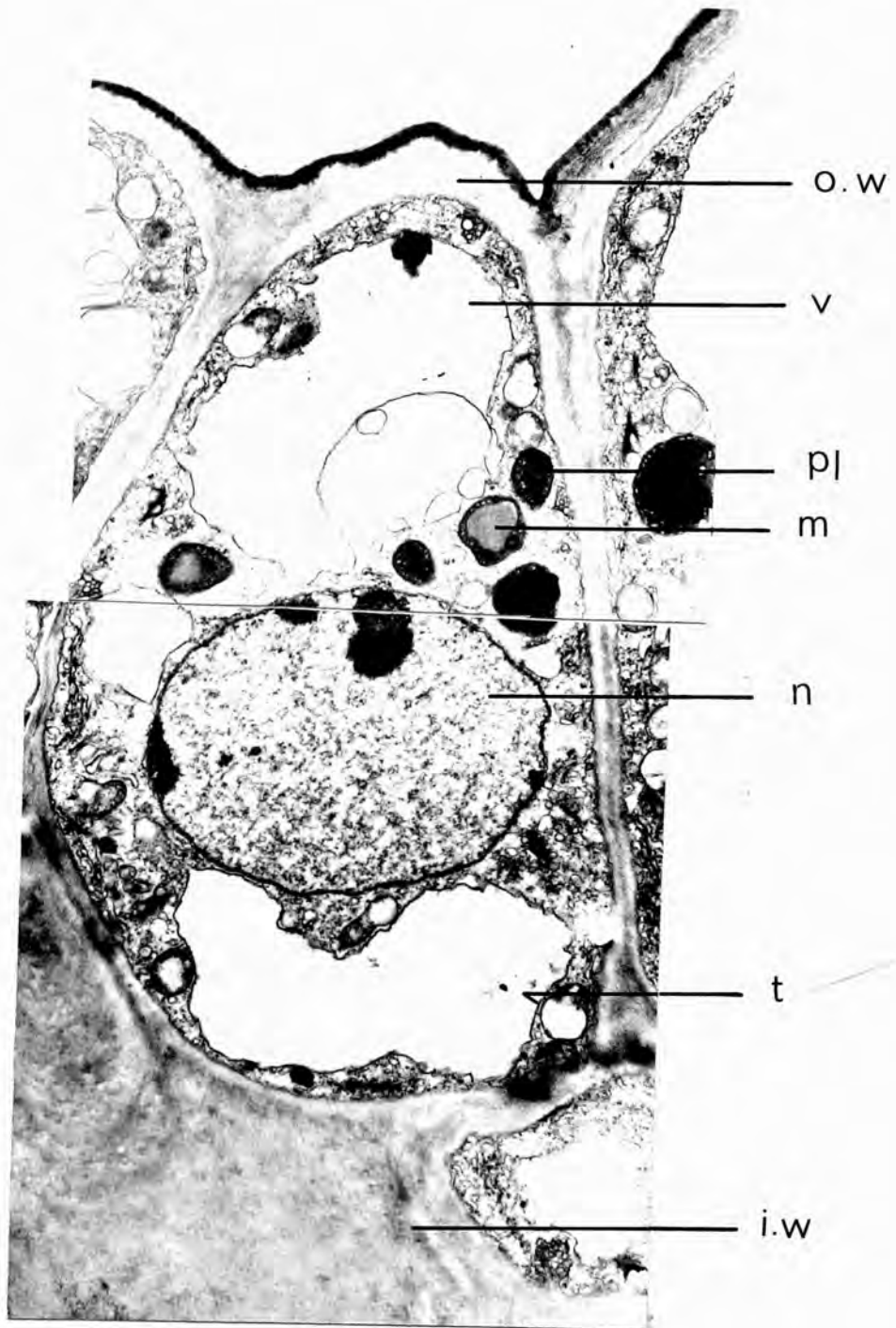
v. vacuole

pl. plastid

m. mitochondria

n. nucleus with distinct nucleoli

i.w. thicker inner wall of the cell



SECTION 5

GENERAL DISCUSSION AND BIBLIOGRAPHY

GENERAL DISCUSSION

This study of leaf movement in Linum usitatissimum has shown not only some points of similarity between the leaves of Linum, which are studied here for the first time, and the better known pulvinate leaves, but has also revealed several important differences. These differences are not shared with the few other non-pulvinate leaves which have been studied so far. The study has also touched on several problems that are worthy of further investigation.

As commonly found, the initial nyctinastic movement (a rising movement in Linum) is reversed after some hours despite the continuing dark; and likewise a rising movement will occur, though at a delayed time, if light is continued through the usual dark period. Such observations are the first clues to the participation of an endogenous circadian oscillation in the regulation of the leaf movement rhythm. It was found that the rhythmic leaf movement persists through a period of 48 h of continuous light and also that a rhythm in the nyctinastic response is evident when various longer periods of continuous light are brought to an end. Although not every possible test has been applied, the above mentioned observations together with the nature of the phase-shifts achieved with short periods of light given at various times, and the entrainment of the rhythm to a non-diurnal cycle, constitute adequate proof of the existence of a circadian oscillation regulating leaf movement in Linum.

Unless light is given no significant leaf movement takes place. Thus prior exposure to light is required for the nyctinastic response. A light period of at least 8 h is required to produce an immediate

response on transfer to darkness,<sup>1</sup> but a 4 h light period (red or white light) is sufficient to induce a rhythmic nyctinastic movement that commences 10 hours after the termination of the light period.<sup>2</sup> Rhythmic nyctinasty can also be induced by as little as 15 min red light given at the 7th or 9th hour of the subjective day, while a weaker reaction follows induction at the 14th hour.<sup>3</sup>

The course of nyctinastic movement following a 14 h light period corresponds closely to the course of the movement of rhythmic nyctinasty induced by a shorter light period. On the other hand, light periods of 10 h or 12 h produce a movement that takes a rather different course. After the initial rising movement the leaves are held steady for a few hours before a final small further rise takes them to the maximum position, the point at which reversal of movement occurs.<sup>4</sup> The nyctinastic leaf movement may be resolved into two components: one, an immediate rise which takes place when plants are darkened after 8 or more hours of light<sup>5</sup> and appears to be conditioned solely by the light-dark transition; and another, a rhythmic component which determines the maximum leaf position and reversal of movement. The two become coincident when the light period is 14 h long.

It appears that light given during but confined to the middle of the established light period is not only less effective for the induction of rhythmic nyctinasty but may actually be inhibitory.<sup>6</sup> A 7 h light period is less effective than a 4 h light period when they both commence at the established dawn.<sup>7</sup> Although a normal rhythmic movement can be induced by a 15 min exposure to red light at this time,<sup>8</sup> a longer exposure to red light in the middle only of the established light period is without such effect.<sup>9</sup> It was further found that this latter 15 min

---

1)Fig. 1-5; 2)Fig. 3-7 C and D; 3)Fig. 3-9; 4)Fig. 1-2; 5)Fig. 1-5;

6) Fig. 1-7 B; 7) Fig. 1-4 A and B; 8)Fig. 3-9; 9)Fig. 3-8 C



red light treatment was nullified if preceded by 4 h white light<sup>10</sup>. Apparently it is not only the wavelength of light received in the middle of established light period which is important, but also the length of time (or phase of the rhythm) during which the plant is exposed to light.

No similar finding exists in the literature and it is difficult to interpret the results so far obtained. It is well known that the middle of the established light period (subjective mid-day) is neutral so far as phase shifting by light is concerned, as is shown in the several phase-response curves that have been published, so it would not be expected that the phase of the movement has been shifted to beyond the sampled times. The conditions for induction of nyctinasty in leaves other than Linum have not, however, been subject to enquiry along the lines pursued herein as the rhythmic nyctinastic movement in Albizzia, for example, takes place without induction by light in each cycle.

Phase control and entrainment.

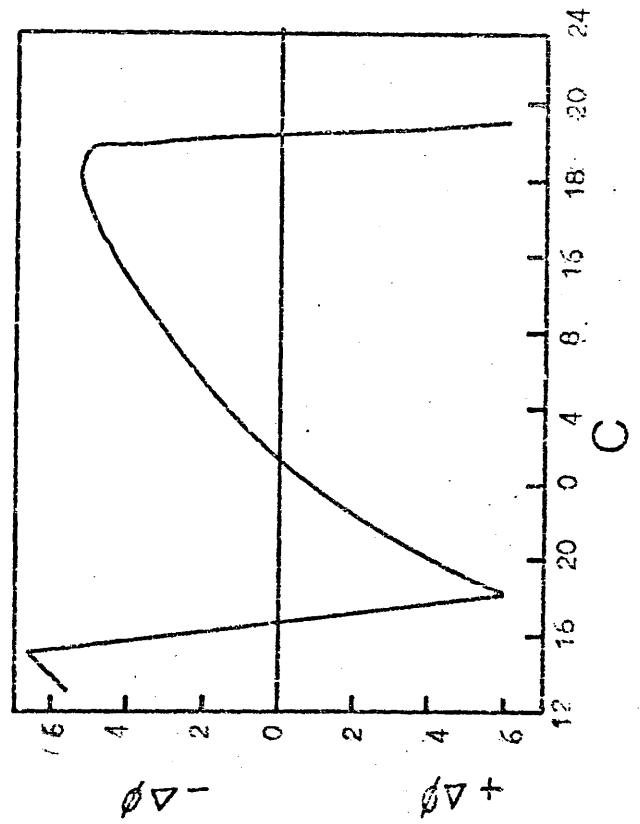
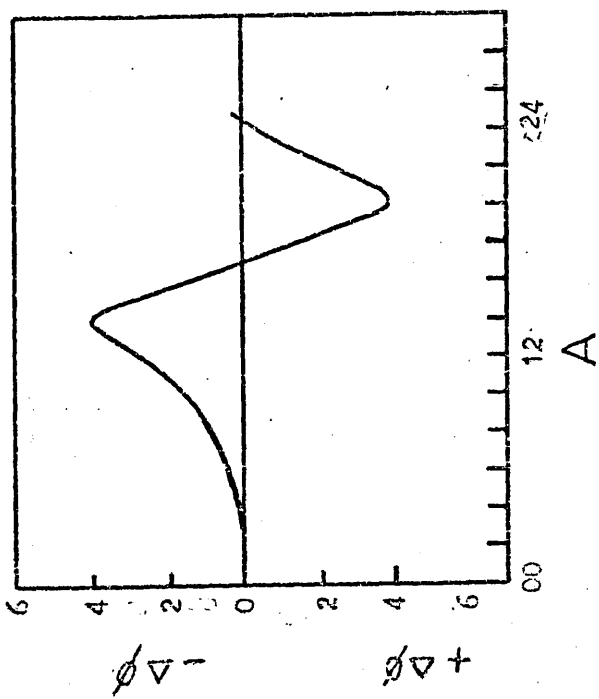
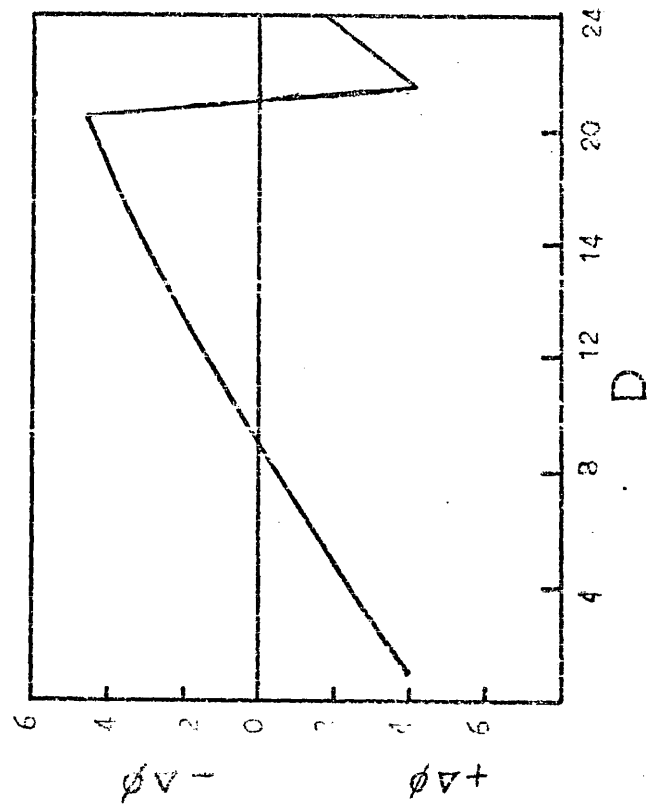
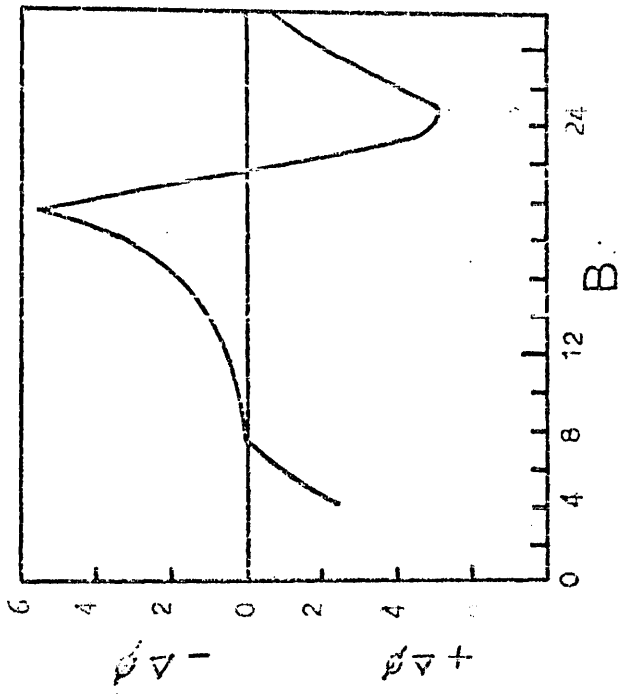
The present investigations of the properties of the endogenous rhythm are of course only of a preliminary nature, but nevertheless permit some conclusions to be drawn about the phase responses of the system. The most reliable indication of the phase response are the results of the experiments in which rhythmic nyctinastic movement was induced by 4 h light given at different times in the 24 h cycle of darkness, summarized in Section 2 - Comments. These results have been plotted as a phase response curve (Fig. 5 - 1 A).

---

10) Fig. 3-11 B

Fig. 5-1 Phase response curves.

- A. Linum usitatissimum. Leaf movement induced by 4 h light period in first cycle of darkness.
- B. Phaseolus multifloris. Leaf movement. 3 h higher intensity light periods (150--1500 lux.).  
Phase shift in first cycle. (Moser 1962)
- C. Coleus blumei. Steady-state phase shifts for 4 h periods of higher intensity light (170--22100 lux.) (Halaban 1968).
- D. Kalanchoe blossfeldiana. Petal movement. Based on phase shifts in first cycle in darkness produced by 2 h red light periods (Zimmer 1962).



Phase response curves strictly plot the phase-advances and phase-delays that are brought about by short, single perturbations of a steady state free-running rhythm and show that the phase response depends on the phase of the steady state rhythm that is perturbed (Pittendrigh 1965).

Although at present only four points are available for Linum they give an outline for a curve that closely resembles previously published phase response curves (Pittendrigh 1965). It should be emphasized that the Linum phase response curve refers specifically to movement during the first cycle of darkness and not to steady state phase shifts of a dark free-running rhythm. This first cycle would normally be regarded as a transient to the steady state. A comparable phase response for leaf movement of Phaseolus multiflorus has been plotted (Fig. 5 - 1 B) from the data of Moser (1962). This refers to the shifts in the "night" minimum leaf position brought about by raising the light intensity from 150 lux to 15000 lux for 3 h at different times during the 24 h cycle, and like Linum curve, refers to the shift in the first cycle only. It will be seen that there is very close agreement in the shapes of the curves.

The only other available curve for plant leaf movement is that for Coleus (Halaban 1968 b). This is given as Fig. 5 - 1 C and refers to the steady state (4th - 5th cycle) phase shifts in the free-running rhythm in dim white light brought about by 4 h white light of higher intensity.

Finally, for comparison, the curve showing the shifts in phase of minimum opening of petals of Kalanchoe blossfeldiana (Zimmer 1962) is given (Fig. 5 - 1 D). This refers to the first cycle (perturbed cycle) in darkness following a 2 h treatment with red light. Although further

advances had occurred by the second and third cycles the general shape of the curve remains as shown in the figure.

The phase response curve of Linum can be used to make clear the view taken of the mechanism of entrainment of the leaf movement to a non-diurnal  $10:\overline{10}$  cycle<sup>11</sup>. The free-running rhythm may be represented by a succession of these curves (as in Fig. 5 - 2 A).

This, of course, is hypothetical for Linum because no free-running rhythm has been tested for its phase response beyond the first cycle. A similar rhythm would exist when the system is entrained to a  $12:\overline{12}$  or a  $14:\overline{10}$  light:dark cycle. A difference would be expected in the period length, which would be almost exactly 24 h in the entrained plants but a natural free-running period length in those not so entrained. But in each case these may be presented on the 24 h Circadian Time scale (CT).

Referring now to Fig. 5 - 2 B, the effect on the rhythm of a transfer to the  $10:\overline{10}$  light:dark cycle is shown. After the initial cycle in which the phase remains unaffected the next "dawn" of the 10 h light period falls at the 20th hour and results in a phase advance of 4 h. A similar re-setting of the phase now occurs in each cycle. Repetitive phase setting has been suggested as the mechanism of entrainment of the circadian rhythms of Gonyaulax to non-diurnal cycles (Hastings and Sweeney 1959; Hastings 1964).

Confirmation of this interpretation requires measurement of the phase responses of the rhythm following the dawn signal at the 20th hour by the method used for the 24 h cycle, i.e., assaying the leaf movement after short periods of light given at different times through the cycle.

---

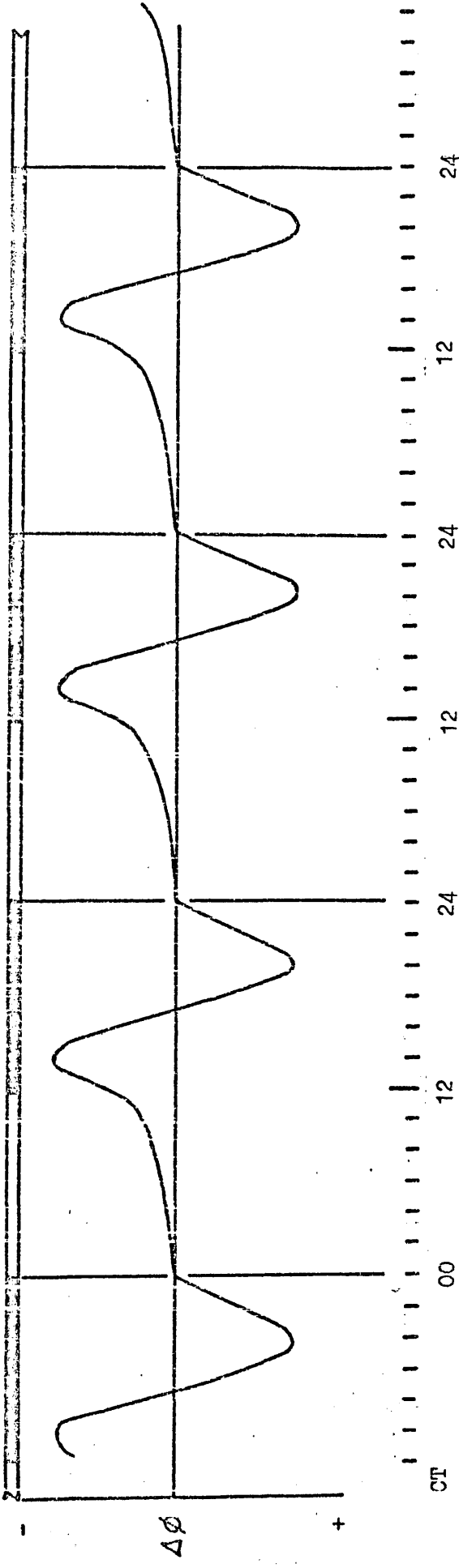
11) Fig. 2-3

Fig. 5-2

- A. A representation by phase response curve of the rhythm entrained to  $\overline{12:12}$  or  $\overline{14:10}$  light:dark cycles.
- B. A representation by phase response curve of the rhythm entrained to  $\overline{10:10}$  light:dark cycles.  
After the initial cycle, a phase advance of 4 h occurs with each "dawn."

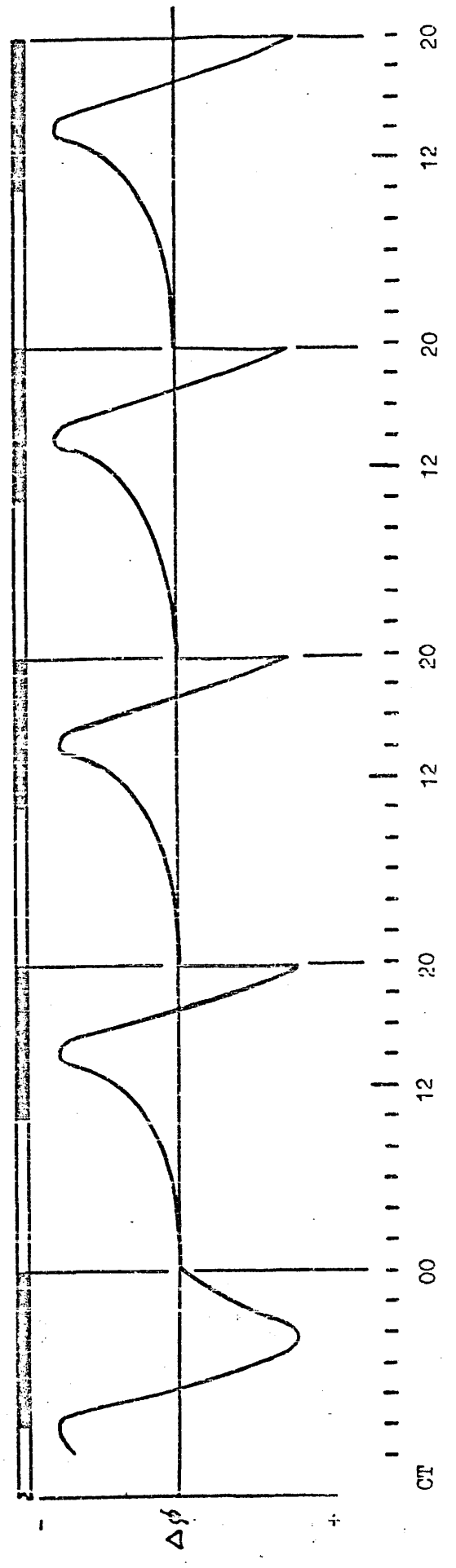
A

12:12



B

10:10



Direct investigation of the promotive effect of light on the downward movement of Linum leaves was not included in the present study. There is, therefore, no basis for detailed comparison with previous work on this aspect of the opening movements of pulvinate leaves. The pulvinate leaves of several species may be closed and opened repeatedly by alternation of light and darkness throughout the day, but there comes a time later in the day when re-opening will not immediately take place on re-exposure to light; this is the time when rhythmic control is greatest. When the phase of rhythmic activity is past the leaves become responsive to light once more so that exposure to light hastens their opening (Burkholder and Pratt 1936; Palmer and Asprey 1958).

With Linum it would be possible to investigate the effects of re-exposure to light at times after transfer to darkness at the 8th hour of the light period or later, and also the effects of re-exposure to light at times throughout the cycle of rhythmic nyctinastic movement.

It has been shown in Linum that light holds the leaves down until rhythmic control completely overrides this effect and that it is the blue end of the spectrum which operates here: plants transferred to red light behave almost as if they had been placed in the dark<sup>12</sup>. In this respect the leaves of Linum are identical to pulvinate leaves of Leguminosae and Oxalis whose direct simple responses to blue and red light have been known since the investigations of Sachs. It must, however, remain for further study to determine whether the actual downward movement is promoted by blue light in Linum as is the opening movement of the leaves of Mimosa (Burkholder and Pratt 1936; Fondeville et al. 1967) and Albizzia (Evans and Allaway 1972). This may be contrasted

---

12) Fig. 3-14



with the photohyponastic effect of blue light exhibited both by leaves of Phaseolus (Brauner 1959) which are lowered at night and by Linum cotyledons (Brett 1970) which are raised at night. Perhaps this is a long-term photonastic effect rather than one which involves the daily periodic movements.

The phytochrome system.

The phytochrome system is involved in the regulation of the leaf movements in Linum, and there are some obvious points of similarity between the results described herein and the results of experiments with Albizzia (Hillman and Koukkari 1967; Satter and Galston 1971, 1973; Evans and Allaway 1972).

Far-red light slows the rising leaf movement at the end of a 10 h light period<sup>13</sup> but not at the end of a 14 h light period; it also has no effect on the rising movement of rhythmic nyctinasty when it is given later in the day. Pinnule closure can be evoked in Albizzia at all times throughout the light period and far-red light greatly inhibits this closure in the early hours of the light period but then has progressively less effect until there is no effect at the end of the light period when rhythmic control of the pinnule closing mechanism is complete.

In Linum leaves do not respond to darkness in the early hours of the light period. Nevertheless the course of nyctinasty following 10 h light is interpreted as having two components and it is the non-rhythmic response to darkness which is inhibited by treatment with far-red light. In Albizzia the reason for this inability of the phytochrome to exert control later in the circadian cycle appears to be that both  $P_{fr}$  and

the endogenous rhythmic system regulate (though by different means) one and the same mechanism of turgor changes viz. potassium flux out of the ventral cells into the dorsal cells of the pulvinule (Satter and Galston 1971). There seems good reason to conclude therefore that a similar mechanism may exist in Linum.

The effect of far-red light on the reverse, downward leaf movement in Linum is consistently to speed it up. This occurs whether far-red is given at 10 h or 14 h light period; and the effect is a long-lasting one because when far-red is given after a 10 h light period the effect is not manifested till some 8 h later, and an even longer delay may occur between a far-red break at the dark after a 4 h inductive light period and the rhythmic movement<sup>14</sup>. This timing of the reverse downward movement is, of course, always in a definite phase relationship to the rhythm, and no variation can exist depending solely on the length of the light period. The pinnule opening movement of Albizzia is promoted by far-red light (Evans and Allaway 1972). The rhythmic opening in prolonged darkness is associated with a large flow of potassium into the ventral cells and a relatively small efflux from the dorsal cells.

The efflux from the dorsal cells, however, is considerably enhanced by blue light which consequently promotes opening and maintains the open position during daylight hours. If the mechanism in Linum is at all comparable to that in Albizzia there are three possibilities for the far-red promotive effect:

- (1) Expansion of ventral cells (K influx)
- (2) Contraction of dorsal cells (K efflux)
- (3) A combination of both (1) and (2).

---

14) Fig. 3-5 B

Anatomically there is a great difference between the base of the Linum leaf and a pulvinule of Albizzia. The only cells that look as if they might be specialised for turgor changes are in a small group in the ventral side of the leaf base.

is a  
scattered  
omitted here?

In comparison with the Albizzia mechanism this is entirely understandable for what is normally promoted by darkness may be expected to be promoted also by brief exposure to far-red radiation if the phytochrome system is involved. Such an explanation, however, also involves the supposition that rhythmic control only lasts through the rising phase of the leaf movement of Linum and that the reversal of movement occurs when that control is relaxed. Perhaps this interpretation is supported by the results of experiments which demonstrate that the rising movement is considerably weakened as the transition from light to darkness is delayed beyond the point of strongest rhythmic control.

As previously demonstrated by Halaban (1969), red and blue light have opposite effects on the leaf movement rhythm: red light advances the rhythm, blue light delays it. The red light advancing action in Linum comes at a time in the cycle when phytochrome control of leaf movement induction can be demonstrated whereas the system appears to have been released from phytochrome by the time the delaying effect of blue light is shown.

Halaban's (1969) suggestion that the circadian rhythm is periodically responsive to blue or red radiation can be followed for Linum leaf movement, but definite evidence for phytochrome control in Coleus leaf movement was not shown. Halaban put this forward as a point of difference between the entrainment of the circadian rhythm and the photoperiodic induction which is mediated by phytochrome.

The overwhelming evidence that now exists in favour of the participation of the circadian rhythm in photoperiodic induction (King and Cumming 1972 a, b) makes this a very important issue. Much further study is required to clarify the situation in rhythmic leaf movement and photoperiodic induction where a periodicity in phytochrome activity is implicated.

Bibliography

- Alford, D. K. and T. W. Tibbitts (1970). Circadian rhythm of leaves of Phaseolus angularis plants grown in a controlled carbon dioxide and humidity environment. *Pl. Physiol.*, 46: 99-102.
- Asprey, G. F. and J. H. Palmer (1955). A new interpretation of the mechanics of pulvinus movement. *Nature*, 175: 1122..
- Bailey, N. T. J. (1959). *Statistical Methods in Biology*. The English University Press Ltd. London.
- Barber, H. N., Halsall, D. M. and J. H. Palmer (1968). Genetic, environmental and physiological control of leaf orientation in Plantago lanceolata. *Aust. J. biol. Sci.*, 21: 641-648.
- Borthwick, H. A. (1964). Phytochrome action and its time displays. *Amer. Nat.*, 98: 347-355.
- Borthwick, H. A., Hendricks, S. B., Parker, M.W., Toole, E. H. and V. K. Toole (1952). A reversible photoreaction controlling seed germination. *Proc. Natl. Acad. Sci. U.S.A.*, 38: 662-666.
- Brauner, L. (1959). Phototropismus und Photonastie der Laubblätter. *Handbuch der Pflanzenphysiologie*, XVII/1: 472-491.
- Brauner, L. and N. Arslan (1951). Experiments on the auxin reactions of the pulvinus of Phaseolus multiflorus. *Rev. Fac. Sci. Univ. Istanbul*, 16/B:257-300.

- Brauner, M. (1932). Untersuchungen über die Lichtturgorreaktionen des Primärblattgelenkes von Phaseolus multiflorus.  
 Planta (Berl.), 18: 288-337.
- Brett, D. W. (1970). Shoot auxin and light in the mechanism maintaining the posture of the cotyledons of Linum usitatissimum.  
 J. Exp. Bot., 21: 432-445.
- Bünning, E. (1944). Die allgemeinen Grundlagen der photoperiodischen Empfindlichkeit.  
 Flora, 38: 93-112.
- Bünning, E. (1957). Endogenous diurnal cycles of activity in plants.  
 In: "Rhythmic and Synthetic Processes in Growth", ed. Rudnick.  
 Princeton University Press.
- Bünning, E. (1959). In: "Photoperiodism and Related Phenomena in Plants and Animals", ed. R. B. Withrow.  
 A.A.A.S., Washington, D.C.
- Bünning, E. (1960). Cold Spring Harbor Symp. Quant. Biol., 25:249-256.
- Bünning, E. (1967). The Physiological Clock.  
 Longmans, Springer-Verlag.  
 New York.
- Bünning, E., Kurras, S. and V. Vielhaben (1965). Phasenverschiebungen der endogenen Tagesrhythmik durch reduktion der Atmung.  
 Planta (Berl.), 64:291-300.
- Bünning, E. and V. Lörcher (1957). Regulierung und Auslösung endogen-tagesperiodischer Blattbewegungen durch verschiedene Lichtqualitäten.  
 Naturwiss., 17:472.

- Bünning, E. and I. Moser (1966). Response-kurven bei der circadianen Rhythmik von Phaseolus.  
 Planta (Berl.), 69:101-110.
- Bünning, E. and K. Stern (1930). Ueber die tagesperiodischen Bewegungen der Primärblätter von Phaseolus multiflorus. II. Die Bewegungen bei Thermokonstanz.  
 Ber. dtsh. bot. Ges., 48: 227-252.
- Burkholder, P. R. and R. Pratt (1936). Leaf movements of Mimosa pudica in relation to light.  
 Am. J. Bot., 23:46-52.
- Cumming, B. G. and E. Wagner (1968). Rhythmic processes in plants.  
 A. Rev. Pl. Physiol., 19:381-416.
- Darwin, C. and F. Darwin (1880). The Power of Movements in Plants, pp. 407-408.  
 Appleton and Co., New York.
- De Candolle, A.D. (1832). In "Physiologie vegetale ou exposition des forces et fonctions vitales des vegetaux", 2, Book 4, Cap. 6.  
 Bechet, Lib. Fac. Med.  
 Paris.
- De Mairan (1729). Proc. Roy. Acad. Sci. (Paris). 35 pp.
- Duncan, D.B. (1955). Multiple range and multiple F tests.  
 Biometrics, 11:1 - 42.
- Fondeville, J.C., Borthwick, H. A. and S. B. Hendricks (1966). Leaflet movement of Mimosa pudica L. indicative of phytochrome action.  
 Planta (Berl.), 69:357-364.

- Fondeville, J.C., Schneider, M. J., Borthwick, H.A. and S. B. Hendricks (1967). Photocontrol of Mimosa pudica L. leaf movement. *Planta* (Berl.), 75: 228-238.
- Flügel, A. (1949). Die Gesetzmässigkeiten der endogenen Tagesrhythmik. *Planta* (Berl.), 37:337-375.
- Fredericq, H. (1964). Conditions determining effects of far-red and red irradiation on flowering response of Pharbitis nil. *Pl. Physiol.*, 39: 812.
- Guttenberg, H. V. and L. Kröpelin (1947). Ueber den Einfluss des Heteroauxins auf das Laminargelenk von Phaseolus coccineus. *Planta* (Berl.), 35:257-280.
- Halaban, R. (1968 a). The circadian rhythm of leaf movement of Coleus blumei X C. frederici, a short day plant. I. Under constant light conditions. *Pl. Physiol.*, 43:1883-1886.
- Halaban, R. (1968 b). II. The effects of light and temperature signals. *Pl. Physiol.*, 43:1887-1893.
- Halaban, R. (1969). Effects of light quality on the circadian rhythm of leaf movement of a short-day plant. *Pl. Physiol.*, 44:973-977.
- Halberg, F., Halberg, E., Barnum, C. P. and J. J. Bittner (1959). In "Photoperiodism and Related Phenomena in Plants and Animals", ed. R.B. Withrow. A.A.A.S., Washington, D.C., pp. 803-878.
- Hastings, J.W. (1964). In "Photophysiology", Vol. I, pp. 333-361. Ed. A.C. Giese. Academic Press, New York, London.



- Hastings, J. W. and B. M. Sweeney (1959). The Gonyaulax clock.  
In "Photoperiodism and Related Phenomena in Plants and  
Animals", ed. R. B. Withrow.  
A.A.A.S., Washington, D.C.
- Hastings, J.W. and B. M. Sweeney (1960). The action spectrum for  
shifting the phase of the rhythm of luminescence in  
Gonyaulax polyedra.  
J. Gen. Physiol., 43:697-706.
- Hill, H.D. (1757). The Sleep of Plants.  
Baldwin, London. 60 pp.
- Hillman, W. S. and W. L. Koukkari (1967). Phytochrome effects in the  
nyctinastic leaf movements of Albizzia julibrissin and some  
other legumes.  
Pl. Physiol., 42: 1413-1418.
- Holdsworth, H. M. (1960). The spectral sensitivity of light-induced  
leaf movement.  
J. Exp. Bot., 11:40-44.
- Hoshizaki, T. and K. C. Hamner (1964). Circadian leaf movements:  
Persistence in bean plants grown in continuous high-  
intensity light.  
Science, 144:1240-41.
- Hoshizaki, T., Brest, D. E. and K. C. Hamner (1969). Xanthium leaf  
movement in light and dark.  
Pl. Physiol., 44:151-152.
- Inada, K. (1969). Effect of blue light on the photonastic reaction of  
rice leaves.  
Plant and Cell Physiol., 10:845-854.

- Jaffe, M. J. and A. W. Galston (1967). Phytochrome control of rapid nyctinastic movements and membrane permeability in Albizzia julibrissin.  
 Planta, 77:135-141.
- Kadman, S. and A. Zahari (1972). Persistence of  $P_{fr}$  and other manifestations of phytochrome in the nyctinastic leaflet movement of peanut (Arachis hypogoea L.).  
 Israel J. Bot., 21:142-149.
- Karve, A. D. and S. G. Jigajinni (1965). Circadian rhythm in Portulaca.  
 Z. Pflanzenphysiologie, 53:169-172.
- Karve, A. D. and S. G. Jigajinni (1966). Leaf movement of Portulaca under different photoperiods.  
 Z. Pflanzenphysiologie, 54:270-274.
- Kleinhoonte, A. (1929). Ueber die durch das Licht regulierten autonomen Bewegungen der Canavalia Blätter.  
 Arch. neerl. Sci. ex. et nat. IIIb, 5:1-110.
- Kleinhoonte, A. (1932). Untersuchungen über die autonomen Bewegungen der Primärblätter von Canavalia ensiformis.  
 Jahrb. f. wiss. Bot., 75:679-725.
- Könitz, W. (1958). Blühhemung bei Kurztagpflanzen durch Hellrot und Dunkelrotlicht in der photo- und scotophilen Phase.  
 Planta, 51:1-29.
- Koukkari, W. L., Halberg, F. and S. A. Gordon (1973). Quantifying rhythmic movements of Albizzia julibrissin.  
 Pl. Physiol., 51:1084-1088.

- Koukkari, W. L. and W. S. Hillman (1968). Pulvini as photoreceptors in the phytochrome effect on nyctinasty in Albizzia julibrissin.  
Pl. Physiol., 43:698-704.
- Leinweber, F. J. (1956). Ueber die Temperaturabhängigkeit der Periodentage bei der endogenen Tagesrhythmik von Phaseolus.  
Z. Bot., 44:337-364.
- Linnaeus, C. (1751). Philosophia botanica. 274 pp.  
Stockholm.
- Linnaeus, C. (1755). Somnus plantarum.  
Amoenitat Academicae 4:333.
- Lörcher, L. (1958). Die Wirkung verschiedener Lichtqualitäten auf die endogene Tagesrhythmik von Phaseolus.  
Z. Bot. 46:209-242.
- Lyon, C. J. (1964). Auxin transport in leaf epinasty.  
Pl. Physiol., 39:567-74.
- Metzner, P. (1934). Untersuchungen über den Pflanzenschlaf.  
Forsch. u. Fortsch., 10: 172-173.
- Moser, I. (1962). Phasenverschiebungen der endogenen Tagesrhythmik bei Phaseolus durch Temperatur und Lichtintensitätsänderungen.  
Planta (Berl.), 58:199-219.
- Palmer, J. H. (1964). Comparative study of the effects of applied indoleacetic acid and horizontal orientation of the primary shoot, upon internode extension and petiole orientation in Helianthus annuus, and the modifying influence of gibberellic acid.  
Planta (Berl.), 61:283-297.

- Palmer, J. H. and G. F. Asprey (1958). Studies in the nyctinastic movement of the leaf pinnae of Samanea saman (Jacq.) Merrill. I - A general description of the effect of light on the nyctinastic rhythm. *Planta* (Berl.), 51:757-769.
- Palmer, J. H. and D. M. Halsall (1969). Effect of transverse gravity stimulation, gibberellin and indoleacetic acid upon polar transport of IAA-<sup>14</sup>C in the stem of Helianthus annuus. *Physiol. Plantarum*, 22:59-67.
- Pfeffer, W. (1875). Die periodischen Bewegungen der Blattorgane. Engelmann, Leipzig, 176 pp.
- Pfeffer, W. (1915). Beiträge zur Kenntnis der Entstehung der Schlafbewegungen. *Abhandl. Math. Phys. Kl. Kön. Säch. Ges. d. Wiss.*, 34: 1-154.
- Pittendrigh, C. S. (1954). On temperature independence in the clock system controlling emergence time in Drosophila. *Proc. Natl. Acad. Sci. U.S.A.*, 40:1018-1029.
- Pittendrigh, C. S. (1960). Cold Spring Harbor Symp. Quant. Biol., 25:159-182.
- Pittendrigh, C. S. (1965). On the mechanism of the entrainment of a circadian rhythm by light cycles. In "Circadian Clocks", ed. J. Aschoff, pp. 277-297. North-Holland.
- Portheim, L. (1941). Further studies on the action of heteroauxin on Phaseolus vulgaris. *Ann. Bot., N.S.* 5:35-46.

- Sachs, J. (1887). Lectures on the physiology of plants.  
Trans. H. M. Ward.  
Clarendon Press,  
Oxford.
- Satter, R. L. and A. W. Galston (1971). Phytochrome controlled  
nyctinasty in Albizia julibrissin. III. Interaction between  
an endogenous rhythm and phytochrome in control of potassium  
flux and leaflet movement.  
Pl. Physiol., 48:740-746.
- Satter, R. L., Marinoff, P. and A. W. Galston (1970). Phytochrome  
controlled nyctinasty in Albizia julibrissin. II. Potassium  
flux as a basis for leaflet movement.  
Amer. J. Bot., 57:916-926.
- Semon, R. (1905). Ueber die Erbllichkeit der Tagesperiode.  
Biol. Zbl., 25:241-252.
- Sweet, H. C. and S. W. Hillman (1969). Phytochrome control of nyctinasty  
in Samanea as modified by oxygen, submergence and chemicals.  
Physiol. Plantarum, 22:776-786.
- Wilkins, M. B. (1962). An endogenous rhythm in the rate of CO<sub>2</sub> output  
of Bryophyllum. IV. Effect of intensity of illumination on  
entrainment of the rhythm by cycles of light and darkness.  
Pl. Physiol., 37:735-741.
- Wilkins, M. B. (1969). Circadian Rhythms in Plants. In "Physiology  
of Plant Growth and Development", ed. M. B. Wilkins, pp.  
647- 671.  
McGraw-Hill, London.

- Williams, C. N. and V. Rhagavan (1966). Effects of light and growth substances on the diurnal movements of the leaflets of Mimosa pudica.  
J. Exp. Bot., 17:742-749.
- Yin, H. C. (1941). Studies on nyctinastic movement of the leaves of Carica papaya.  
Am. J. Bot., 28:250-261.
- Zinn, J. G. (1759). Von dem Schläfe der Pflanzen.  
Hamburg Mag., 22:40-50.
- Zimmer, R. (1962). Phasenverschiebung und andere Störlichtwirkungen auf die endogen tagesperiodischen Blütenblattbewegungen von Kalanchoë blossfeldiana.  
Planta (Berl.), 58:283-300.

Additional references:

- Evans, L. T. and W. G. Allaway (1972). Action spectrum for the opening of Albizzia julibrissin pinnules, and the role of phytochrome in the closing movements of pinnules and of stomata of Vicia faba.  
Aust. J. Biol., 25:885-893.
- King, R. W. and B. G. Cumming (1972 a). Rhythm as photoperiodic timers in the control of flowering in Chenopodium rubrum.  
Planta (Berl.), 103:281-301.
- King, R. W. and B. G. Cumming (1972 b). The role of phytochrome in photoperiodic time measurement and its relation to rhythmic timekeeping in the control of flowering of Chenopodium rubrum.  
Planta (Berl.), 108:39-57.
- Satter, R. L. and A. W. Galston (1973). Leaf movements: Rosetta stone of plant behaviour?  
BioScience, 23:407-415.
- Satter, R. L., Sabnis, D. D. and A. W. Galston (1970). Phytochrome controlled nyctinasty in Albizzia julibrissin. 1. Anatomy and fine structure of the pulvinule.  
Amer. J. Bot., 57:374-381.
- Weintraub, M., (1952). Leaf movements in Mimosa pudica L.  
New Phytol., 50:357-382.

SECTION 6

APPENDIX



APPENDIXShadowgraph Technique

This technique was used in earlier experiments. The plants were cut and arranged on a photographic plate, which was then exposed to light for 3 seconds. The plate was developed in I.D. 36 and fixed in Kodafix. Angles were measured by enlarging the shadowgraphs of the leaves on a scale ruled in degrees.

A contact print of a typical plate is shown in Fig. 6 - 1.

The angles obtained by shadowgraph method were not significantly different from those measured by hand as mentioned in Materials and Methods Section.

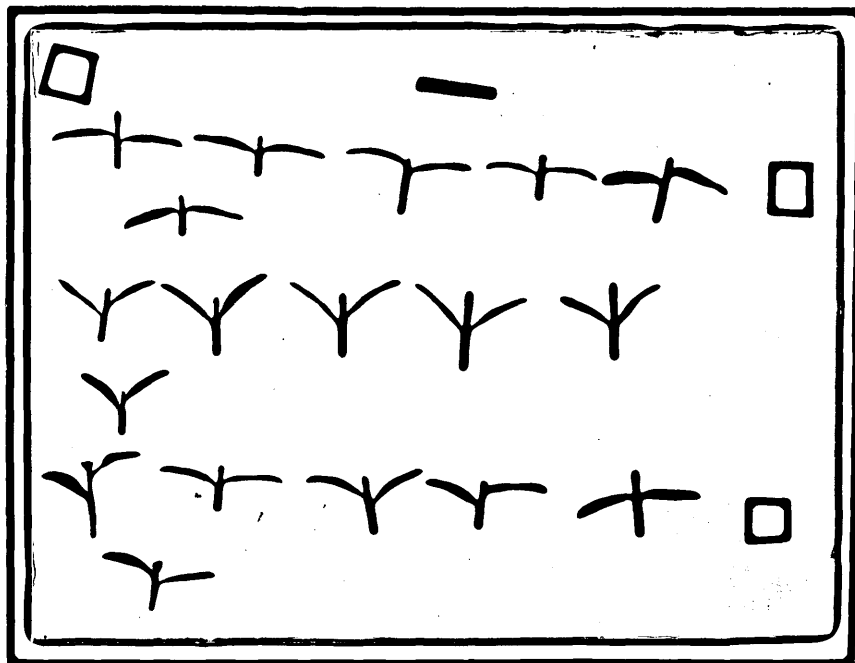


Fig. 6 -1. Contact print of the photographic plate showing the shadowgraphs of the leaves.

Effect on leaf movement of excision of the shoot and application of  
Indole Acetic Acid (IAA).

Preliminary experiments showed that decapitation of the shoot of Linum seedling resulted in a hyponastic reaction of the remaining leaves, following which no further movements appear to take place. In the experiments described here the shoot above the measured leaves was replaced with various concentrations of IAA in lanolin. The shoot was excised and IAA was applied immediately prior to the 14 h light period and intact plants were kept as the control. After the 14 h light period, measurements were taken at the 14th and the 20th h of the established cycle.

Brett (1970) has shown that certain concentrations of applied IAA prevent the long term epinastic reaction of Linum cotyledons in the prolonged darkness. In the present experiment it can be seen that IAA prevents the hyponastic reaction of the leaves in the light, the angle of leaves on decapitated shoots (Fig. 6 - 2 A) being over  $30^{\circ}$  higher at the end of light period than those on intact plants (Fig. 6 - 2 D) and over  $25^{\circ}$  above the leaves of decapitated plants treated with 5 ppm IAA (Fig. 6 - 2 C). Greater and smaller concentrations of IAA permit hyponasty but while the 10 ppm concentration leads to epinasty (Fig. 6 - 2 E), 1 ppm allows the nyctinastic movement.

Fig. 6 - 2 also shows that plants with 5 ppm IAA (curve C) showed nyctinastic movement which corresponded closely to that of the intact control (curve B). It is not clear, however, whether or not the shoot apex (or IAA) is necessary for nyctinastic movement to occur. There is in fact a small nyctinastic rise seen in the decapitated plant without IAA and it is a possibility that the full nyctinastic response is only

prevented by the initially high angle of the leaf which is therefore physically incapable of rising further. Thus, although IAA at physiological concentration may have a regulatory influence on the posture of the leaves in both light (by preventing hyponasty) and dark (by preventing epinasty), its role, if any, in the nyctinastic rising movement of the leaf of Linum is not definitely proved. Further discussion of the role of auxin in leaf movement is given in the general introduction of this thesis.

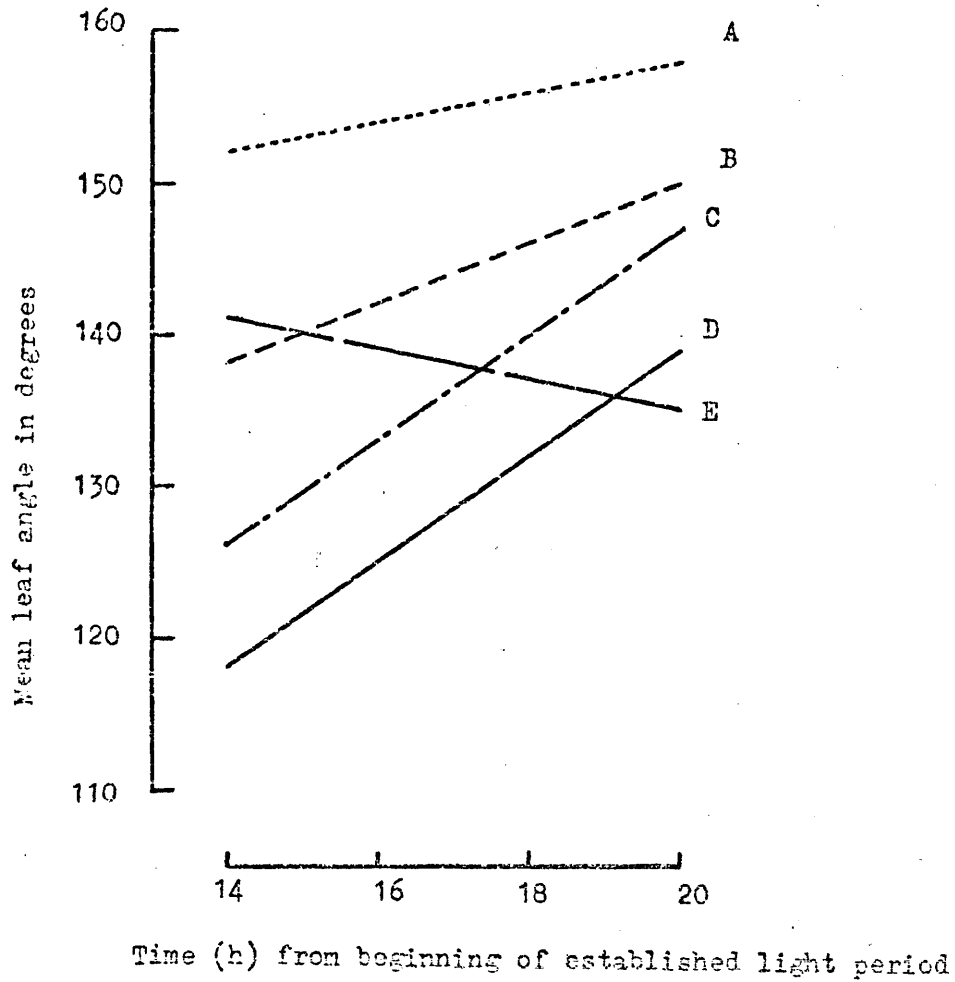


fig. 6-2. Effect of various concentrations of IAA on nyctinastic leaf movement.

Concentrations given are, 0ppm (lanolin) A; 1ppm B; 5ppm C; intact plants D; 10 ppm E. All treatments were given at the beginning of established light period and measurements taken 14 h after during the dark at the 14th and 20th h.

Values are means of 20 replicates.

Effect on nyctinastic movement of painting the leaf with India ink.

Since intact plants are routinely used in all the main investigations the question arises whether the effect of light on the leaf movements is to be attributed to the action on the leaf itself or whether the light is perceived by the rest of the plant and a stimulus transmitted to the site of action, i.e., the leaf. Darkening the leaves individually could not be achieved by any means other than painting them, and India ink was used for this purpose. The upper surface only was painted.

Figures 6 - 3 and 6 - 4 show the results of two experiments. In the first experiment, both the measured pair of leaves on each plant were either painted or unpainted. The second set of unpainted plants remained in darkness while the other plants were then transferred to light for the full 14 h light period. The angle of the leaves was measured at the end of the light period and thereafter in the dark.

Fig. 6 - 3 shows that the nyctinastic movement of the painted leaves after the 14 h light period is very little compared to the unpainted leaves which received full light. Plants that were in uninterrupted darkness also showed little response (curve B).

In the second experiment only one of the measured pair of leaves was painted and the other leaf was left as a control which received the full light period. It is seen in Fig. 6 - 4 that the painted leaf showed very little movement and the normal movement occurred in the unpainted leaf.

The results of the experiments appear to indicate that the perception of light for induction of nyctinastic movement takes place in the leaf itself and does not appear to be transmitted from one leaf to another. Any further conclusions require further study of this aspect of the mechanism of leaf movement.

Fig. 6 - 3. Effect of painting the upper surface of both measured leaves with India ink.

Light control received 14 h white light (A); uninterrupted dark control (B); and both leaves painted (C).

The leaves were painted at the end of the dark period, after which the plants were transferred to the 14 h light period. Measurements were taken through 8 h of darkness, 14 h from beginning of established light period.

Values are means of 12 replicates. Vertical bars indicate 95% confidence limits.

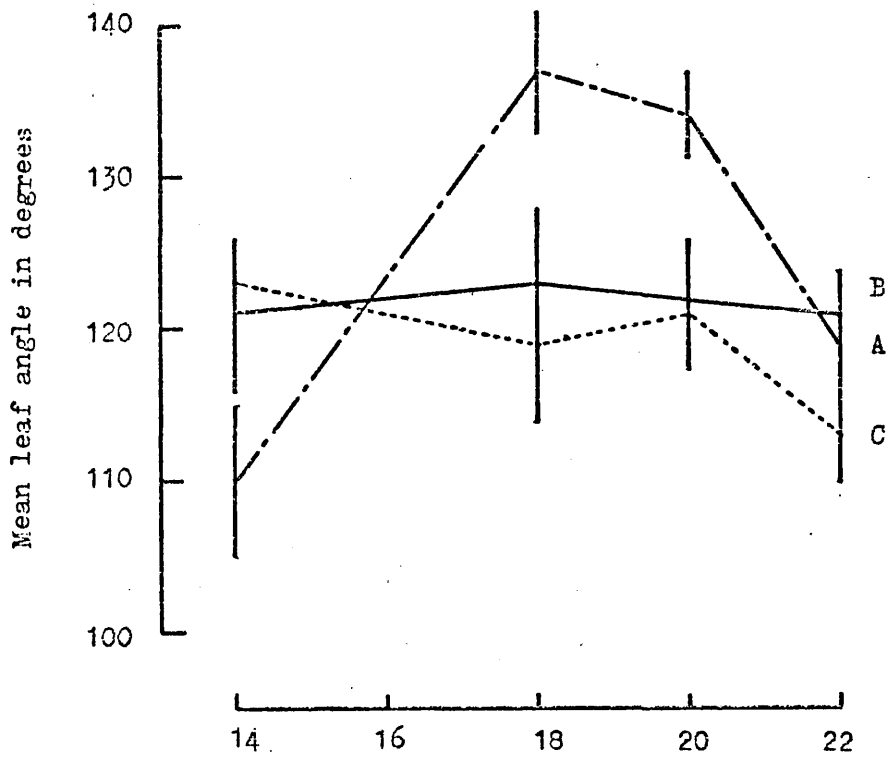
Fig. 6 - 4. Effect of painting the upper surface of only one of the measured leaves with India ink.

One leaf unpainted as a control (A); and the other leaf on the same plant, painted (B).

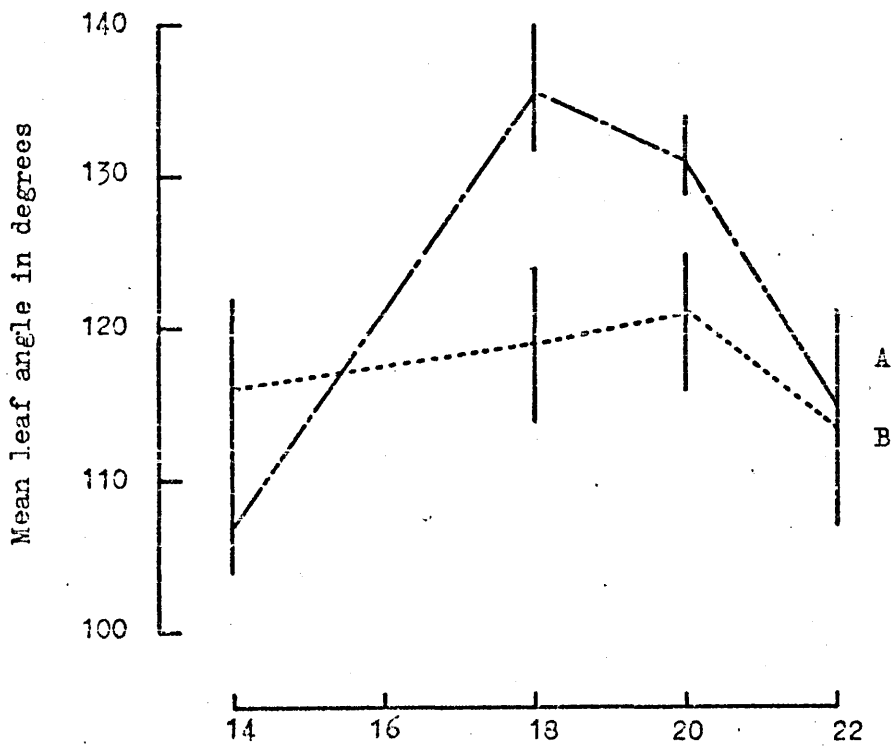
Experimental procedure as given above for Fig.

6 - 3.

Values are means of 12 replicates. Vertical bars indicate 95% confidence limits.



Time (h) from beginning of established light period



Time (h) from beginning of established light period

Table 6 - 1

Effect of painting of two leaves on a plant with India ink.

Light control received 14 h light (A); uninterrupted dark control (B); and two leaves painted (C). Measurements were taken during the dark 14 h from beginning of established light period.

Values are means of 12 replicates with 95% confidence limits.

(Data of Fig. 6 - 3).

Treatment	Time (h) from beginning of established light period			
	14	18	20	22
A	110.40 <sub>±</sub> 5.06	137.05 <sub>±</sub> 4.16	134.15 <sub>±</sub> 2.82	119.75 <sub>±</sub> 4.71
B	122.50 <sub>±</sub> 5.50	123.50 <sub>±</sub> 5.26	122.05 <sub>±</sub> 3.94	121.85 <sub>±</sub> 4.18
C	123.50 <sub>±</sub> 4.46	119.55 <sub>±</sub> 5.15	121.45 <sub>±</sub> 3.35	113.95 <sub>±</sub> 4.61

<sub>±</sub> 95% confidence limits.



Table 6 - 2

Effect of painting of only one leaf on a plant with India ink.

One leaf unpainted as a control (A), and the other leaf painted on the same plant (B).

Values are means of 12 replicates with 95% confidence limits.

(Data of Fig. 6 - 4).

Treatment	Time (h) from beginning of established light period			
	14	18	20	22
A	108.75 $\pm$ 3.06	137.05 $\pm$ 4.27	131.65 $\pm$ 3.13	115.00 $\pm$ 6.05
B	116.65 $\pm$ 6.82	119.15 $\pm$ 5.56	122.90 $\pm$ 5.15	113.00 $\pm$ 6.68

$\pm$  95% confidence limits.