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EFFECTS OF LIGHT QUALITY
ON FLOWERING AND MORPHOGENESIS
IN *HYOSCYAMUS NIGER* L.

H. EL HATTAB

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EFFECTS OF LIGHT QUALITY
ON FLOWERING AND MORPHOGENESIS
IN *HYOSCYAMUS NIGER* L.

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWWETENSCHAPPEN
OP GEZAG VAN DE RECTOR MAGNIFICUS, DR. IR. F. HELLINGA,
HOOGLEERAAR IN DE CULTUURTECHNIEK,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP DONDERDAG, 21 NOVEMBER 1968, TE 16.00 UUR

DOOR

A. H. EL HATTAB

THEOREMS

I

Flower induction in *Hyoscyamus niger* requires the phytochrome system, although this is not fully characterized by reversibility during the dark period.

This thesis

II

The sensitivity of *Hyoscyamus* plants to red and far-red irradiation during the night is connected with some sort of endogenous rhythm.

This thesis

III

Blue light plays an important role in flowering of *Hyoscyamus* plants.

This thesis

IV

Optimum flower induction in long- and short-day plants is likely to require a specific ratio between the amounts of the two forms of phytochrome.

This thesis

CUMMING, B. G. Can. J. Botany, **41**,
1963: 901-926

V

Complete reconciliation between the photoperiodic behaviour in long-day plants and the simpler formative reactions in relation to photoperiod appears difficult.

VI

Morphological characteristics of a plant largely determine the degree of herbicide absorption.

AUDUS, L. J. The physiology and biochemistry of herbicides,
1964.

VII

Carbohydrate level may greatly influence leaf shape, although it does not hold in all cases.

ALLSOPP, A. Encyclopedia of plant physiology **15** (1), 1965.
SÁNCHEZ, R. A. Meded. Landbouwhogeschool Wageningen **67**
(16), 1967.

VIII

The promotion of the use of herbicides in Egypt necessitates a thorough reconsideration of agricultural research and educational program objectives and direction.

IX

Cooperation between mutation breeders and agronomists offers better chances to reveal the usefulness of mutant types.

X

Sugar beet may compete successfully with sugar cane in Egypt.

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CHAPTER I

INTRODUCTION AND SCOPE OF THE INVESTIGATION

A. INTRODUCTION

Floral initiation is the transition from vegetative to reproductive growth in seed plants. The initiation of flower primordia is generally visualised as an interaction between the genetic constitution and the environmental factors as experienced by the plant during its life cycle.

The main factors that influence plant growth and development are temperature, light, and water.

Light not only supplies the energy required for the assimilation of carbon dioxide in the photosynthetic process, but also provides the stimulus for the photomorphogenic reactions that are responsible for the appearance of the plants. Light functions in green plants, therefore, can be divided into two groups: the photo-energetic reactions, and the photostimulus functions (WASSINK, 1954). Photomorphogenesis and photoperiodism which belong to the second group can be initiated with very low light intensities.

Photomorphogenesis is the resultant of the control which is exerted by visible radiation over growth, development and differentiation of a plant (MOHR, 1964). The reaction of plants to the length of the photoperiod (TOURNOIS, 1912) is called photoperiodism (GARNER and ALLARD, 1920). Flower induction caused by the duration of the photoperiod is located in the leaves (KNOTT, 1934), and results in the production of the floral stimulus. These phenomena show that in plants a variety of photochemical reactions occur as a result of light absorption in pigment molecules.

Most plants possess several photomorphogenetic reaction systems by which visible light of both short and long wavelengths can influence the growth of plants. These systems depend on the physiological state of the plant and may react differently to time, duration, intensity, and colour of the applied radiation.

Action spectra are useful in the analysis of photochemical activities. Each one of the plant growth processes has its own spectral sensitivity ranges and most of these ranges are specifically different as far as the different processes are concerned (LEOPOLD, 1964). The action spectra may reveal the nature of the responsible pigment, but for most light-driven plant responses the action spectra have not yet permitted a precise identification of the pigment system, at least of its specific reactive state.

B. SCOPE OF THE INVESTIGATION

Much of the earlier work on photoperiodism was concerned with effects of white light on flowering, which clearly consists of the combined effects of a large

variety of wavelengths within the visible spectrum. However, in the analysis of photobiological reactions, work with restricted spectral regions has proved to yield more valuable information.

The purpose of this investigation is to compare the relative effectiveness of short days of different light sources of well defined spectral composition on the flowering and morphogenesis of *Hyoscyamus niger*, when these short days were extended by periods of similarly well defined qualities of light, or linked up with a night interruption of a specific colour at various specific moments during the long dark period.

CHAPTER II

COLOURED LIGHT EFFECTS ON FLOWERING; REVIEW OF LITERATURE

A. GENERAL

A series of reviews on coloured light effects on flowering have been published in recent years (STOLWIJK, 1954; WASSINK and STOLWIJK, 1956; MEIJER, 1959; VAN DER VEEN and MEIJER, 1959; DE LINT, 1960; BORTHWICK and HENDRICKS, 1961; HILLMAN, 1962; MOHR, 1962; ZEEVAART, 1962; HENDRICKS and BORTHWICK, 1963; SALISBURY, 1963; MOHR, 1964; LANG, 1965).

Many investigators consider the critical daylength as rather fixed, notwithstanding the fact that it was known to be dependent on certain conditions (LANG and MELCHERS, 1943). Coloured light studies, however, have shown this notion to be rather irrelevant. In an article on the effects of light quality on plant growth, WASSINK and STOLWIJK (1956) argued that the term 'critical daylength' is to a high degree senseless in view of recent photoperiodicity research. This idea has been confirmed by the work of DE LINT (1958, 1960) showing that a short-day treatment including 2 hours of low intensity far-red provoked shooting in *Hyoscyamus niger* (a qualitative long-day plant), while WASSINK, STOLWIJK, and BEEMSTER (1951) reported an equal result for *Brassica rapa* (a quantitative long-day plant). This indicates that no fixed critical daylength exists and that this is similarly valid for plants denoted as 'qualitatively sensitive' as for those denoted as only 'quantitatively sensitive'.

Evidence was presented to show that the spectral composition of the light used during the main light period may strongly influence the critical daylength, so that even a long-day plant may fail to flower for extended periods in continuous light if this light lacks certain spectral regions. STOLWIJK and ZEEVAART (1955) observed promotion of *Hyoscyamus* flowering by far-red at the beginning of the dark period. They also found that this long-day plant entirely failed to flower when grown in long or even continuous days of high intensity green or red irradiations, although it flowers rapidly in white light. Rapid flowering was observed in the violet and blue regions and in red with admixtures of small amounts of far-red irradiation. Flowering of plants in continuous red light was greatly speeded up by intercalation of exposures to blue light for 9 hours once a day or once every two or three days, while plants in short days of blue (9 hours) did not flower, but their leaf petioles were considerably elongated. It was suggested that possibly the slight contamination of far-red in the blue might have been responsible for the original effects reported, but much purer sources have since been shown to provoke the same effects (CURRY and WASSINK, 1956; WASSINK, BENSINK and DE LINT, 1957). Therefore, in *Hyoscyamus niger*, blue or far-red irradiations are necessary and may be physiologically equivalent for

flower formation. This conclusion has been confirmed by MEIJER (1959) who extensively studied the effects of different qualities of light in the main light period and their relation to the effects of light given during parts of the dark period on photoinduction. He studied many species, including both long- and short-day plants such as *Hyoscyamus*, *Kalanchoë*, but the main plant studied was *Salvia occidentalis*, a short-day plant. In spite of the existence of qualitative differences in the behaviour of different species, the main results were similar and may be summarized in that the long-day effect was mainly caused by:

- 1) blue light, or
- 2) the addition of far-red radiation to a long light period, or
- 3) blue light when given as extended irradiation following a short main light period, or
- 4) red light if given as a night break during the long dark period, provided the high intensity light period contains blue or far-red light (MEIJER and VAN DER VEEN, 1960).

These observations show that the light quality given in the main light periods of photoperiodic cycles may affect the daylength response of the plant and also its response to light applied in the dark period.

Information concerning the nature of this photoreaction is obtained from action spectrum determinations. PARKER et al. (1946); BORTHWICK et al. (1948); PARKER et al. (1950); BORTHWICK et al. (1952b); HENDRICKS and BORTHWICK (1963) found that wavelengths in the region of 660 nm were the most effective in nullifying the effectiveness of a long dark period. With lettuce seed, they further found that the effect of brief treatments with wavelengths of 660 nm could be annihilated by immediate subsequent treatment with wavelengths of 730 nm. Upon repeated treatment with wavelengths of 660 nm, germination was stimulated again and could be annihilated again, and so on. They postulated that this reaction upon alternating red and far-red irradiations involved a pigment, phytochrome (BUTLER et al., 1959), occurring in two interconvertible forms. When this pigment is exposed to white or red light, it is converted to the far-red absorbing (P_{fr}) form and when the P_{fr} -form of the pigment is exposed to far-red light it is converted to the red absorbing (P_r) form (HENDRICKS, 1960). The far-red absorbing form (P_{fr}) may also revert to the red absorbing form (P_r) in darkness.

It may be asked which of the two forms of phytochrome exerts a specific physiological effect. The data of DOWNS (1956) in *Xanthium* indicate that when the long dark period was interrupted by red light, inhibition of flowering took place, while the addition of far-red immediately after the red repromoted flower induction. If far-red radiation followed the red only after 20 min of darkness, the extent of reversal decreased to about 50% of the maximum; when it was given after 60 min, far-red had lost its effectiveness. Moreover, when the temperature during the intervening dark period was lowered, reversion by far-red was reduced. Similar results were obtained in soybean (DOWNS, 1956) and *Chrysanthemum* (CATHEY and BORTHWICK, 1957; BORTHWICK 1959).

Thus, it seems that the far-red absorbing (P_{fr}) form of phytochrome is phy-

siologically active, and it seems that the results of its action are processes which inhibit or prevent flower initiation in short-day plants and lead to flower initiation in long-day plants.

Furthermore, if the duration of far-red irradiation is extended, its effect begins to resemble that of red light, as is shown in *Chrysanthemum* (CATHEY and BORTHWICK, 1957). This may be due to the overlapping absorption of the P_r - and P_{fr} -forms of phytochrome (HENDRICKS and BORTHWICK, 1959). Because of this overlapping, even in pure red light there will be always some 20% of the phytochrome in P_r -form (BUTLER, HENDRICKS and SIEGELMAN, 1964). Thus, light used to produce a red effect converts some P_{fr} to P_r and light used to produce a far-red effect converts some P_r to P_{fr} , so that in either case a certain characteristic equilibrium between the two pigment forms is established. It was suggested additionally, that far-red somehow promotes the action of phytochrome P_{fr} (EVANS, 1964). This suggestion was made to account for the superiority, for many long-day plants, of daylength extensions containing a certain proportion of far-red energy.

Experiments with etiolated *Pisum* tissue (FURUYA and HILLMAN, 1964) showed that the standard red source brought about a photostationary equilibrium of roughly 90% of the far-red absorbing form (P_{fr}), already within 5 min, the blue light gave a photostationary state of roughly 50% P_{fr} , within 45 min, a 15 min blue exposure gave 25–35% P_{fr} . These results confirm the observations of BORTHWICK and HENDRICKS (1960) that both forms of phytochrome have some absorption in the blue, so that the effects of blue are relatively small and slow. These findings are also similar to those of ODA (1962) who reported that long exposures of *Lemna perpusilla* 6746 to far-red light resembled blue light in their effect; these, like blue, would be expected to maintain a fair proportion of the phytochrome in the P_{fr} -form.

Up to now, however, the way in which phytochrome interferes with the morphological and biosynthetic mechanisms in the plant is unknown. It is, now, evident that the far-red absorbing (P_{fr}) form of phytochrome reverts to the red absorbing (P_r) form in darkness. This dark reaction was postulated to be the basis of the time measuring mechanism of photoperiodism (BORTHWICK et al., 1954). Upon this assumption, HENDRICKS (1960) has estimated the half life of the dark reversion to be about 2 to 3 hours for most plants. It has been proposed that a red light break in the middle of the night, or after 4 hours of darkness, causes the conversion of P_r (the presence of which is considered to be due to reversion of P_{fr} to P_r in the dark) to P_{fr} and that inhibition of flowering in short day plants is a result of the presence of P_{fr} . Furthermore, it was suggested that the critical dark period was measured by the time required for the conversion of phytochrome (BORTHWICK and HENDRICKS, 1960; HENDRICKS and BORTHWICK, 1963). Moreover, HENDRICKS (1963) suggested that metabolic reserves for the reaction system in which phytochrome is involved are also time-dependent. Thus, both the changing amounts of pigment and of reaction substrates are considered to be essential features in the timing system. This idea arose from the observation that during darkness, an interaction between availability of

photosynthetic products of the preceding day and presence of P_{fr} is evident in the varying effectiveness with time of night breaks.

Not only which fraction of phytochrome is in the P_{fr} -form seems to be important, but also the amount of P_{fr} is essential. STOLWIJK and ZEEVAART (1955) reported that shooting of *Hyoscyamus* plants, grown in long days of red light, is inhibited, while a far-red admixture (30%) to the long days in red light permitted rapid flowering. We should like to mention that this observation could be interpreted on the basis of phytochrome action. In red long days, P_{fr} is present to inhibit flowering, but the additional far-red decreases the P_{fr}/P_r ratio, permitting flowering. Moreover, FRIEND, HELSON and FISHER (1961); VINCE, BLAKE and SPENCER (1964) found that the floral induction of long-day plants was highly promoted by certain mixtures of red and far-red irradiations. Also, a requirement for far-red energy in the light source for an optimum photoperiodic response in barley was observed (PALEG and ASPINALL, 1964). Further, it was reported that both *Lemna perpusilla*, a short-day plant, and *Lemna gibba*, a long-day plant, flower in continuous red light only if blue or far-red is admixed at proper ratios (ESASHI and ODA, 1966). BUTT (1968) demonstrated the importance of maintaining a red : far-red energy ratio of adequate value for a certain minimum photoperiod for the onion plant (LD) to initiate bulb formation. Recently, it was also reported again that a small amount of incandescent light added to weak fluorescent light greatly enhanced both flowering and stem lengthening in *Hyoscyamus niger* (SCHNEIDER, BORTHWICK and HENDRICKS, 1967). The latter added that flowering and stem-lengthening responses of *Hyoscyamus niger* to light show control by phytochrome and high energy reaction, with an action maximum at 710–720 nm. According to an interpretation by HARTMANN (1966), the high energy reaction of photomorphogenesis (B–FR reaction) could be mediated solely by the phytochrome system, in such a way that it establishes a definite P/P_{fr} ratio which is essential for the reaction.

A certain concentration of P_{fr} or a narrow range of P_{fr}/P ratios seem required for optimum initiation of flowers in *Chenopodium rubrum*, and also the concentration of floral hormone seems directly dependent on the P_{fr} -situation (CUMMING, 1963). CUMMING reported that when plants of two short-day selections Nos 372 and 374 of *Chenopodium rubrum* are exposed to different photoperiods, including continuous light, progressively longer photoperiods are required for optimum (earliest) floral initiation when the red to far-red spectral energy ratio (R/FR) is lowered during the terminal hours or the whole of each photoperiod. He concluded that the constant presence of P_{fr} in continuous light resulted in earlier floral initiation than in photoperiods of identical R/FR ratios but with a daily dark period. Conversely, with photoperiods of high R/FR ratios, floral initiation was earliest in 8-hours daily photoperiods and latest in continuous light. Altogether, it appeared that darkness was not essential for early flowering of the mentioned short-day selections. The results clearly show that long photoperiods are promotive when light of low R/FR ratios is used.

Some remarks should be made with respect to BÜNNING's concept (1936) who proposed that the photoperiodic induction of flowering may be controlled by an

endogenous rhythm. In the initial absence of substantial supporting evidence, his theory was not immediately accepted. However, observations are available now supporting his hypothesis in the short-day plants *Glycine Max* (NANDA and HAMNER, 1959; COULTER and HAMNER, 1964); *Chenopodium rubrum* (CUMMING, HENDRICKS, and BORTHWICK, 1965) and *Pharbitis nil* (TAKIMOTO and HAMNER, 1965). The reports on rhythm experiments with long-day plants have not been conclusive. However, some data have been reported which indicate that one long-day plant, *Hyoscyamus niger* may have a rhythmic flowering response (CLAES and LANG, 1947; CLAUSS and RAU, 1956; FINN, 1958, and HSU and HAMNER, 1967).

The results of DE LINT (1960), however, suggest that the assumption of a rhythmic light sensitivity is not necessary to interpret cyclic functions in flowering of *Hyoscyamus*, because some consecutive days of darkness are inductive when following any daylength treatment and because far-red irradiations are inductive, within certain limits, irrespective of the duration of its daily application. Moreover, far-red irradiation directly following a short day was found promotive for flowering, while red night interruptions can be either inhibitive or promotive, dependent on their duration (DE LINT, 1959). DE LINT (1960) suggested an interpretation on the idea that an inhibitive irradiation and the subsequent dark period constitute an entity with respect to the physiological response of the plant, the effect of which can be modified, within the limits of reaction of the plant, by additional irradiation.

B. EFFECTS OF SPECTRAL REGIONS AS SUPPLEMENTARY LIGHT OR NIGHT BREAK IRRADIATIONS

In many plants, flower bud initiation is daylength dependent. The light energy required for daylength extension is relatively low, as was found, e.g. by PARKER et al. (1946). The spectral dependence of this light reaction has been studied by several investigators. Floral initiation can be induced in long-day plants by supplementing the light period of short days with light of very low intensities (WITHROW and BENEDICT, 1936; FABIAN, 1938) or by interrupting the long dark period with brief irradiations (NAYLOR, 1941; BORTHWICK et al. 1948, 1950). There seem to be two distinct light functions, one which directly promotes the formation of the floral stimulus and another which removes the inhibitory effects of darkness and requires only small amounts of light energy (LANG, 1952). It may be concluded from the experiments applying supplementary light or night breaks with long-day plants that the main light period of high intensity has some preparatory role via photosynthesis and does not enter in a direct way into the formation of the floral stimulus. FRIEND, FISHER and HELSON (1963) have suggested that the high energy reaction may participate in the induction in wheat, but there is no evidence that it does so also during the extension of a high intensity light period by low intensity light.

Earlier workers found a maximum for the photoperiodic response in the red part of the spectrum. RASUMOV (1933), working with radiation filtered through

coloured solutions, found a maximum of photoperiodic effectiveness in the red region in inhibiting flowering in short-day plants and in promoting flowering in long-day plants. WITHROW and BENEDICT (1936); WITHROW and BIBEL (1936) confirmed this result and found that blue light was ineffective, except in *Callistephus chinensis*, var. 'Heart of France'. KATUNSKY (1937) and KLESHNIN (1943) reported that all wavelength regions were effective, provided the intensities were sufficiently high, and they only found differences in the degree of effectiveness. The results are in general agreement with those of BORTHWICK et al. (1948, 1952a) and PARKER et al. (1946, 1950) where they found a minimum effectiveness in the blue and violet regions and a broad maximum in the red region of the spectrum. The action spectrum curves obtained in this work are similar, and on the whole confirm the statements made by the authors mentioned above.

Since FUNKE (1936, '37, '38, '39, '48), there has been a great deal of work on the vegetative development and flowering of plants grown with relatively high energies (WASSINK et al., 1950, 1951; STOLWIJK, 1952, '54 and MEIJER, 1959). WASSINK et al. (1951) found that the promotion of flowering in *Brassica rapa* upon blue and far-red day extensions was independent of daylength to a much greater extent than that in white light. They concluded that, in white light, the remainder of the spectrum has an antagonistic effect, destroying the promotion caused by the blue and far-red components in the white light.

Furthermore, it was reported that far-red light resulted in a distinct promotion of flowering when given supplementary to a short day in white light of high intensity (STOLWIJK, 1952; FORTANIER, 1954; STOLWIJK and ZEEVAART, 1955; WASSINK et al., 1957; DE LINT, 1958, 1960, 1961; TAKIMOTO, 1961; LONA, 1963; KANDELER, 1963). Recently, ESASHI and ODA (1966) reported that a red light period supplemented with far-red irradiation was effective for flowering also in *Lemna gibba*.

Far-red irradiation also produces a definite elongation of plant parts (stems, internodes and leaves) in *Cosmos bipinnatus*, *Brassica rapa* var., *Lactuca sativa* cv. 'Wonder van Voorburg', *Solanum lycopersicum* cv. 'Ailsa Craig' and *Hyoscyamus niger* when applied at low intensity supplementary to a basic period in strong white light (DE LINT, 1961). This result confirms the earlier observation of WASSINK and SYTSEMA (1958) that a far-red supplement caused a greater elongation of petiole in *Hyoscyamus niger* than that of the other spectral regions. The latter also found that low intensity red night interruptions in the middle of the dark period decreased the ultimate petiole length, whereas with a brief supply of far-red alone, petioles grew longer than in uninterrupted darkness.

Blue light extensions at low intensities were found to cause promotion of flowering in a variety of plants (WASSINK et al., 1950, 1951; STOLWIJK, 1954). However, further analysis showed that the elongating effects of the quality of blue light used were due to far-red admixture (CURRY and WASSINK, 1956). This conclusion was further confirmed and it was found that blue light without any far-red contamination produced no elongation, and the contamination alone

did (WASSINK et al., 1957 and DE LINT, 1960, 1961). The inactivity of the blue was also reported (BORTHWICK et al., 1952d; DOWNS et al., 1957; VINCE and STOUGHTON, 1957). Moreover, relative ineffectiveness was observed of photoperiod extensions with fluorescent light rich in blue energy in flower promotion of *Lolium temulentum* (EVANS, BORTHWICK and HENDRICKS, 1965). However, CURRY and WASSINK (1956) and DE LINT (1960) found elongation in pure blue light of high intensity in *Hyoscyamus*; only MEIJER (1959) reported elongation at low intensity supplementary blue light in gherkin seedlings. Recently, ESASHI and ODA (1966) reported that blue light was effective when applied supplementary to a red basic photoperiod for the flowering of *Lemna gibba*.

Light from incandescent lamps, which has approximately equal red and far-red energy components frequently is far more effective as photoperiod extension than red radiation or light from fluorescent lamps which has little far-red energy (WASSINK, STOLWIJK and BEEMSTER, 1951; BORTHWICK and PARKER, 1952; TAKIMOTO, 1957; DOWNS, PIRINGER and WIEBE, 1959; PIRINGER and CATHEY, 1960; FRIEND, HELSON and FISHER, 1961; LANE, CATHEY and EVANS, 1965). Similarly, it was reported that a small admixture of far-red radiation with a red light extension or of red radiation with a far-red extension increased their effectiveness. The flowering response fell as the proportions of either red or far-red energy increased (VINCE, BLAKE and SPENCER, 1964; EVANS, BORTHWICK and HENDRICKS, 1965; VINCE, 1965).

The inactivity of red light extensions of a short day of high light intensity may be due to the high concentration of the far-red absorbing form of the phytochrome (P_{fr}) which is maintained during the entire supplementary period (VINCE, 1965), thus inhibiting or delaying floral induction (WASSINK et al., 1950, 1951; STOLWIJK and ZEEVAART, 1955; DE LINT, 1958, 1960; VINCE, BLAKE and SPENCER, 1964; EVANS, BORTHWICK and HENDRICKS, 1965; VINCE, 1965).

STOLWIJK and ZEEVAART (1955), in *Hyoscyamus*, found that when 10-hour days in white light were extended by 4 hours red, followed by 4 hours far-red irradiation or vice versa, at low intensity, elongation and flowering took place irrespective of the sequence of the two wavelength bands; an antagonism between red and far-red was not observed under these conditions.

DE LINT (1960), for *Hyoscyamus*, showed that the duration of the daylength extension with red light has a definite effect on the activity of supplementary far-red irradiation. The more red was intercalated between white and far-red, the sooner elongation started. Therefore, red light was really active as daylength extension, while far-red acts over a broad range of daylengths.

Recently, EVANS, BORTHWICK and HENDRICKS (1965) showed that a brief exposure to far-red irradiation, following a prolonged extension with red light, or a brief exposure to red light, following a far-red extension, both increased the flowering response in *Lolium temulentum*. They concluded that induction in that plant requires the presence of P_{fr} -phytochrome over a prolonged period and, independent of that, over some period, the presence of P_r -phytochrome, which is usually considered inactive.

However, VINCE (1965) showed with a strain of *Lolium temulentum* other than

the one used by EVANS et al. (1965) that far-red for 7 hours followed by one hour of red light as a supplementary treatment to short days in natural daylight, accelerated flowering as much as exposure to red and far-red simultaneously for 8 hours. Furthermore, 8 hours of far-red extension was without effect; 7 hours of red light followed by one hour of far-red had no more effect than 8 hours of red; 7 hours of darkness followed by one hour of red (night break) was almost as effective as 7 hours of far-red followed by one hour of red. These results suggest that the explanation of the difference in effectiveness of red light given as a night break and given as an 8 hour supplement is that the concentration of P_{fr} has to be low during part of the 8-hour period directly after the basic light period for flowering to occur most rapidly. This low concentration of P_{fr} seems to be achieved during the dark period prior to the red night break, as a result of some thermal reaction. Keeping the P_{fr} -level low by exposure to far-red during 7 hours prior the red night break is equally effective in flower promotion.

Another possible explanation for the superiority of photoperiod extensions containing some far-red is the assumption of an endogenous circadian (cycle of about 24 hours) rhythm due to which the sensitivity of the plant to P_{fr} changes such that the proportion of phytochrome in the P_{fr} -form required for optimum induction changes in the course of a daily cycle. Evidence suggestive for such a change in long-day plants has been presented by BÜNNING and KEMMLER (1954). With her strain of *Lolium temulentum*, VINCE (1965) also found a pronounced change in the relative response to red and far-red light during the course of photoperiod extensions. However, the strain of *Lolium temulentum*, used by EVANS et al. (1965) showed no such evidence.

Photoperiod extensions given just before the daylight period were shown to be much more effective than those given at the end of the basic photoperiod. Recently, LANE, CATHEY and EVANS (1965) found that photoperiod extensions with different lamp types were more effective for all species studied (*Anethum graveolens* L.; *Beta vulgaris* L.; *Hordeum vulgare* L.; *Hyoscyamus niger* L.; *Lolium temulentum* L.; *Petunia hybrida* Vilm.) when given in the second half of the long dark period than in the first one, as was also found much earlier by FABIAN (1938). Furthermore, in *Lolium temulentum*, it was observed that red radiation preceding the daylight period was highly promotive, while red light extensions directly after the main light period were ineffective (VINCE, 1965).

It is evident that red light often is ineffective for flower induction when used as a low light intensity extension to a short day of high intensity, but in other cases, it is the most effective daylength extension. In many short-day and long-day plants, the action spectrum for inhibition or promotion of flowering, respectively, by a brief light exposure in the middle of the long dark period shows a peak in the red and a lower one in the blue. This action spectrum has been investigated by the Beltsville group, for 'Wintex' barley (BORTHWICK, HENDRICKS and PARKER, 1948) and *Hyoscyamus niger* (PARKER, HENDRICKS and BORTHWICK, 1950). Thereafter, the same result has been obtained in the experiments of STOLWIJK and ZEEVAART (1955) with *Hyoscyamus niger*. Also, VINCE (1965) reported red light activity as a night break in *Lolium temulentum*. These

results can be interpreted in terms of phytochrome such that processes leading to floral induction require the presence of P_{fr} .

Night breaks are most effective when given near the middle of the dark period (HARDER and BODE, 1943; CLAES and LANG, 1947; BORTHWICK, HENDRICKS and PARKER, 1948; PARKER, HENDRICKS and BORTHWICK, 1950; STOLWIJK, 1954; WAGENAAR, 1954; CLAUSS and RAU, 1956; SALISBURY and BONNER, 1956; SALISBURY, 1961; BHARGAVA, 1964). Such night breaks with red light are not effective unless the high intensity light period contains blue or far-red light (MEIJER and VAN DER VEEN, 1960).

The effectiveness of a night break was decreased when the duration or the intensity of light increased beyond a certain point (WAGENAAR, 1954). Night interruptions with red light, in *Hyoscyamus niger*, can be promotive or inhibitive depending upon their duration (DE LINT, 1959). In *Lolium temulentum*, increasing durations of red light night breaks increased their effect (VINCE, 1965).

In many species of short-day and long-day plants, a short break with white or red light of relatively low energy is quite sufficient to nullify the effect of a long dark period. However, in *Chrysanthemum*, a short-day plant, a brief irradiation even with light of very high energy (sunlight) proved to be entirely ineffective to suppress flower formation. It was necessary to give a relatively extended period of light or else repeated short light breaks spread over a similar period of time and separated by dark periods not exceeding a certain length which depended on the R/FR ratio in the light used for the breaks (BORTHWICK and CATHEY, 1962). It is apparent that phytochrome must be maintained in the P_{fr} -form for a relatively much longer time in *Chrysanthemum* than in the other short-day plants, in order to complete its flowering inhibiting action.

Recently, CHAILAKHYAN and LOZHNIKOVA (1966) mentioned that the response of long-day species to an interruption of the dark period by light is associated with an intensification of metabolism and the formation of gibberellins and auxins, with the resultant formation of flowers. Moreover, they observed that the response of short-day species to an interruption of the dark period by light also is evidently associated with a disturbance of the formation of metabolites essential in the formation of flowers, and as a result of an inhibition of flowering.

Contrary to the red part of the spectrum which is active in the middle and during the second half of the night, far-red irradiation promotes flowering in the long-day plant *Lemna gibba* when given in the early or middle portions of a noninductive night, being considerably less effective in the later hours of the dark period (KANDELER, 1956). Moreover, irradiations with far-red in the early and middle hours of the dark period were highly inhibitory for flowering of the short-day plant *Lemna perpusilla* 6748, and this inhibition decreased with irradiation nearer the end of the night (PURVES, 1961). These results are in close agreement with those obtained by NAKAYAMA (1958), and BORTHWICK and DOWNS (1964) with *Pharbitis nil*. Generally, prolonged irradiation with far-red near the middle of the dark period is inhibitory in many short-day plants (DOWNS, 1956; MANCINELLI, 1963; PIRINGER, DOWNS and BORTHWICK, 1963; KASPERBAUER, BORTHWICK and HENDRICKS, 1963; MANCINELLI and DOWNS,

1967). The degree of flower inhibition in *Xanthium pensylvanicum* Walr. depends on the duration of the photoperiod; the inhibition by far-red irradiation increases with increase in night length (MANCINELLI and DOWNS, 1967). These authors proposed an interpretation assuming that the control of the reaction could be shifted from a condition where P_{fr} is the limiting factor, in shorter nights, to a condition where the substrate for phytochrome action is the limiting factor, in longer nights; the ultimate response intensity being the interplay of both factors: level of substrate and total phytochrome.

These light break experiments have proved very useful for studies on the mechanism of photoperiodism. Especially reactions going on during dark periods have been shown to be very important controlling factors in photoperiodism.

C. PHOTOREVERSIBILITY IN PHOTOPERIODISM

FLINT and MCALISTER (1935, 1937) found the germination of lettuce, *Lactuca sativa*, to be promoted by red light. If seeds, previously exposed to enough red light to cause germination, were exposed to either blue or far-red (700–800 nm) light, red-induced germination was inhibited. This work was taken up by the Beltsville group (BORTHWICK et al., 1952b, 1954). They determined the action spectrum for germination promotion; it shows a peak at about 650 nm and thus resembles the night break action spectra in daylength sensitive plants, while also the action spectrum for the far-red inhibition showed a maximum around 730 nm. Their observations led them to postulate the existence of a red, far-red reversible pigment system, now called phytochrome.

Evidence for the red, far-red reversibility system for photoperiodic night breaks was presented first by BORTHWICK et al. (1952a) using *Xanthium pensylvanicum*. DOWNS (1956) showed the same for the long-day plants *Hyoscyamus niger* and 'Wintex' barley, and the short-day plants *Amaranthus caudatus*, 'Biloxi' soybean and *Xanthium pensylvanicum*; furthermore he was able to demonstrate repeated reversibility. STOLWIJK and ZEEVAART (1955) in their experiments with *Hyoscyamus niger* found that the addition of far-red to the same amount of red night break light decreased the effectiveness of the promotive action of the red. Likewise, for short-day plants, red light inhibition can be reversed by far-red in *Chrysanthemum morifolium* (CATHEY and BORTHWICK, 1957; BORTHWICK, 1959) and also in *Salvia occidentalis* (MEIJER, 1959).

It appears that the far-red reversal of red night break action is not complete, especially when the duration of far-red given after red is extended, or when a dark period is intercalated between the red and far-red, or when the temperature during the intervening dark period was lowered (DOWNS, 1956; CATHEY and BORTHWICK, 1957 and BORTHWICK, 1959).

Thus, the role of phytochrome in photoperiodic control of flowering is demonstrated by the effects of interruptions of the dark period with red light and the reversal by subsequent far-red irradiation. Exceptions, however, have been found. Reversibility could not be observed in *Pharbitis nil* (NAKAYAMA, 1958;

NAKAYAMA, BORTHWICK and HENDRICKS, 1960); the inhibitory action of red light was not reversed by subsequent exposure to far-red. NAKAYAMA et al. (1960) interpreted this result by suggesting failure of P_{fr} to undergo conversion when irradiated by far-red. A short-day strain of *Lemna perpusilla* 6746 seems to exhibit a quite similar behaviour, red night breaks inhibited flower formation but the effect could not be reversed with far-red irradiation (HILLMAN, 1959). In *Lemna*, it was further observed that far-red at the beginning and in the first part of the dark period caused an inhibition of the flowering response and that this inhibition was reversible by red, but the inhibition by red light was not reversible by far-red (PURVES, 1961). Recently, in *Xanthium pensylvanicum*, the flowering response was not reversed when alternating red and far-red irradiations were given in the middle of the night, following a 2 hour photoperiod (BORTHWICK and DOWNS, 1964). Furthermore, EVANS, BORTHWICK, and HENDRICKS (1965) treated *Lolium temulentum* plants under 8 hr main light periods which were extended with low intensity incandescent light to subcritical photoperiod lengths with a brief red night break in the middle of the following dark period. Day extensions of 4 or 6 hrs of incandescent light only caused no inflorescence initiation, extensions of 8 hrs caused 50% initiation and of 10 hrs almost complete initiation. A red night break of 5 min given after photoperiod extensions of 4 or 6 hrs caused initiation in one-third to one-half of the treated plants, and so did a 15 min night break. Exposures to far-red light for 5 min immediately after the red night breaks did not significantly reduce the flowering response.

FREDERICQ (1964), however, did observe far-red reversibility in flowering of *Pharbitis nil* when the red and far-red irradiations in the middle of the dark period were no more than 30 seconds each and were not separated by darkness. He observed failure of reversal when longer irradiations were used or when 3 mins darkness was intercalated between 30-second irradiations. Failure of reversibility was interpreted to be due to very rapid action of P_{fr} .

When in *Begonia evansiana* (ESASHI, 1966), a short-day plant, a 12-hour nyctoperiod was interrupted at the 7.5-hour point by red light, the inhibitory action of the red was partially reversed by irradiation either with blue or far-red irradiations. However, at the middle of a 16-hour nyctoperiod, far-red radiation failed to reverse the red effect. ESASHI assumed that the blue and far-red not only convert a large quantity of P_{fr} into the inactive form P_r , but also exhibit their own action which consists in the inhibition of inductive dark processes.

More recently, HILLMAN (1966) reported that reversal of night interruption depends, to some degree, on the colour of the main photoperiod. Data from experiments with a 10-hour main photoperiod composed of red or white fluorescent light showed that far-red interruptions inhibited flowering in *Lemna perpusilla* 6746 and failed to reverse the inhibition caused by red light. When a 10-hour blue photoperiod was used, however, reversal was obtained. The reversal obtainable with a 10-hour main photoperiod was completely abolished when this main photoperiod consisted of 9 hours of blue followed by one hour of red. Thus, the effect of far-red given as a brief interruption in the dark period is dependent on the quality of the light closing the preceding photoperiod.

Moreover, REID, MOORE and HAMNER (1967) reported in their experiments with *Xanthium pensylvanicum* three distinct responses to red and far-red night breaks in 48-hour dark period: 1) response to red light, 2) response to far-red light, and 3) response to red followed by far-red light. A red light perturbation at the start of the dark period had little effect. Red light was most inhibitory between the 6th and 9th hrs of darkness. Subsequent red light breaks became less inhibitory until the 15th hour, after which the red light perturbations had no significant effect. Far-red light perturbations were most inhibitory when applied early in the dark period. The red followed by far-red treatment elicited a response which has characteristics of both the red response and the far-red response. Another feature still seems more relevant to some results we will discuss later on in this paper. It is the observations that the red light effect tends to decline in the second half of the long dark period whereas the far-red effect tends to increase. Something analogous, but in an opposite direction, will later on be discussed for our object, the long-day plant *Hyoscyamus niger*.

Furthermore, CLELAND and BRIGGS (1968), using *Lemna gibba* G3, observed that the plants were fairly sensitive to low-intensity red light treatments given during a 15-hour dark period, that far-red light was almost as effective as red light, and attempts to reverse the red light response with subsequent far-red light treatment were not successful.

CHAPTER III

MATERIAL AND METHODS

A. PLANT MATERIAL

An annual strain of *Hyoscyamus niger* var. *pallidus* was used throughout this investigation. Seeds were originally obtained from Professor A. LANG (STOLWIJK and ZEEVAART, 1955). The same species was used before in work of this laboratory (CURRY and WASSINK, 1956; WASSINK, BENSINK and DE LINT, 1957; DE LINT, 1958, '59, '60, '61). Before the start of the experiments, the plants were kept in a 9-hour day, in the phytotron at 20°C under high intensity (ca. 30 000 ergs/cm²/sec) fluorescent light (PHILIPS, TL/55/40 W) in order to keep the plants in the vegetative stage. The plants were allowed to develop more than 20 leaves before they were used in the experiments. At the beginning of the treatments the plants were selected for uniformity. A control group for each experiment was kept under these non-flower inducing conditions, and was compared with the treated plants to ascertain that stem elongation had not already been induced before the treatments started.

Various methods have been used by previous workers to determine the degree of flowering response in *Hyoscyamus niger* (CLAES and LANG, 1947; CLAUS and RAU, 1956; FINN, 1958). Among these, are leaf counts, number of flower primordia or flowers, height of the floral stalk and the number of days to bolting.

In the experiments described in this paper, the following characteristics were recorded: the youngest developed leaves were marked at the beginning of an experiment, and at the end of the experiment all newly developed leaves were counted; stem length (in mm) was determined either periodically or at the end of the experiment; plants were examined daily for bolting until the termination of an experiment. Four plants were included in every treatment. Dry weight determinations were made on material kept in a ventilated oven at 70°C for 48 hours, followed by 15 minutes at 105°C.

B. IRRADIATION

1. General

The investigation has been carried out in the phytotron. Air humidity mostly was 70%. Temperature was about 20°C, day and night. Artificial light was supplied in a well defined, broad band, the spectral composition was that obtained from monophosphor fluorescent lamps in combination with filters of specific transmission. The filters were coloured plexiglass, copper sulphate solution, and water. When two or more wavelength regions were compared, they were given in equal intensities on energy basis. An irradiation was ended either by automatically switching off the lights, or by moving the plants into

darkness or into another treatment. Transport was in complete darkness over short distances. Irradiation cabinets were those described before (WASSINK and VAN DER SCHEER, 1950; WASSINK and STOLWIJK, 1952) and also used by STOLWIJK (1954); CURRY and WASSINK (1956); WASSINK, BENSINK, and DE LINT (1957); DE LINT (1958, '59, '60, '61) and LIE (1964) with few modifications. The high and low intensity cabinets are now placed in a compartment of the phyto-tron.

Fig. 1 presents the spectral characteristics of the incident irradiances in the compartments as given by DE LINT (1960). In general, the irradiation in high and low intensity cabinets is of the same spectral composition. The blue light is without any detectable far-red admixture (CURRY and WASSINK, 1956; ZURZYCKI, 1957; WASSINK and SYTSEMA, 1958; DE LINT, 1960, '61).

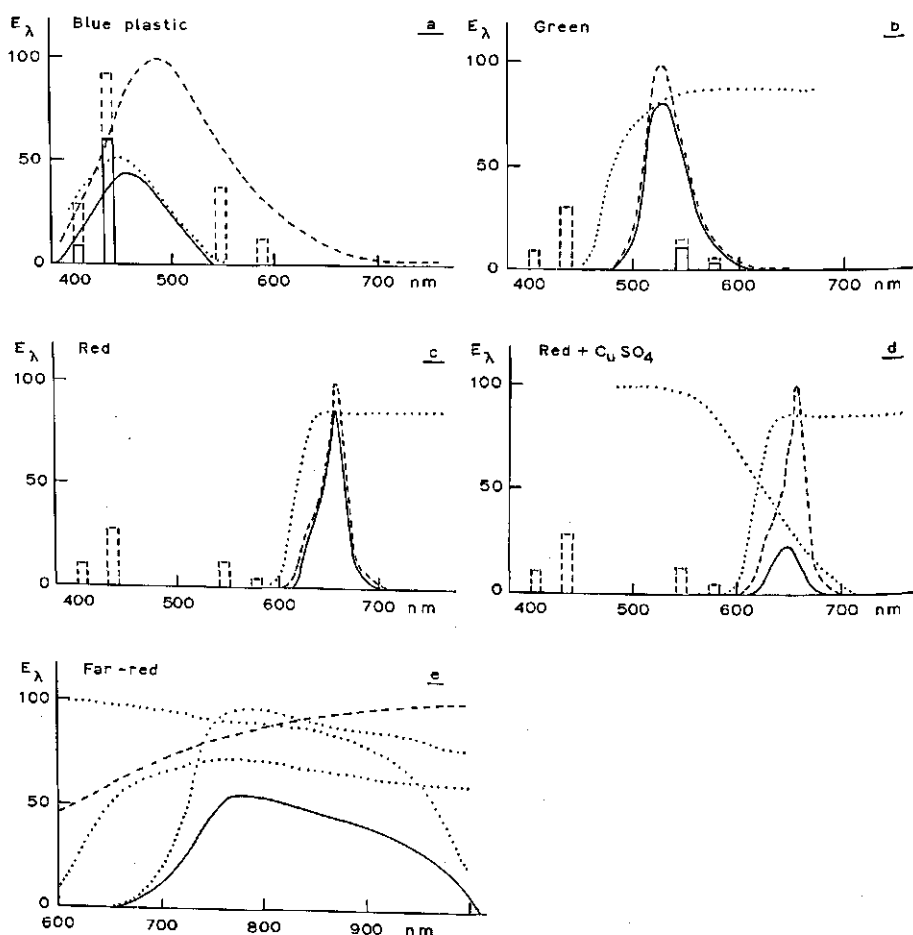


Fig. 1. Spectral characteristics of the equipment for narrow wavelength irradiation; — energy distribution spectra of incident irradiances, --- emission spectra of the lamps, ... transmissions of the filters. (From DE LINT, 1960).

2. The white light equipment

The white light equipment is the one, generally installed in the light rooms of the phytotron of this laboratory. In our experiments the white light was supplied by fluorescent 40 Watt, PHILIPS, TL 55 lamps. Two types of this white lamp have been used. The first one, the original type, which is rich in red emission and contains little far-red (fig. 2a) was used only in one experiment (chapter V, B, 1, p. 48); the second one, recently introduced into this laboratory, emits more far-red (fig. 2b) than the first one as derived from GAASTRA (1966); and was employed for the rest of the experiments.

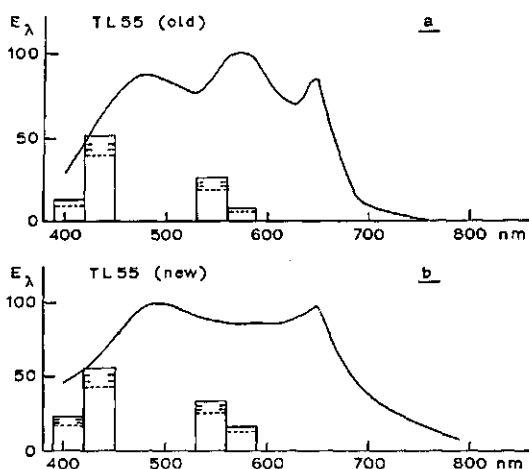


FIG. 2. Spectral composition of the original type of white fluorescent light (a) and the new one (b). (From GAASTRA, 1966).

The fluorescent white light (TL), from both lamp qualities, was also combined with the light from 60 Watt incandescent lamps (TL/IL). The light intensities of white fluorescent (TL) and the mixed source (TL/IL) were kept equal for the fractions between 400–700 nm, so that equal amounts of light energy were available for photosynthesis. Five cm below the lamps, a sheet of colourless plastic is inserted which separates the lamp space and the growth room, and which enables separate ventilation of the lamp space.

3. The coloured light equipment of high intensity

Irradiation in narrow wavelength bands is given in separate cabinets for each colour. The cabinets are uniform and consist of two parts, they were described before (WASSINK and STOLWIJK, 1952). The inner part is the plant chamber, 110 cm long, 35 cm wide and 85 cm high. Two metal doors are at the narrow ends. The door positions are used to correct for temperature differences, resulting from the different number of lamps used in the different cabinets. Entrance of scattered light through the door openings is avoided by black screens mounted between the cabinets. Plexiglass filters are mounted in rims along the edges of the colourless glass of side walls and tops of the plant chambers. For each cabinet lamps and their ballasts are mounted upon a frame, which is placed over the

plant chamber. One of the cabinets serves as a white control. White fluorescent tubes without filtering yield white light of the same intensity as the light in the coloured cabinets.

Because the blue filters usually have low transmission values, the available blue light intensity, being 5000 ergs/cm²/sec, was the limiting factor to obtain equal intensity in all colours.

Plants are placed at the bottom of the cabinets.

Due to the limited capacity of the cabinets and the rather large number of treatments desired for some colours, an additional set-up (chapter V, C) at higher intensities was made in a 20°C compartment of the phytotron. Blue light was applied from three directions. Red and mixed red and far-red (R + FR) irradiations were available only from the top. The mixed irradiation is obtained from a combination of a red filter with red fluorescent tubes and incandescent lamps. Scattering of light is avoided by black screens between the coloured units. The intensity of the photosynthetically active light in this mixed source (8000 ergs/cm²/sec) is the same as in blue and red. The red/far-red ratio is approximately equal. Plants in the additional set-up are placed on trolley cars.

4. The coloured light equipment of low intensity

For low intensity irradiation in narrow wavelength bands another set of cabinets was available; also these have been described before (WASSINK and VAN DER SCHEER, 1950). Each of these cabinets has four monophosphor fluorescent tubes on top; the far-red cabinet has eight 60 Watt incandescent lamps. The inner dimensions of the low intensity cabinets are: 120 cm long, 60 cm wide and 100 cm high. Red light in low intensity is filtered through copper sulphate solution in addition to the red filter (fig. 1d). The source of far-red light consisted of incandescent lamps, the emission of which was filtered by 8 cm of water, 2 layers of red filters and 4 layers of blue filters (fig. 1e). Plants can be placed in the cabinets at various positions of height, i.e. various distances from the light source.

5. Measurement of light intensity

In general, the incident intensities of irradiation have been measured on plant level, in ergs/cm²/sec (1 erg = 0,1 W/cm²) with a cosine corrected photocell (HARTIG and HELWIG, 1955). This cell was calibrated for the wavelength combinations used with the aid of a standardized thermopile. In this way, the incident intensities are measured in correct correspondance to their angle of incidence. The intensities of mixed irradiations were determined by separate determination of the components.

CHAPTER IV

EFFECTS OF BASIC AND SUPPLEMENTARY LIGHT OF DIFFERENT QUALITIES ON GROWTH AND DEVELOPMENT OF *HYOSCYAMUS NIGER* L.

A. PHOTOPERIODIC AND FORMATIVE EFFECTS OF HIGH INTENSITY

1. *Effects of white fluorescent light and of a mixture of white fluorescent and incandescent light*

In this preliminary experiment the effects of different periods of artificial light on stem elongation and flowering in *Hyoscyamus niger* were studied. The aim of this experiment was to compare the effects of two light sources, and was not intended to be a clear experiment on the photoperiodic reaction. Plants, grown in the phytotron under short days of 9 hrs of 20°C, were exposed to photoperiods of 10, 12, 12.5, 13, 13.5, 16, 20 or 24 hrs per day; two sources of light at an intensity of 35 000 ergs/cm²/sec have been used. One of the high light intensity qualities was white light from the new type of fluorescent lamps (TL) and the other light quality was a mixture from the light of the same fluorescent lamps and incandescent lamps (TL/IL). The experiment started 6-7- 1966, and lasted 70 days. The temperature was kept at 20°C, both during the light and the dark period. Representative plants are shown on plate 1. The time to visible shooting in the various photoperiods is shown in fig. 3.

TABLE 1. Days to bolting and flowering in *Hyoscyamus* under various photoperiods of two sources of light, white fluorescent light and a mixture of white fluorescent and incandescent light, at an intensity of ca. 35 000 ergs/cm²/sec. The experiment started 6-7-1966 and ended after 70 days.
Averages of 10 plants.

| Photoperiods in hrs | Days to stem elongation | | Days to flowering | |
|------------------------|-------------------------|-----|-------------------|----|
| | TL/IL | TL | TL/IL | TL |
| 24 | 17 | 19 | 29 | 31 |
| 20 | 19 | 22 | 32 | 33 |
| 16 | 26 | 28 | 38 | 40 |
| 13.5 | 30 | 31 | 45 | 50 |
| 13 | 32 | 36 | 45 | b |
| 12.5 | 37 | 41 | 51 | b |
| 12 | 46 | 60 | b | b |
| 10 | >70 | >70 | * | * |

b = after 70 days only flower buds.

* = vegetative plants.

TL = white fluorescent light.

IL = Incandescent light.

TL/IL = Mixture of white fluorescent and incandescent light.

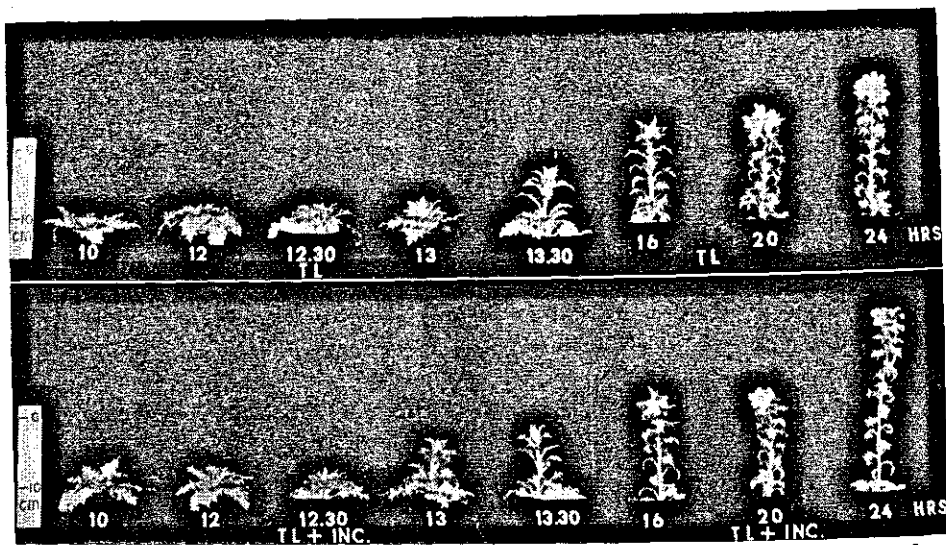


PLATE 1. Stem elongation and flowering under various photoperiods (indicated in hours) of two sources of light; white fluorescent (TL) light or a mixture from the same fluorescent and incandescent lamps (TL + INC.). See figure 3. Photographed 12-8-1966, after 38 days.

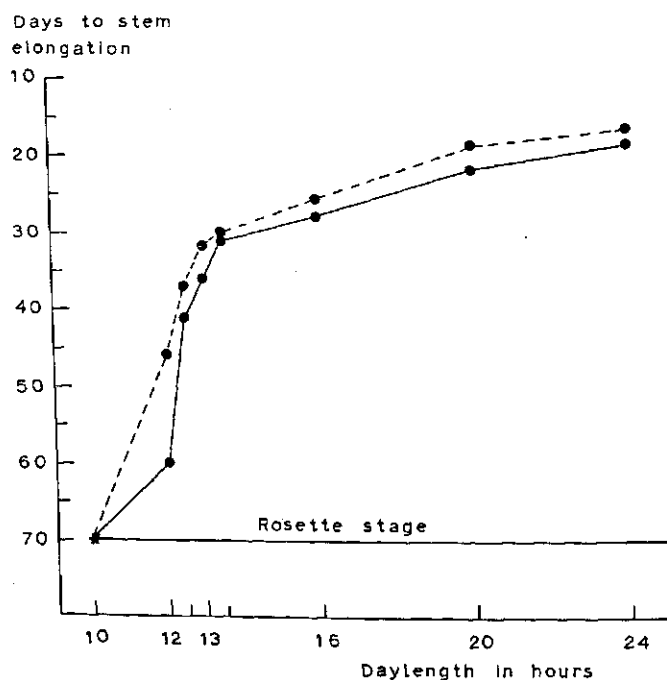


FIG. 3. Days to bolting in *Hyoscyamus* under various photoperiods of two sources of light, viz., white fluorescent light (—) or a mixture of white fluorescent and incandescent light (---), at an intensity of ca. 35 000 ergs/cm²/sec. The experiment started 6-7-'66, and ended after 70 days. Average of 10 plants.

Plants in 10 hr photoperiods remain in the rosette stage under both sources of light. Stem elongation and flower formation is most rapid in continuous light. Increasing the light period speeds up shooting of *Hyoscyamus*. There is not much difference of effect between the two sources of light on elongation, although plants in 12 hr photoperiods of TL/IL start to shoot after 46 days and in case of TL only after 60 days. Data on days to flowering as presented in table 1 indicate that addition of some far-red energy from incandescent lamps to light of TL alone causes somewhat faster flowering irrespective of the photoperiod.

It can be concluded that in 12 hr photoperiods, under the experimental conditions used, stem elongation and flower bud formation are induced and that therefore the approximate critical daylength lies between 10 and 12 hrs.

The improved growth of the plants kept under the mixture of fluorescent and incandescent light must be due to the addition of far-red energy. From these data it cannot be established, of course, whether it is the absolute increase in the far-red energy or the reduction in the R/FR ratio that speeds up shooting and flower formation (HELSON, 1965).

From this experiment, it can be concluded that *Hyoscyamus* reacts as a typical LDP under artificial light. The daylength reaction is the same under fluorescent light and under a mixture of fluorescent and incandescent light; the response being slightly faster under the last quality.

2. Effects of broad band coloured radiation

The first aim of this experiment was to test the behaviour of *Hyoscyamus* plants exposed to high intensities of restricted spectral regions, and to reproduce the results obtained earlier by STOLWIJK and ZEEVAART (1955).

Selected sets of uniform plants, previously grown in 9-hr days of the new TL light at intensity of ca. 35 000 ergs/cm²/sec, were exposed to light of different spectral regions, (exclusively, without any white light) during 16 hrs per day at an intensity of 5000 ergs/cm²/sec. The remaining 8 hrs were in complete darkness. The temperature was kept at 20°C both during the light and the dark period. The experiment was discontinued after 47 days.

TABLE 2. Development and formative effects in *Hyoscyamus niger* in different colours of light at an intensity of 5000 ergs/cm²/sec during 16 hrs/day. Measurement after 47 days. Average of 10 plants.

| Spectral region | Stem length in mm | Days to stem elongation | No. of newly expanded leaves to stem elongation | Petiole length in mm | Dry weight per plant in mg | | | | |
|-----------------|-------------------|-------------------------|---|----------------------|----------------------------|--------|-------|-------|----------------------|
| | | | | | stem | leaves | roots | total | Top/Root ratio (T/R) |
| Blue | 302 | 19 | 4 | 65 | 160 | 250 | 100 | 510 | 4.10 |
| Green | 0 | >47 | — | 34 | 0 | 440 | 315 | 755 | 1.40 |
| Red | 0 | >47 | — | 34 | 0 | 419 | 310 | 729 | 1.35 |
| White | 85 | 30 | 7 | 34 | 45 | 460 | 200 | 705 | 2.53 |

Plants exposed to far-red radiation and the control group that received 24 hrs of complete darkness, died after 18 days due to the lack of photosynthesis.

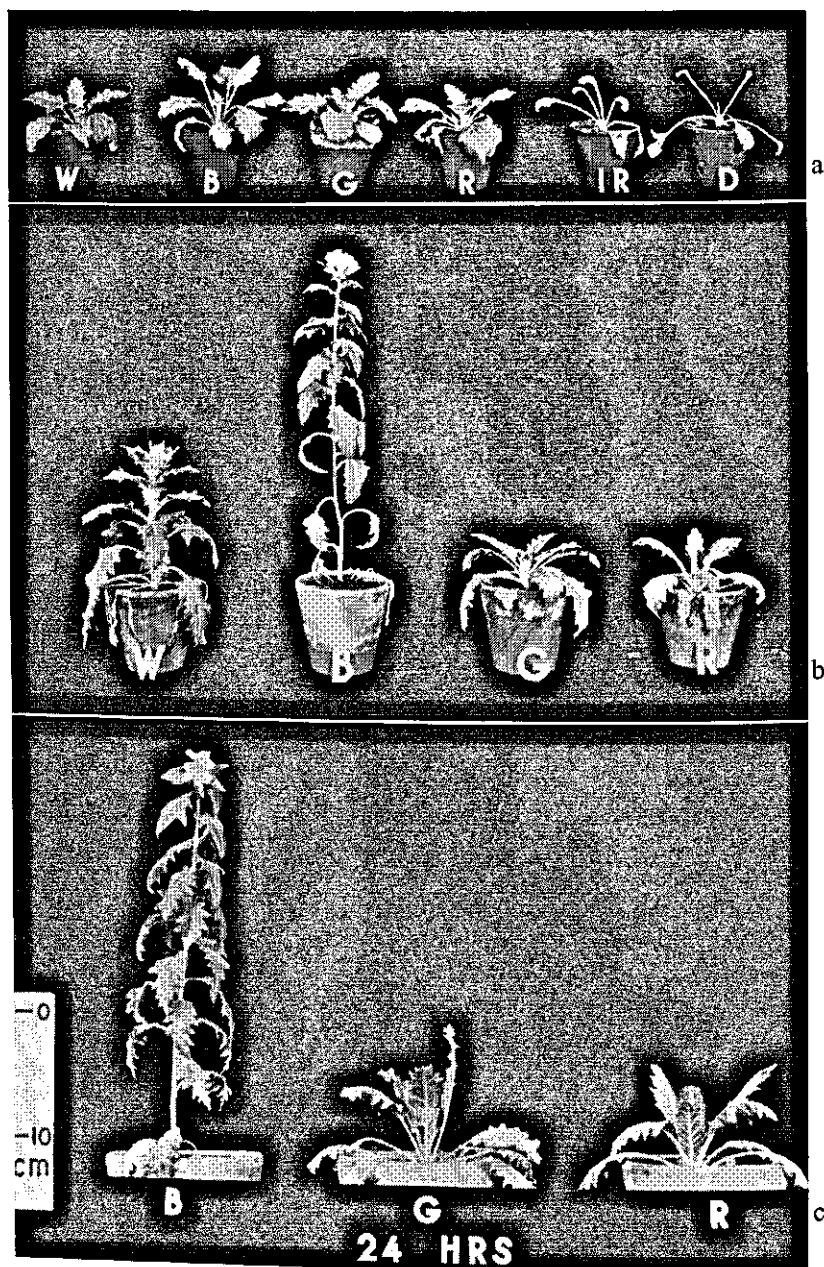


PLATE 2a. A daily treatment of 16 hours in monochromatic light of blue (B), green (G), red (R) and far-red (IR) compared to white (W) light. D = in complete darkness. See table 2, p. 21. Photographed 24-9-1965, after 16 days.

PLATE 2b. Representative plants after 42 days of treatment in (a). See table 2, p. 21. Photographed 20-10-1965.

PLATE 2c. Effect of different spectral regions in a 24-hour day (started 9-11-1966) on flowering. Photographed 15-12-1966, after 37 days.

Leaf petioles elongated excessively in blue, in far-red and in complete darkness; length after 16 days being 61, 92 and 104 mm, respectively; while it was about 31 mm in green, red and white light. Some epinasty was obvious in the leaves of plants that were irradiated with blue light. On plate 2, a, b representative plants of each treatment are shown.

From table 2 it is evident that plants in daily cycles of 16 hrs in blue light produced stems after 19 days, after expanding 4 new leaves. Plants in green and red light remained in the rosette stage throughout the experiment. Plants in white light elongated after 30 days, after having produced 7 new leaves. Clearly the blue irradiation is more elongative than white light. This seems logic since white light is a mixture of blue and the inhibitive red and green wavelengths (WASSINK, STOLWIJK, and BEEMSTER, 1951).

Even after continuous irradiation with green and red light for 37 days no stem elongation could be observed, while quick bolting and flowering occurred in blue light; see plate 2, c.

From table 2 it is evident that after 47 cycles, for dry weight, plants in blue and white light have higher top/root ratios than plants exposed to green and red light. Probably, these differences are a result of differences in shooting and flowering. It is clear that a large portion of the top/root differences in the various light qualities is due to differences in root growth; blue and white light reducing it, green and red light stimulating it. The explanation probably lies in the fact that nutrients are fully consumed in the shooting and flowering stem itself. Also, it might be possible that a relatively decreased formation of phloem tissues, associated with elongation and flowering, plays a role in restricting the flow of sugars into the root system.

In addition to the colour influence on shooting and flowering, it is clear that elongation of petioles occurred as a result of exposure to blue light, whereas this excessive elongation is absent in the other wavelength bands, and in white light.

From plate 2, a, b, there seems a correlation in wavelength dependence between elongation of petioles of the basal leaves and stem elongation. The results of this experiment are in accordance with the data published by CURRY and WASSINK (1956).

B. PHOTOPERIODIC AND FORMATIVE EFFECTS OF MAIN AND SUPPLEMENTARY LIGHT OF VARIOUS WAVELENGTH REGIONS

1. *Effects of coloured basic illumination supplemented by various colours of light*

a. *Effects of high intensity supplementary irradiation*

The results presented by STOLWIJK and ZEEVAART (1955); CURRY and WASSINK (1956); MEIJER (1959) and DE LINT (1960) demonstrate that blue and far-red irradiations are more active in causing a long-day effect than red or green light.

The following experiment has been designed to determine the influence of

different sequences of various light colours on the photoperiodic and formative effects of *Hyoscyamus*.

Plants were exposed to blue, green, red or white light in 14-hour days as a main light period. Additional to the basic light period, the plants received blue, red or far-red irradiation during two hours, and darkness during the remaining hours, or darkness immediately. The intensity of both the main light period and the supplementary irradiation was 5000 ergs/cm²/sec. The experiment started 27-12-'65 and was closed after 62 days. Average plants are presented on plate 3.

Bolting data are presented in fig. 4. The plants receiving basic days in red light remained in the rosette stage till the end of the experiment. Daylength extension with red light of the same intensity has no additional effect. However, a far-red supplement causes rapid bolting (after 23 days); blue causes also bolting but considerably slower (after 58 days). These data are in full agreement with those of STOLWIJK and ZEEVAART (1955); MEIJER (1959) and DE LINT (1960).

The reactions under basic days in green light are quite similar to the ones obtained with basic days in red light, but blue supplementary irradiation causes faster stem elongation than in the red group, whereas the far-red supplement causes rather quicker shooting in the first group than in the second one.

Concerning the result of the group with basic blue light, stem elongation occurs in all four supplementary treatments. In this case, the inhibitory action of 2 hrs of red light was insufficient to overcome the bolting function of the blue main light period. This result is in accordance with the observations of DE LINT (1960) which oppose MEIJER's supposition (1959) that during the first hours after blue irradiation the plants are highly sensitive to the inhibitive action of red irradiation.

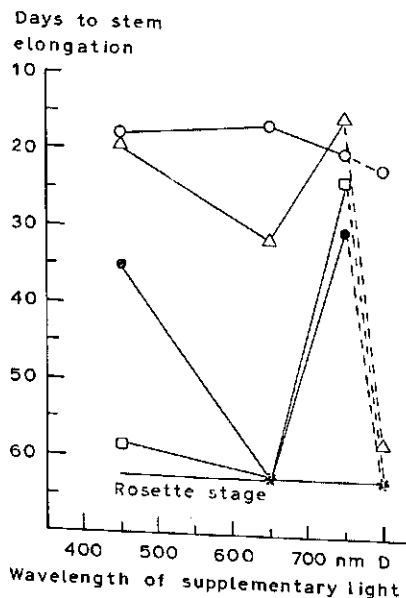


FIG. 4. Days to stem elongation in *Hyoscyamus* under a daily irradiation of 14 hours blue (○), green (●), red (□) or white (△) light (5000 ergs/cm²/sec) followed by 2 hours blue, red, far-red radiation (5000 ergs/cm²/sec), or darkness. Plants previously grown in 9 hour short days in white fluorescent light. Treatment started 27-12-'65, and ended after 62 days. Averages of 4 plants.



PLATE 3. Stem elongation and flowering under a daily schedule of 14 hours blue (B), green (G), red (R), or white (W) light followed by 2 hours B, R, far-red (FR), or darkness (D). The light intensity in both the basic and the supplementary light period was nearly equal (5000 ergs/cm²/sec). See figure 4, p. 24. Photographed 24-1-1966, after 29 days.

With a white basic irradiation, all supplementary treatments used resulted in stem elongation. However, plants under the far-red supplement reacted faster than all others. Different from its normally inhibitive function, the red supplementary radiation seems to be definitely promotive instead of causing some retardation relative to the dark control. This observation closely resembles the one by DE LINT (1958) that *Hyoscyamus* plants, receiving 8 hrs white light daily remained in the rosette stage, whereas plants receiving 8 hrs white light supplemented by two hours of far-red bolted.

The preceding experiment also seems to confirm the results obtained by STOLWIJK and ZEEVAART (1955) in which *Hyoscyamus* plants were irradiated with red light of high intensity. One lot of plants received continuous red light, and three other lots received nine hours of blue light either daily, once in two days or once in three days, respectively, while red light was given for the remaining hours. Only plants in continuous red and those receiving a 9-hr day in blue light daily remained vegetative. Plants of all other treatments produced stems and more rapidly so the more blue light they received. It is obvious that blue light, like far-red irradiation, has the ability to annihilate red light inhibition.

Stem lengths of the plants after 62 days of treatment, as presented in fig. 5, show the same trend as the figures of days to bolting. Generally, upon far-red supplementary irradiation stem length is ahead of all others, probably because of excessive cell elongation.

The petiole length data of the foregoing experiment after 21 days are shown in fig. 6. In all plants receiving differently coloured basic periods followed by

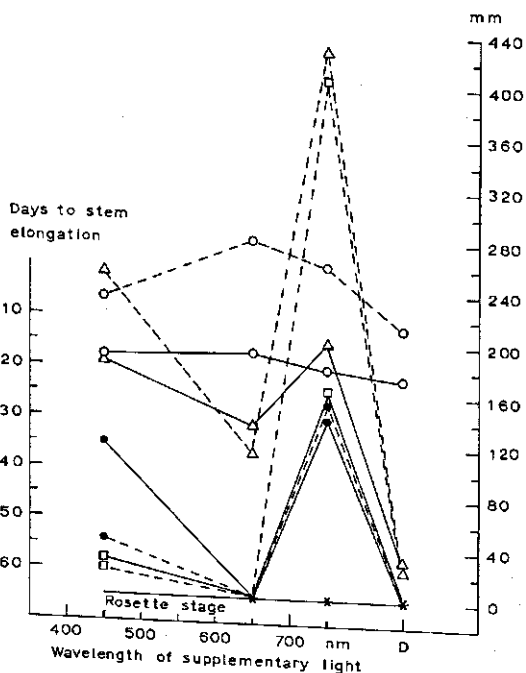
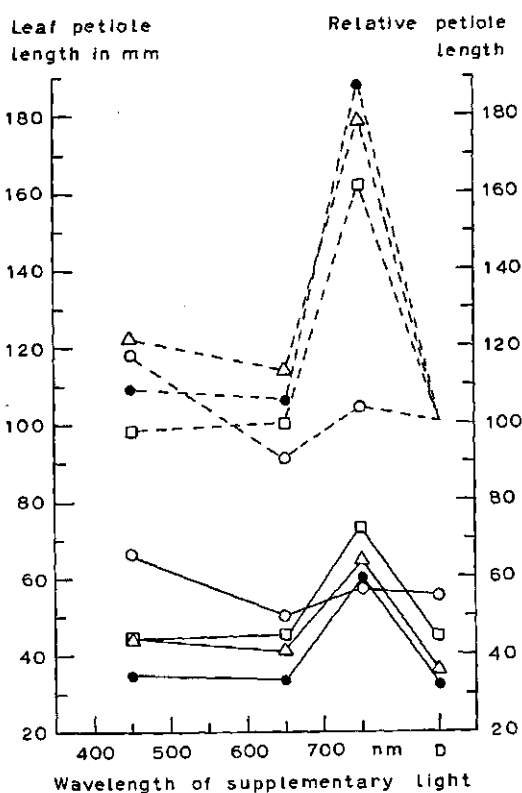


FIG. 5. Stem length (---) correlated to days to stem elongation (—) in *Hyoscyamus* under a daily irradiation of 14 hours blue (○), green (●), red (□), or white (△) light (5000 ergs/cm²/sec) followed by 2 hours blue, red, far-red radiation (5000 ergs/cm²/sec), or darkness. The treatment ended after 62 days. Averages of 4 plants.

FIG. 6. The average of leaf petiole length (—) and its relative values (---) in *Hyoscyamus* upon a daily irradiation of 14 hours blue (○), green (●), red (□), or white (△) light (5000 ergs/cm²/sec) followed by 2 hours blue, red, far-red radiation (5000 ergs/cm²/sec), or darkness. Measurement after 21 days. Averages of 4 plants.



far-red irradiation, the average length of leaf petioles is greater than that of the dark controls. Leaf petioles of plants irradiated with green, red or white light in the main light period show a stronger response to the far-red supplement than those with a blue basic period.

With blue supplementary irradiation, the average lengths of petioles are greater with basic periods of blue and white light than in combination with the green and red main light periods.

Considering red supplementary light, there is not much elongation observed at all.

The influence of a coloured supplement to a basic light period of various colours is seen easier when presented as relative petiole lengths, fig. 6. From these data it is clear, that far-red supplementary irradiation produces distinctly more elongation than blue.

Concluding we may say that *Hyoscyamus* reacts to supplementary irradiation in the blue and far-red regions with a marked elongation of stem and petiole lengths. This indicates that this plant has the mechanism that is called the blue-far red reaction (MOHR, 1959; MOHR, and NOBLE, 1960).

Ef. bfects of low intensity supplementary irradiation

According to the available literature and the observation presented in the preceding experiments, it is clear that blue and far-red supplementary irradiations are more active in producing a long-day effect than green and red light. However, other data indicate that for some other plants supplementary or night break irradiation with red light is more active.

Therefore, the following experiment was performed to study the influence of light quality of both the main and the supplementary light periods on the photo-periodic and formative reactions of *Hyoscyamus niger*.

Plants were exposed daily to 10 hrs of blue, green, red, or white light at an intensity of about 5000 ergs/cm²/sec. After this main light period, plants of each group were exposed for another 6 hrs to supplementary light at low intensity of 1000 ergs/cm²/sec followed by darkness, or to darkness immediately. Average plants of this experiment are presented on plate 4. The experiment started 12-9-1966 and lasted 60 days. The results are shown in fig. 7.

Short day treatments of 10 hrs in the various colours all are ineffective in inducing stem elongation and flower initiation.

Supplementary light, during 6 hrs of red or blue following a short day in green or red light does not produce any long-day effect. Far-red combination with the same two colours does cause shooting.

After a short day in blue light, supplementary treatments with blue, red or far-red are elongative. The effects of red and far-red are equal and probably somewhat earlier than that of the blue supplement; elongation starts after 22 days from the beginning of the treatment with red and far-red, and only after 35 days with blue light.

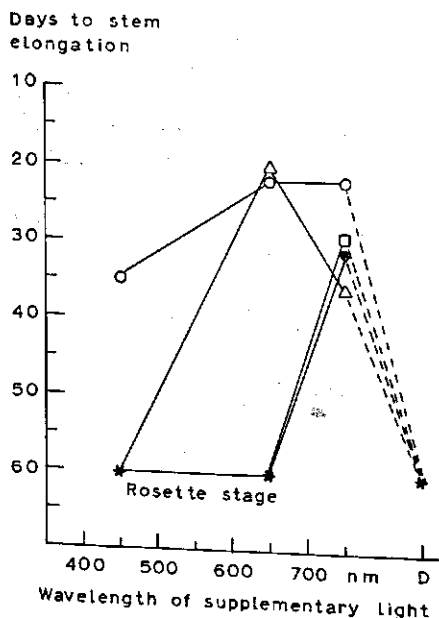


FIG. 7. Days to stem elongation in *Hyoscyamus* upon a daily irradiation of 10 hours blue (○), green (●), red (□) or white (△) light (5000 ergs/cm²/sec) followed by 6 hours blue, red, far-red radiation (1000 ergs/cm²/sec), or darkness. Treatment started 12-9-'66, and lasted 60 days. Averages of 4 plants.

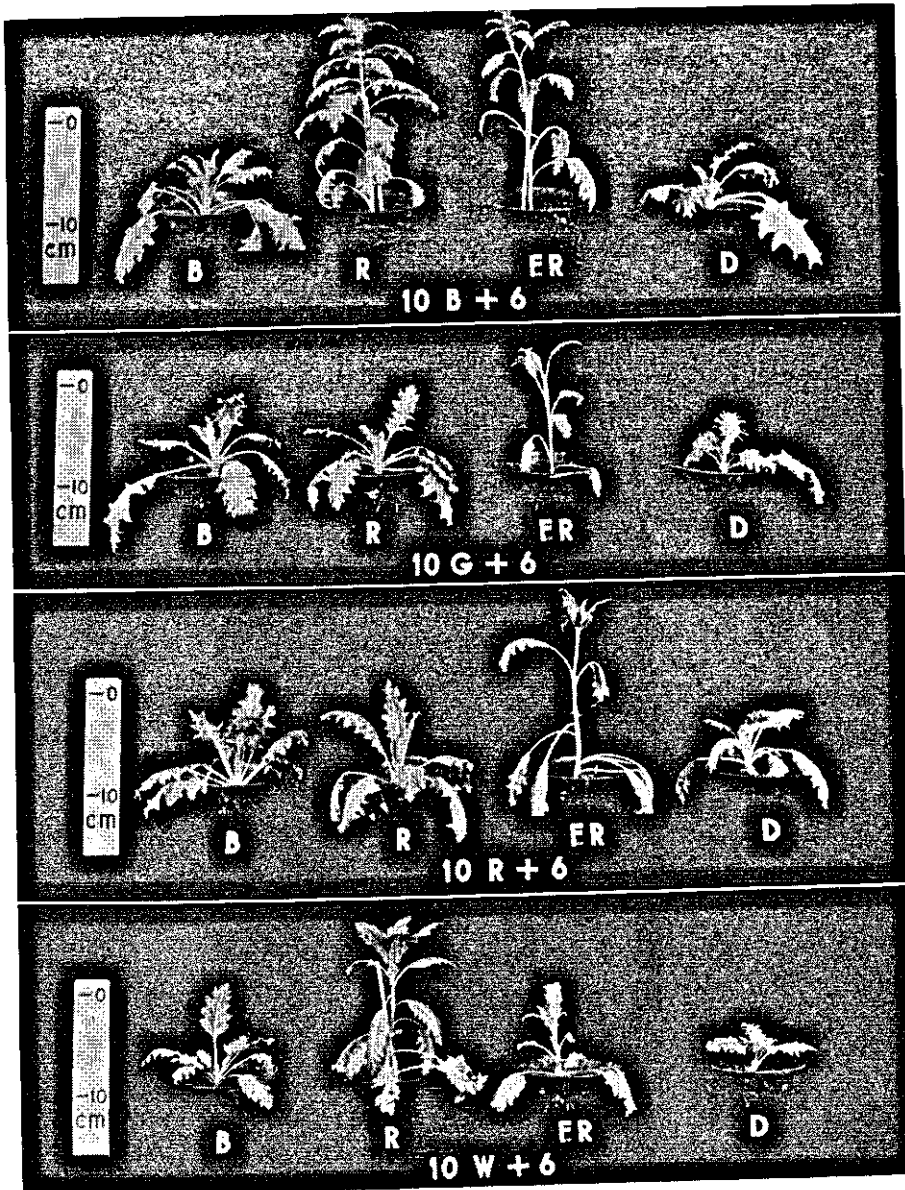


PLATE 4. Continued treatment with 10 hour days in blue (B), green (G), red (R), or white (W) light supplemented with 6 hours B, R, or far-red (FR) light (1000 ergs/cm²/sec), or darkness (D). See figure 7, p. 28. Photographed 27-10-1966, after 46 days.

After a short day in white light, 6 hrs of blue light are non-inductive, whereas red and far-red are.

The result that in *Hyoscyamus*, red light supplementary to a basic irradiation with the new type of white fluorescent light is more active than far-red, has not been reported before. This difference with earlier work may be due to the higher fraction of far-red present in the white light used as compared with the original type of white light containing very little far-red.

This result, and the long-day effect observed when 6 hrs of red light were given after a short day in blue light, may be interpreted on the basis of the phytochrome system, assuming that the red supplementary radiation acts as a main light period, and that, therefore the phytochrome at the close of the red light period is in the far-red absorbing form (P_{fr}), which is gradually reversed to the red absorbing form in the following dark period. After the decrease to a lower level of the far-red absorbing form it is raised again by the subsequent periods of either blue or white light, which then act as supplementary light, leading to stem elongation and flower bud initiation.

This result suggests that induction in *Hyoscyamus niger* requires intermediate phytochrome P_{fr} -levels, while higher levels are inhibitive. A similar assumption was proposed by LANE, CATHEY and EVANS (1965), who worked with long-day plants. This same idea was also presented by CUMMING (1963) who suggested that intermediate phytochrome P_{fr} -levels may be optimum for induction in the short-day plant *Chenopodium rubrum*.

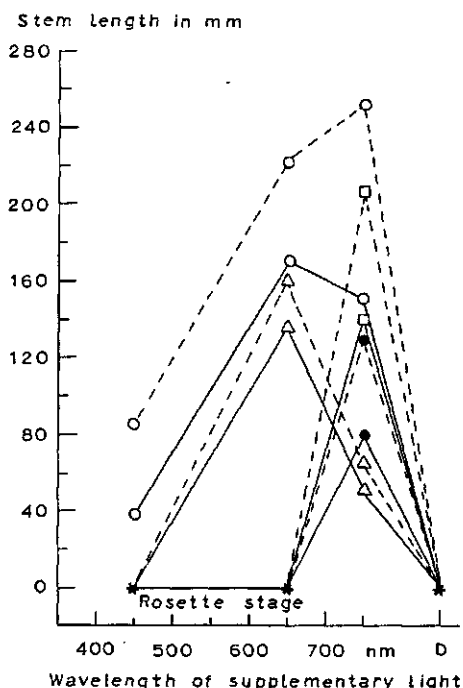
Thus, the results of the experiments carried out in long days indicating that blue and far-red radiation are more active than red light in inducing the long-day effect, are in agreement with those of the experiments with supplementary light to a short-day period. Moreover, the results obtained in the foregoing experiment show that the effect of a supplementary irradiation with respect to the photoperiodic reaction is specifically dependent on the colour of the basic irradiation.

It is evident that plants receiving basic days in red light (14 or 16 hrs) remained in the rosette stage, while 2 hrs of far-red supplemented to 14 hrs red light cause bolting. This observation may be explained on the basis of the phytochrome system, assuming that with red light only P_{fr} is present in too high a concentration to permit flowering, and that the additional far-red decreases the P_{fr}/P_r ratio, and then permitting flowering. This insight reminds of the data of STOLWIJK and ZEEVAART (1955) who reported that shooting of *Hyoscyamus* plants, grown in long days of red light, is inhibited, while a far-red admixture (30%) to the red long days permitted flowering.

Again, 2 hrs blue supplemented to 14 hr basic days in red light cause bolting, but obviously more slowly than a far-red supplement. Thus, it is clear that blue light resembles far-red in its effect.

Moreover, it is clear that distinct stem elongation results from far-red supplementary irradiation after short-day treatments in all colours. This observation suggests that either far-red radiation somehow promotes the action of P_{fr} (EVANS, 1964) if this is at a low level, or that *Hyoscyamus* plants require a specif-

FIG. 8. Dependence of stem length in mm in *Hyoscyamus* after 40 (—) or 55 (---) days upon a daily irradiation of 10 hours blue (○), green (●), red (□), or white (△) light (5000 ergs/cm²/sec) followed by 6 hours blue, red, or far-red radiation (1000 ergs/cm²/sec), or darkness. Averages of 4 plants.



ic ratio between the amounts of the two pigment forms. However, supplementary light, during 6 hrs of red or blue following a short day in green or red light do not produce any long day-effect.

In addition, plants receiving long days in blue light (14 or 16 hrs) or 10 hrs blue followed by 6 hrs blue light at lower intensity gave bolting. It is not established whether both forms of phytochrome have an absorption in the blue (BORTHWICK and HENDRICKS, 1960) or whether in blue light action another pigment is involved.

Stem length after 40 and 60 days from the beginning of the experiment just described are shown in fig. 8.

It is observed that short days of 10 hours produce no stems.

Blue supplementary light, given after green, red or white short days does not produce stems, while after a basic period of blue it does.

Red supplementary irradiation gives stems after main periods of blue and also of white light. With white light, however, stem lengths are less than with blue light, whereas after green and red short days the plants remained vegetative.

Distinct stem elongations result from far-red supplementary irradiation with short-day treatments in all colours. The elongation response to a far-red supplement of the main light period is very clear with a main period in blue, and increasingly weaker with red, green and white light.

Considering red or far-red supplements in combination with a blue basic period, stems with the red supplement after 40 days are distinctly longer

than in case of far-red, while after 55 days, with far-red irradiation, stem lengths definitely surpass those of plants that received red.

As has been mentioned above, bolting upon red radiation starts earlier than upon far-red following white short days, stems are also longer with the former than with the latter.

The trend of these results is quite similar to that of the data about days to shooting. A clearer picture about the relationship between stem length after 60 days and the shooting data can be obtained from fig. 9.

Generally, as the plants advanced in age (after 60 days of the beginning of the treatment) stem length under the effective supplementary wavelengths continued to increase very gradually.

Although stem elongation in short days of blue light is absent, leaf petioles elongate as compared to the other short-day treatments. This result is in agreement with the data of STOLWIJK and ZEEVAART (1955). There seems a distinct formative effect on petiole length as is presented in fig. 10.

With regard to blue supplements, petiole length shows pronounced elongation after blue and white short days, while no elongation occurs after green and red as compared with the control treatments.

Concerning red supplements, it is observed that distinct petiole elongation

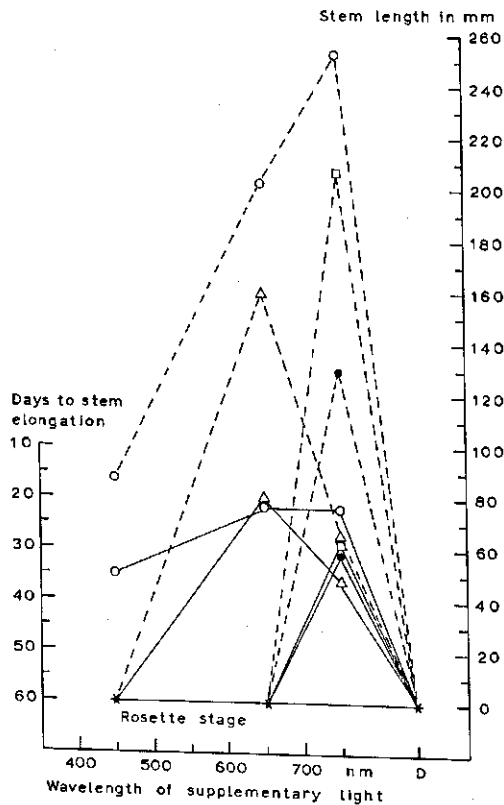


FIG. 9. The relationship between the photoperiodic reaction (—) and stem length (---) in mm in *Hyoscyamus* under a daily treatment of 10 hours blue (○), green (●), red (□), or white (△) light (5000 ergs/cm²/sec) followed by 6 hours blue, red, or far-red radiation (1000 ergs/cm²/sec), or darkness. The experiment started 12-9-'66. Measurement after 60 days. Averages of 4 plants.

FIG. 10. The average of petiole length in mm in *Hyoscyamus* upon a daily irradiation of 10 hours blue (○), green (●), red (□), or white (△) light (5000 ergs/cm²/sec) followed by 6 hours blue, red, or far-red radiation (1000 ergs/cm²/sec), or darkness. Measurement after 21 days. Averages of 4 plants.

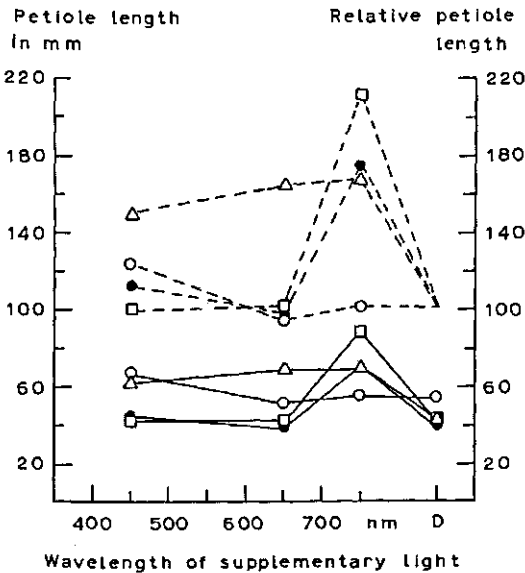
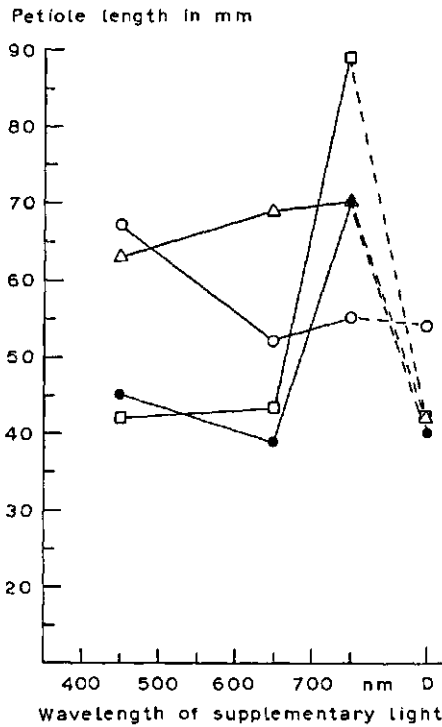


FIG. 11. The relationship between leaf petiole length in mm (—) in *Hyoscyamus* and its relative values (---) upon a daily treatment of 10 hours blue (○), green (●), red (□), or white (△) light (5000 ergs/cm²/sec) followed by 6 hours blue, red, far-red radiations (1000 ergs/cm²/sec), or darkness. Measurement after 21 days. Averages of 4 plants.

takes place after short days in white light, whereas after the other short days petioles do not excessively elongate.

With far-red supplementary irradiation, distinct petiole elongation occurs after green, red and white short periods, while no clear elongation is observed after blue light.

Petiole lengths in mm and in relative values (fig. 11) may provide a clearer picture about the influence of low intensity supplementary irradiation. It seems justified to suggest that *Hyoscyamus* plants have the mechanism responsible for the blue – far-red reaction, also known as the high-energy reaction (MOHR, 1959; MOHR and NOBLE, 1960).

From these observations, it is not easy, however, to completely separate flowering effects and the simpler formative reactions in relation to photoperiod.

2. Effects of coloured illumination with supplementary far-red radiation of different durations

The data obtained from the previous experiments indicate that far-red radiation has a formative and photoperiodic effect. From the experiments of STOLWIJK and ZEEVAART (1955) it seemed that *Hyoscyamus* had a daylength reaction different from that of other plants, e.g. *Brassica*. With supplementary irradiation, stem elongation proceeds most quickly in far-red. WASSINK, SLUIJSMANS and STOLWIJK (1950) observed that *Cruciferae* react clearly to the formative elongation reaction, but they seem to be more or less indifferent to the photoperiodic flowering reaction. In addition, DE LINT (1959) demonstrated that the long-day plant *Hyoscyamus niger* reacts in the same way, and that the long-day requirement is not essential. Adding 2 hrs of far-red radiation to 8 hrs of white light in high intensity, stem elongation and flower bud formation occur irrespective of daylength.

The object of the following experiment is to confirm DE LINT's observation and to see whether the critical daylength of *Hyoscyamus* can be considerably reduced by far-red supplementary radiation, given after short days in light of various colours as when given after white light.

Plants were irradiated daily with 10 hrs of blue, green, red or white, at an intensity of about 5000 ergs/cm²/sec. Following the main light periods, plants of each treatment received daily and immediately different durations of far-red radiation (1, 2, 3, 4, 5, or 6 hr; 1000 ergs/cm²/sec) or were moved into darkness directly.

The results are presented in fig. 12. Again, a short-day treatment of 10 hrs is not elongative, in any of the colours.

In general, increasing durations of far-red result in sooner elongation with the three colours: blue, green, or red.

Upon short-day treatment in blue, plants are relatively more responsive to far-red supplements than after treatment with the other colours. Retardation of stem elongation was observed when the duration of far-red increased, after a white period, from 1 to 6 hours.

Moreover, one hour of far-red radiation is fully sufficient to induce stem elongation and flower bud formation after all colours in short days. Supple-

FIG. 12. Photoperiodic effect of a supplementary irradiation with 1, 2, 3, 4, 5, or 6 hours of far-red (1000 ergs/cm²/sec) given after a main light period of 10 hours blue (○), green (●), red (□), or white (△) light (5000 ergs/cm²/sec). The experiment started 12-9-'66, and ended after 60 days. Averages of 4 plants.

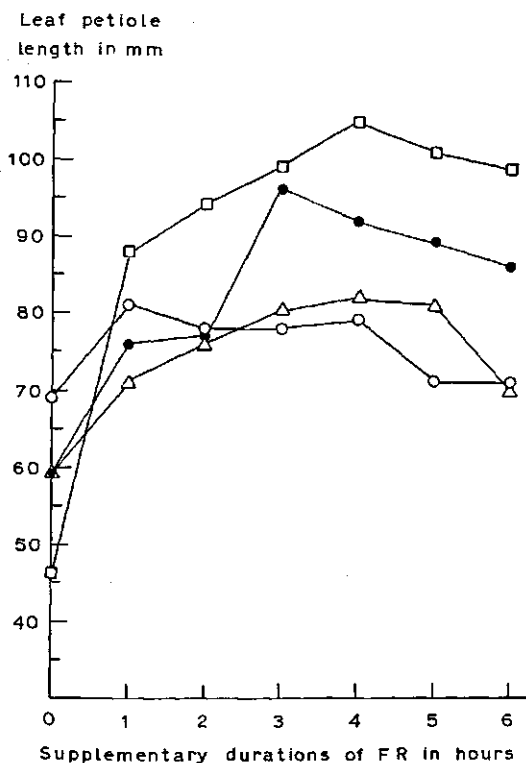
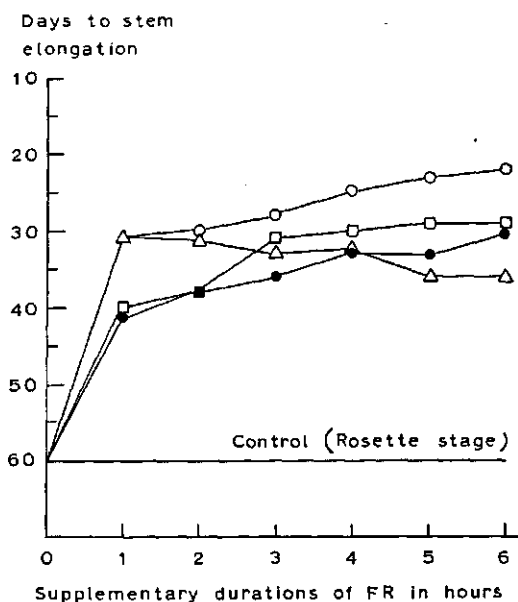


FIG. 13. Leaf petiole length in mm as a result of exposure to different durations (1, 2, 3, 4, 5, or 6 hours) of supplementary far-red irradiation (1000 ergs/cm²/sec) given after a main light period of 10 hours blue (○), green (●), red (□), or white (△) light (5000 ergs/cm²/sec). Measurement after 21 days. Averages of 4 plants.

menting 60 mins of far-red, induces stem elongation after 31 days with the blue and the white main light periods; after about 40 days, bolting starts in the case of green and red days.

In the preceding experiment on the photoperiodic reaction, a formative effect was observed. There also seems to be a distinct effect of the duration of far-red supplementary radiation on petiole length as shown in fig. 13. Increase in the duration of far-red results in longer petioles except after the blue main light period. In combination with the blue main light period, plants are more responsive to far-red with respect to bolting than with the other main light periods; with respect to petiole lengths, however, a short day of red light is more effective than all other colours.

In comparison with the control treatments, clear leaf petiole elongation is shown upon 60 mins far-red supplements. Average petiole lengths in 10 hr-days of blue, green, red and white light are 69, 59, 46, 59 mm, whereas supplementing one hour of far-red shifted the lengths to 81, 76, 88, 71 mm, respectively. However, the elongation does not increase so much with more far-red after blue and white periods, as it does after green and red days. Relative petiole lengths, as percentages of the zero far-red controls, demonstrate the same result clearer, as is shown in fig. 14.

The foregoing experiment indicated that already a supplementary far-red radiation of 1 hour induced a promotion of flowering and a distinct formative effect, confirming the observations of DE LINT (1959).

An attempt was made to see how far this period could be further reduced. *Hyoscyamus* plants were given supplementary far-red radiation at an intensity of 1000 ergs/cm²/sec for 0,3, 10, 30, 60 or 360 mins, immediately after a daily 10 hour period of blue, green, red or white light at an intensity of 5000 ergs/cm²/sec. Plate 5 shows the aspect of representative plants.

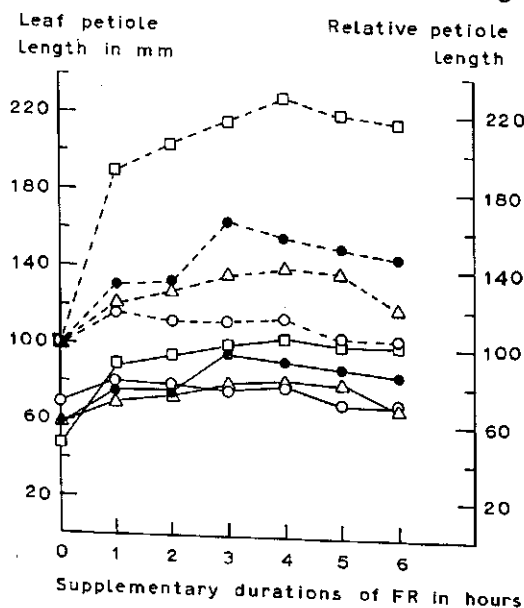


FIG. 14. The relationship between the leaf petiole length in mm (—) in *Hyoscyamus* and its relative values (---) upon a daily treatment of 10 hours blue (○), green (●), red (□), or white (△) light (5000 ergs/cm²/sec) followed by various durations (1, 2, 3, 4, 5, or 6 hours) of far-red radiation (1000 ergs/cm²/sec). Measurement after 21 days. Averages of 4 plants.

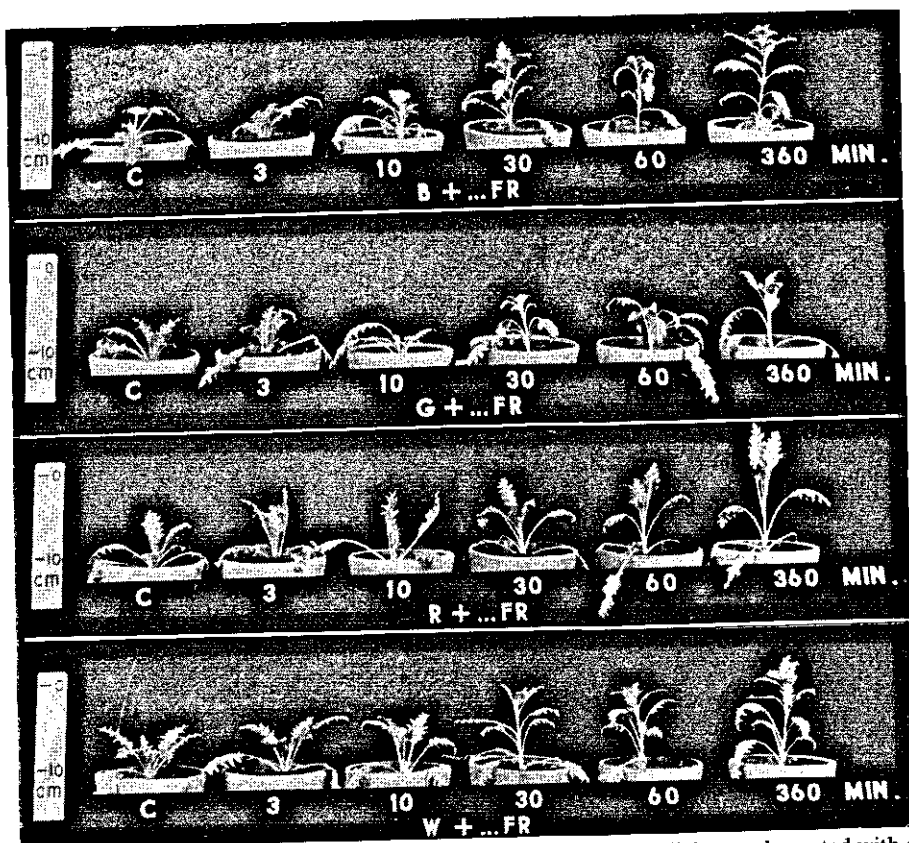


PLATE 5. Continued treatment with 10 hour days in B, G, R or W light supplemented with a series of (0, 3, 10, 30, 60, or 360) minutes of far-red radiation. C = control treatment. See figure 15, p. 38. Photographed 20-3-1967, after 56 days.

From fig. 15, in which the results on visible shooting are presented, it can be seen that the effect of 60 mins is more or less equal to the effect of 360 mins, irrespective of the main light period colours. This trend is the same as obtained in the preceding experiment. Also, it is evident that even 30 mins of far-red supplement are still considerably promotive, when it is given after the various colours of the basic periods. Again, the blue light period is more sensitive to far-red extension than the other colours of the main period. In the case of blue days, stem elongation started after 37 days, while using green, red or white basic periods first shooting appeared after about 42 days. Blue and green basic light periods have a response to the addition of 10 mins of far-red radiation, bolting after 41 and 49 days respectively, whereas this duration is ineffective after red and white light. Supplementary irradiation for only 3 mins has no effect after any of the main light treatments; after 60 days, they were still in the rosette stage as the control ones.

From the preceding experiment, it is evident that 30 mins of far-red radiation supplemented to 10 hours of high intensity light provoke a long-day reaction. It

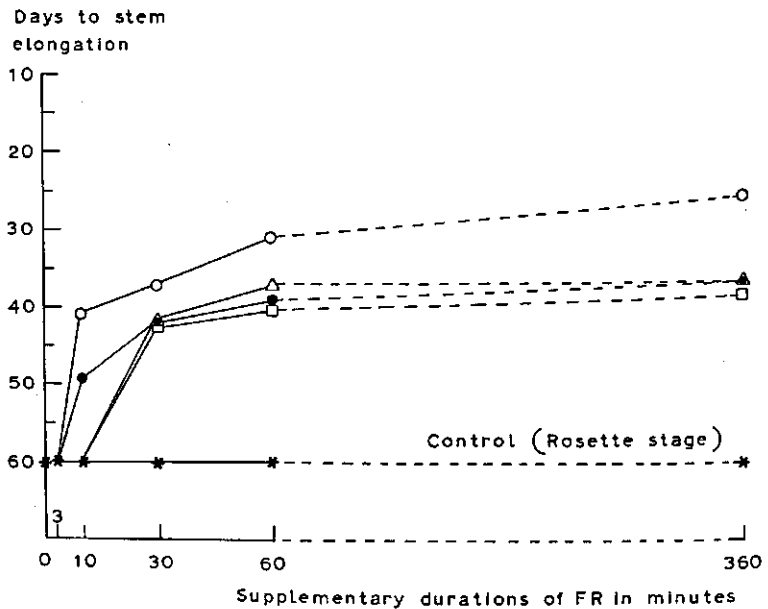


FIG. 15. Days to stem elongation upon a daily irradiation of 10 hours blue (○), green (●), red (□), or white (△) light (5000 ergs/cm²/sec) followed by various durations (0, 3, 10, 30, 60, or 360 mins) of supplementary far-red radiation (1000 ergs/cm²/sec). The experiment started 24-1-'67, and lasted 60 days. Averages of 4 plants.

means that stem formation in *Hyoscyamus niger* can occur in short days. The data, thus, are another example for the statement that it is senseless to use the term 'critical daylength' when working with separate spectral regions (WASSINK and STOLWIJK, 1956).

Another example has been presented by BORTHWICK et al. (1952a) for the short-day plant *Xanthium pensylvanicum* which can be brought to flower in too long days, as compared with white light treatment under the same conditions, by supplying half an hour of far-red at the end of a white day.

Recently, BORTHWICK and DOWNS (1964) reported that flowering of *Xanthium pensylvanicum* was inhibited by far-red at the close of 1.5–2 hr photoperiods.

It can be seen from fig. 16 that average stem lengths plotted against the duration in mins of supplementary far-red radiation confirm the feature of the photoperiodic reaction shown in the previous figure. In general, it seems that increasing far-red doses cause longer stems.

Moreover, another formative effect has been affected by supplementary far-red irradiation. Fig. 17 presents data on petiole lengths in mm. It is evident that the petiole is sensitive to elongation by a few minutes of far-red. Already as little as 3 mins of far-red cause elongation, but increasing the supplement duration further causes the petioles to lengthen much more. It seems reasonable to suppose that the excessive elongation of the petioles in far-red supplementary irradiation is mainly due to increased cell elongation, but observations to this point so far have not been made.

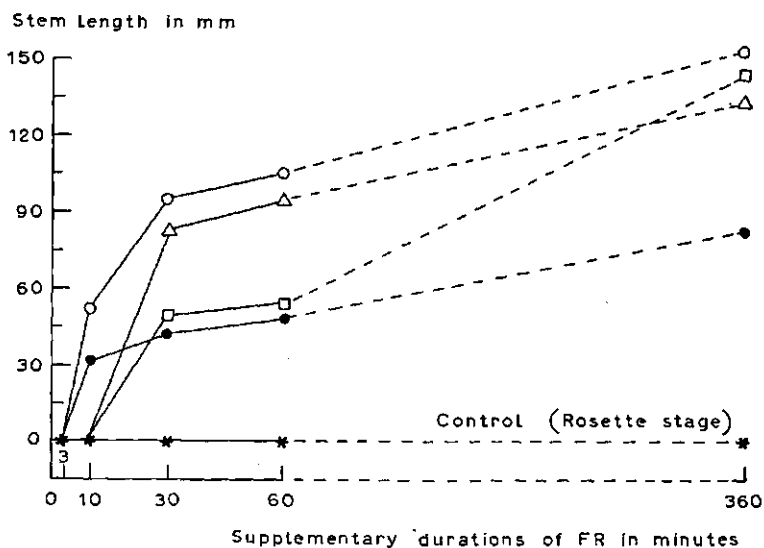


FIG. 16. Dependence of stem length in *Hyoscyamus* after 60 days, upon a daily irradiation of 10 hours blue (○), green (●), red (□), or white (△) light (5000 ergs/cm²/sec), followed by various durations (0, 3, 10, 30, 60, or 360 mins) of far-red radiation (1000 ergs/cm²/sec). The experiment started 24-1-'67, and lasted 60 days. Averages of 4 plants.

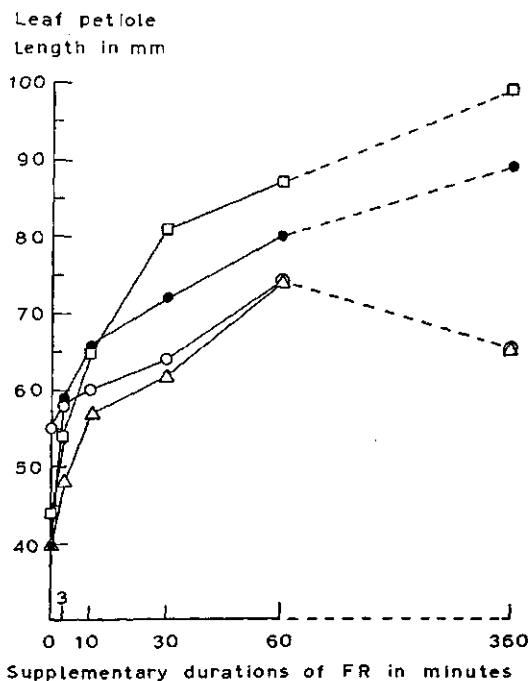


FIG. 17. Leaf petiole length in mm in *Hyoscyamus* as a result of exposure to different durations (0, 3, 10, 30, 60, or 360 mins) of far-red radiation (1000 ergs/cm²/sec) given after 10 hours blue (○), green (●), red (□), or white (△) light (5000 ergs/cm²/sec). The experiment started on 24-1-'67, and ended after 60 days. Averages of 4 plants.

It may be concluded that elongation of the petiole is more sensitive than the flowering reaction.

It is obvious from fig. 15, that for stem elongation 30 mins of far-red are promotive, when it is given after variously coloured main photoperiods. Blue and green basic light periods have a response to the addition of 10 mins of far-red radiation, whereas this duration is ineffective after red and white light irradiation, and 3 mins only has no effect after any of the main light treatments.

However, the data for petiole elongation, fig. 17, indicate that petioles do elongate even upon 3 mins supplementary far-red, irrespective of the colour of the basic light period, and increasing the supplementary dose caused the petiole to elongate much further.

3. *Effects of white fluorescent light and of a mixture of white fluorescent and incandescent light, supplemented by light of well defined spectral regions*

Although red light may be the most effective spectral region for a brief night break, it is often relatively ineffective in prolonged photoperiod extensions, especially as compared with far-red radiation which may be far more effective (STOLWIJK and ZEEVAART, 1955; DE LINT, 1958; TAKIMOTO, 1961; VINCE, 1965). VINCE, BLAKE and SPENCER (1964) found that even a small admixture of far-red radiation to a red light extension, or of red radiation with a far-red extension, increased their effectiveness. Similarly, light from incandescent lamps, which has approximately equal amounts of red and far-red energy, or with mixtures of red and far-red radiations, are frequently far more effective when used as a photoperiod extension than is light from fluorescent lamps which contains little far-red energy (WASSINK et al., 1950, 1951; BORTHWICK and PARKER, 1952; TAKIMOTO, 1957; DOWNS, BORTHWICK and PIRINGER, 1958; DOWNS, PIRINGER and WIEBE, 1959; PIRINGER and CATHEY, 1960; FRIEND, HELSON and FISHER, 1961; EVANS, BORTHWICK and HENDRICKS, 1965).

Moreover, adding incandescent light to fluorescent lamps causes a marked increase in the growth and development of certain plants (DUNN and WENT, 1959; PALEG and ASPINALL, 1964; HELSON, 1965; SCHNEIDER, BORTHWICK and HENDRICKS, 1967).

In addition, it was reported that blue light extensions at low intensities, caused elongation of the plants (WASSINK et al., 1950, 1951; STOLWIJK, 1954). However, CURRY and WASSINK (1956) determined that these elongating effects of blue light were due to a small far-red admixture. This conclusion was further confirmed, and it was found that day extensions with blue light without any far-red contamination under these circumstances produced no elongation (WASSINK et al., 1957 and DE LINT, 1960, 1961). Inactivity of blue light when used as only radiation was generally reported (VINCE and STOUGHTON, 1959; DOWNS et al., 1957).

Therefore, it was of considerable interest to see whether the above phenomena could be repeated under the present experimental conditions.

Uniformly selected sets of *Hyoscyamus* plants were daily exposed to 10 hours of the light sources at ca. 30 000 ergs/cm²/sec. The first quality is white light only

(TL) and the other is composed of the mentioned white light combined with incandescent lamps (TL/IL). Supplementary to these two white light qualities, the plants received blue, red, or far-red radiation at low intensities (1000 ergs/cm²/sec). The following supplementary light treatments were given: a) 6 hours blue, b) 6 hours red, c) 6 hours far-red, d) 6 hours blue followed by 2 hours red, e) 6 hours red followed by 2 hours far-red, f) 6 hours far-red followed by 2 hours red. The treatment lasted 60 days. Plate 6 shows the aspect of representative plants.

Fig. 18 gives the flowering response of *Hyoscyamus* with respect to wavelengths of the supplementary radiation. Plants receiving blue light after both of the two sources remain vegetative, as well as the control treatment. However, plants receiving red or far-red do react with stem elongation and flower bud formation, and faster with red than with far-red.

This observation is in agreement with data presented earlier in this paper, where lower light intensities were used during the coloured and white basic periods, as is shown in fig. 5. It is, however, in contrast to the available literature which indicates that red extensions mostly are inactive. Short days of 10 hours in both sources of light do not produce any shooting reaction.

In daylength extensions, consisting of far-red followed by red, and in those consisting of red followed by far-red, the two colours increase each others effectiveness. On the other hand, extensions consisting of blue followed by red cause a long-day effect more or less equal to the action of extensions of 6 hours red alone. This result suggests that blue radiation at low intensity in this combination acts as darkness, and the red light after the blue acts as a night break.

There is a positive correlation between the flowering response and stem length

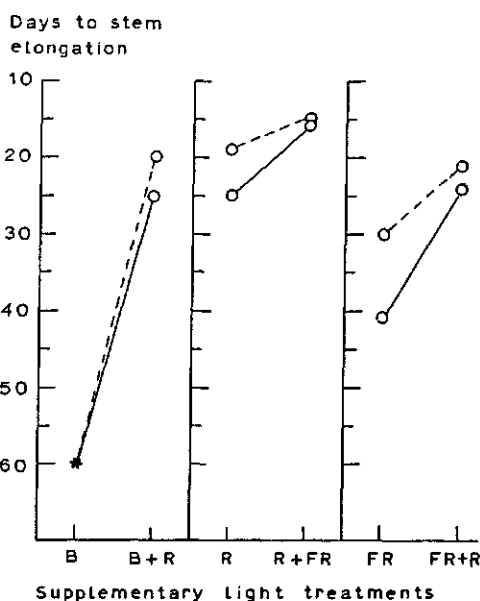


FIG. 18. Photoperiodic effect of a supplementary irradiation with 6 hours of blue (B), red (R) or far-red (FR) radiation, or 6 hours B + 2 hours R, 6 hours R + 2 hours FR, or 6 hours FR + 2 hours R at an intensity of 1000 ergs/cm²/sec given after 10 hours white fluorescent light (—) or a mixture of white fluorescent plus incandescent light (---) at an intensity of ca. 30 000 ergs/cm²/sec. The experiment started 28-3-'66, and lasted 60 days. Averages of 4 plants. Control or rosette stage = ★



PLATE 6. Stem elongation and flowering response as a result of exposure to supplementary irradiation of 6 hours blue (B), red (R), far-red (FR), 6 hours B + 2 hours R, 6 hours R + 2 hours FR, or 6 hours FR + 2 hours R at the intensity of 1000 ergs/cm²/sec, given after continued treatment with 10 hour days in white fluorescent light (TL) or a mixture of the same fluorescent and incandescent light (TL + INC). See figure 18, p. 41. Photographed 3-5-1966, after 37 days.



(Plate 6, Continue)

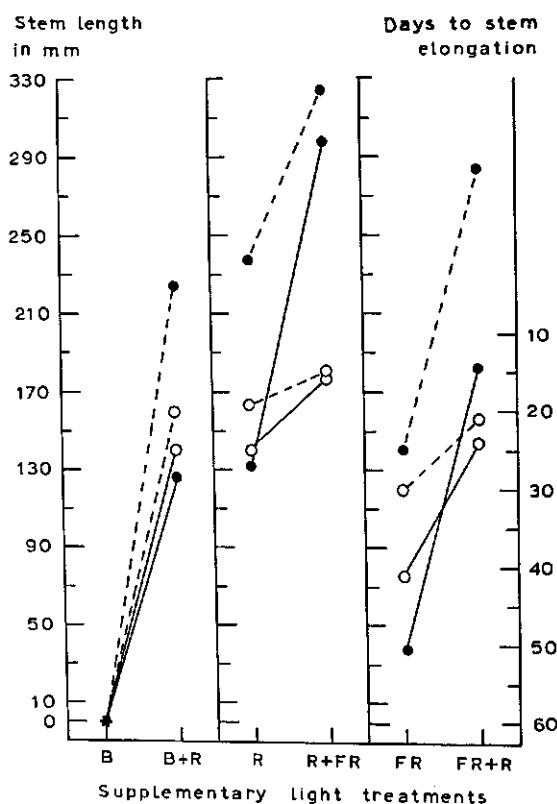


FIG. 19. Correlation between the flowering response (○) and stem length in mm (●) in *Hyoscyamus* as a result of exposure to supplementary irradiation of 6 hours blue (B), red (R), far-red (FR), 6 hours B + 2 hours R, 6 hours R + 2 hours FR, or 6 hours FR + 2 hours R at an intensity of 1000 ergs/cm²/sec given after 10 hours white fluorescent light (—) or a mixture of white fluorescent plus incandescent light (---) at an intensity of ca. 30000 ergs/cm²/sec. The experiment started 28-3-'66, and lasted 60 days. Averages of 4 plants. Control or rosette stage = ★

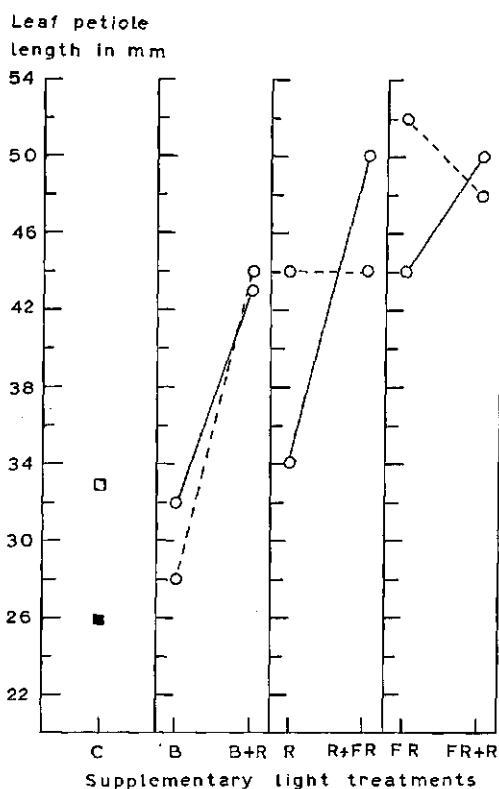
in mm after 60 days as can be seen in fig. 19. It is clear that exposure to red followed by far-red and far-red followed by red radiation accelerates flowering and enhances stem lengthening. It seems likely that red radiation is essential for flower induction and far-red for elongation.

Very clearly, the light source containing incandescent light causes faster shooting and elongation than the other, this may be due to the larger total amount of far-red energy.

Not only the shooting reaction has been affected by the wavelengths of the supplementary light, but also the petiole length is affected. Fig. 20 shows that blue supplementary irradiation does not too much affect the petiole length compared to the control one, while 2 hours of red after a blue extension causes considerable elongation. With fluorescent light as a basic light period, the average petiole length with a blue supplement is 32 mm, hence, altered to 43 mm after adding 2 hrs of red radiation to the blue extension, while petiole length, with regard to the other basic period is 28 and 44 mm respectively. Thus, there is no influence of the main light period on the effect of the blue supplementary irradiation or of the red illumination after the blue supplement.

Both red and far-red supplementary irradiation seem to cause clear elongation of the petiole; however, the latter has a stronger action than the former.

FIG. 20. Leaf petiole length in mm as a result of a supplementary irradiation with 6 hours of blue (B), red (R), or far-red (FR) radiation, or 6 hours B + 2 hours R, 6 hours R + 2 hours FR, or 6 hours FR + 2 hours R at an intensity of 1000 ergs/cm²/sec given after 10 hours white fluorescent light (—) or a mixture of white fluorescent plus incandescent light (---) at an intensity of ca. 30000 ergs/cm²/sec. The experiment started 28-3-'66. Measurement after 21 days. Averages of 4 plants. Control treatment (C) of (—) = ■ and of (---) = □.



The main light period plays a role in petiole lengthening, it is clear that petiole length under red and far-red supplements is longer in the case of the mixed basic period than with white light poor in far-red.

With fluorescent light during the main light period, a further addition of 2 hours of far-red radiation to a red extension or of red illumination to a far-red extension cause distinct elongation of the petiole. However, in case of the mixture, there is no effect of far-red, following a red extension, while a reduction in petiole length occurs when red radiation follows a far-red extension.

To obtain a clearer idea, fig. 21 indicates the petiole lengths in mm and also their relative lengths. The petiole under red and far-red supplements is longer in the case of the mixed basic period than with white light poor in far-red. However, in the case of the mixture there is no effect of far-red, following a red extension, while a reduction in petiole length occurs when red radiation follows a far-red extension, whereas basic fluorescent light periods combined with these treatments cause manifest elongation of the petiole.

These data may be summarized in that a basic light period obtained from fluorescent lamps, with little far-red, results in effects which are well in harmony with the available literature. Adding far-red from incandescent lamps during the basic light periods results in a more complicated picture, as has been shown

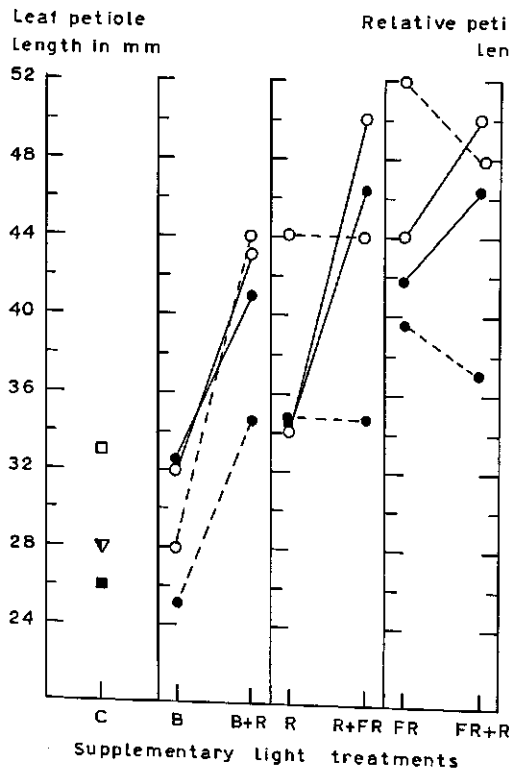


FIG. 21. Relationship between leaf petiole length in mm (○) and its relative length (●), as a result of a supplementary irradiation with 6 hours blue (B), red (R), or far-red (FR) radiation or 6 hours B + 2 hours R, 6 hours R + 2 hours FR, or 6 hours FR + 2 hours R at an intensity of 1000 ergs/cm²/sec given after 10 hours white fluorescent light (—) or a mixture of white fluorescent and incandescent light (---) at an intensity of ca. 30000 ergs/cm²/sec. The experiment started 28-3-'66. Measurement after 21 days. Petiole length in mm of control treatment of (—) = ■, and of (---) = □. Relative length of control treatment of (—) = ▼, and of (---) = ▽ (= 100).

above, which can be understood by assuming that the effect of far-red applied during the basic light period acts in much the same way as far-red applied as daylight extension.

CHAPTER V

MORPHOGENETIC EFFECTS OF LIGHT COLOUR DURING THE MAIN LIGHT PERIOD IN CONJUNCTION WITH VARIOUS PATTERNS OF INTERRUPTION OF THE DARK PERIOD BY LIGHT OF VARIOUS WAVELENGTHS

A. INTRODUCTION

The effect of white light interruptions in the long dark period with long-day plants is to induce flowering, a response opposite to that of the short day plants; these effects are the same as obtained with each type of plant by extending the length of the photoperiod with weak supplementary white light. We have already seen in the previous chapter that this apparently simple situation does not hold anymore in a number of cases when light of restricted spectral regions is applied.

In the present chapter we will discuss experiments in which the action spectrum for night interruption has been investigated. In some short-day and long-day plants, the Beltsville group (PARKER et al. 1946, 1950) observed a main peak effectiveness in the red (660 nm) and a lower peak in the blue, using cv. 'Wintex' barley and *Hyoscyamus niger*. The same result was reported by STOLWIJK and ZEEVAART (1955) with *Hyoscyamus niger*. Recently, VINCE (1965) found that red light is effective for such a break also in *Lolium temulentum*.

The effectiveness of night break depends on the time of application which suggests a connection with endogenous or circadian rhythms.

Light breaks are reported to be most effective when given near the middle of the dark period (CLAES and LANG, 1947; BORTHWICK, HENDRICKS and PARKER, 1948; PARKER, HENDRICKS and BORTHWICK, 1950; STOLWIJK, 1952; WAGENAAR, 1954; CLAUSSE and RAU, 1956; SALISBURY, 1961; BHARGAVA, 1964).

Whereas brief light breaks usually are highly effective in inhibiting the inductive effect of a long dark period in short-day plants, they fail to induce flowering in long-day plants, such as dill (NAYLOR, 1941), spinach (WITHROW and WITHROW, 1944), and *Lolium temulentum* (EVANS et al. 1965).

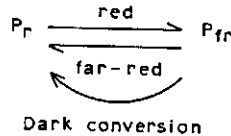
MEIJER and VAN DER VEEN (1960) report that a night break with red light is not effective unless the high intensity light period contains blue or far-red light.

Moreover, it was found that the effect of the night break increased with its duration (VINCE, 1965). However, WAGENAAR (1954) found that the effectiveness of a night break decreased when the duration or the intensity of light increased beyond a certain point.

It has also been shown that the red light effect could be completely reversed with far-red. Evidence for the red, far-red reversibility of photoperiodic night breaks was presented first by BORTHWICK et al. (1952a), in *Xanthium*. Somewhat later, DOWNS (1956) showed that the effect of night breaks were also far-red

reversible in the LDPs *Hyoscyamus niger* and 'Wintex' barley, and the SDPs *Amaranthus caudatus* and 'Biloxi' soybean, and he was able to demonstrate repeated reversibility. MEIJER (1959) showed the same reversibility, using *Salvia occidentalis*.

The pigment involved in these changes was named phytochrome, the reactions of which are summarized in the following scheme:



HENDRICKS (1960) proposed that P_{fr} (the far-red absorbing form of phytochrome), in darkness is slowly converted into P_r (the red absorbing form of phytochrome), and that the P_{fr} -concentration must be reduced below a critical level to allow photoperiodic induction of a short-day plant.

Since far-red radiation reverses the process, its simultaneous presence reduces the effectiveness of the red, owing to mutual antagonism. Light from tungsten-filament lamps contains both wavelength regions of radiation, and, therefore, is less effective than pure red light (CHANHAM, 1966).

STOLWIJK and ZEEVAART (1955) already found in their experiments with *Hyoscyamus niger* that the addition of far-red to the same amount of red night break light decreased the effectiveness of the promotive red radiation.

NAKAYAMA (1958); NAKAYAMA, BORTHWICK and HENDRICKS (1960) observed in *Pharbitis nil* that the inhibition of red light given in the middle of a 16-hour dark period was not reversed by subsequent exposure to far-red. Also, an exception to such reversibility was found in *Lemna perpusilla* 6746 (HILLMAN, 1959). Recently, (FREDERICQ, 1964) using *Pharbitis nil*, however, reported some reversibility in the middle of the dark period (about 8 hours after the beginning of the dark) if the red and far-red irradiations did not last longer than 30 seconds each, and were not separated by darkness. Failure of reversal occurred when irradiations of longer duration were used. He suggested that P_{fr} acts very rapidly, and that if the exposure to red is prolonged, P_{fr} -mediated processes have gone to completion before the far-red radiation was given, so that flowering then could no more be repromoted.

Moreover, it was reported that reversal of night interruption depends on the colour of the main light period (HILLMAN, 1966).

Before entering into a more detailed discussion of these effects, our experimental results will be considered.

B. EFFECTS OF WHITE FLUORESCENT LIGHT AND OF A MIXTURE OF THIS WITH INCANDESCENT LIGHT

1. Effects of different durations of well defined wavelength bands applied around the middle of the long dark period

As has been shown in the last experiment of the previous chapter, 2 hours of red radiation after an (inactive) blue extension cause a long-day effect. Because

of this observation it became of interest to study the relation between the various durations of red and far-red radiation, given in the middle of the long dark period, as well as the precise effect of the colour of the main light period on the photoperiodic response.

Selected *Hyoscyamus* plants were exposed daily to short-days of 10 hours of two sources of light at the same light intensity of ca. 25000 ergs/cm²/sec; the first light source is the original type of fluorescent lamp (PHILIPS, TL/55/40W) which emits little far-red and considerable amounts of red radiation, and the other one is composed of the same type of fluorescent lamp combined with incandescent light to increase the far-red energy.

At the 7-hour point of the dark period, the plants were exposed for varying times (0, 1, 10, 50, or 100 mins) to red or far-red irradiations with intensities of 600 ergs/cm²/sec. Immediately after the breaks, the plants were placed in darkness to follow the short day routine. The experiment started 11-11-1965 and ended after 103 days. Representative plants are shown on plate 7. Days required to bolting are plotted against the time series in minutes of red or far-red radiation, as shown in fig. 22.

Plants in short day in both light sources remain in the rosette stage. Also, 1 min of red or far-red light applied in the middle of the dark period does not visibly promote shooting.

Concerning the fluorescent main light period (FL), a night interruption

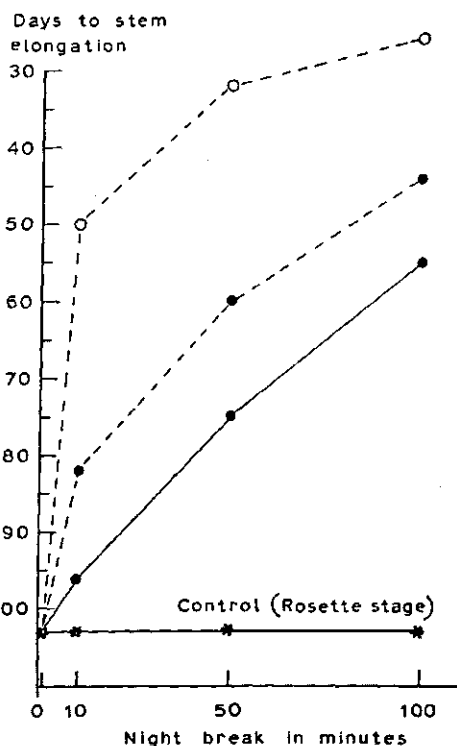


FIG. 22. Number of days to stem elongation of *Hyoscyamus* as affected by red (O), or far-red (●) night interruption (1000 ergs/cm²/sec) of varying durations, given at the middle of the 14 hour dark period, and combined with 10 hours of two types of basic light period: 1 (—) the original white fluorescent light, 2 (---) the same white fluorescent plus incandescent light, both at an intensity of ca. 25000 ergs/cm²/sec. The experiment started 11-11-'65, and ended after 103 days. Averages of 4 plants.

with red light even of 100 minutes is not effective in producing stems. However, plants under night breaks with far-red radiation show increasing stem elongation with increasing duration of the night breaks.

With regard to the mixed source (FL/IL), red light is more active in causing bolting than far-red radiation. The longer the duration of red or far-red radiation, the sooner stem elongation occurs; for instance, 100 mins of red or far-red radiation produce stems after 26 or 44 days, respectively, whereas 10 mins of the same radiations induce bolting only after 50 and 84 days respectively.

From these results it must be concluded that the inclusion of far-red energy in the main light period has a remarkable influence on the photoperiodic effect of a red night interruption. This result is in full agreement with the observation of MEIJER and VAN DER VEEN (1960) who stated that a night break with red light is not effective unless the high intensity light period contains blue or far-red light. In addition, MEIJER (1959) using *Salvia occidentalis* (SDP) which were grown in white fluorescent light found that even a night interruption with 2 hours of red light was not completely effective in preventing the initiation of flowers.

The results of the preceding experiments suggest that far-red energy either in the main light period or during the dark period is essential for stem elongation and flower bud formation in *Hyoscyamus niger*.

With the mixed white light source even only 10 mins of red exposure resulted in the formation of well opened flowers, while the various durations of far-red radiation produced only flower buds, which never became open flowers (only the calyx being well shaped).

It is known that a determination of the number of leaves that in the various treatments develop until shooting is a good indication for the intensity of the flowering response in *Hyoscyamus niger* (CLAES and LANG, 1947; CLAUSSE and RAU, 1956 and FINN, 1958). Fig. 23 shows the relationship between the number of newly expanded leaves until stem elongation and the various durations of red or far-red light, given in the middle of the dark period. With increasing duration of the night breaks stem elongation takes place more rapidly and consequently, the number of newly formed leaves is smaller. In other words, the number of days to shooting and the number of newly formed leaves indeed are inversely correlated. This guarantees, that the growth rates of the plants under the various light treatments were very similar (compare figs. 22 and 23).

As was obvious from the previous experiment, the quality of the main light period plays an important role in the flowering inducing effect of night interruptions by coloured light. In the following experiments, another type of white fluorescent lamp was used, the light of which contains more far-red than that in the former one which was used only in that experiment.

Hyoscyamus plants were illuminated with two types of white light daily during 10 hrs (ca. 25 000 ergs/cm²/sec). The two light qualities were obtained from the new type of fluorescent lamps (PHILIPS, TL/55/40W), which emits about 18% far-red energy, and the other one of that same white light combined with incandescent light.

In the middle of the long dark period during 65 days, plants were subjected to

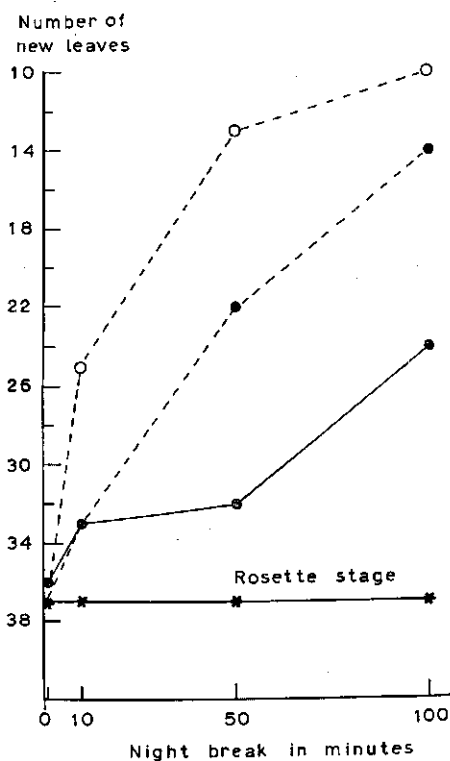


FIG. 23. Number of newly expanded leaves of *Hyoscyamus* as affected by red (○) or far-red (●) night interruption (1000 ergs/cm²/sec) of varying durations given at the middle of the 14-hour dark period, combined with 10 hours of two types of basic light period: 1 (—) the original white fluorescent light, 2 (---) the same white fluorescent plus incandescent light, both at an intensity of ca. 25 000 ergs/cm²/sec. The experiment started 11-11-'65 and ended after 103 days. Averages of 4 plants.

a range of night interruptions of red or far-red radiation (1000 ergs/cm²/sec). Except for the night breaks, the plants were kept in darkness when not under the white irradiation. The effect of these treatments on the start of stem elongation is presented in fig. 24.

In general, it is evident that both sources of basic white light used in this experiment are effective. However, the mixed source causes elongation somewhat more quickly than the other.

Night interruptions of 30 mins and more, with either red or far-red radiation, are elongative and increasingly so when longer. Night interruption with 10 mins of far-red light in combination with both sources of the basic light are inhibitive, as is 10 mins of red radiation associated with a white fluorescent day; promotive is only 10 mins red with the mixed white short day.

Finally, 3 mins night breaks of either quality are inactive, irrespective of the white light quality.

In addition to shooting day data, stem length observations on the plants, after 31 and 51 days under the experimental conditions, are presented in fig. 25. The results confirm the previous data indicating that only 10 mins of red night breaks combined with the mixed white light is sufficient to induce stems.

Thus, the quality of the main light period has an effect on the photoperiodic reaction of coloured night interruptions.



PLATE 7. Stem elongation and flowering as affected by red and far-red night interruption of varying durations (0, 1, 10, 50, or 100 mins) at the middle of 14 hour darkness, combined with daily 10 hours of two basic light periods. The main photoperiods were either the original type of white fluorescent (FL) or a mixture of the same fluorescent plus incandescent light (FL + INC.). See figure 22, p. 49. Photographed 24-12-1965.

FIG. 24. Days to shooting in *Hyoscyamus* as affected by red (○) or far-red (●) night interruption (1000 ergs/cm²/sec) of varying durations given around the middle of the 14-hour dark period, combined with 10 hours of two types of basic light period: 1 (—) the new white fluorescent light, 2 (---) the same white fluorescent plus incandescent light, both at the intensity of ca. 25000 ergs/cm²/sec. The experiment started 8-3-67, and ended after 65 days. Averages of 4 plants.

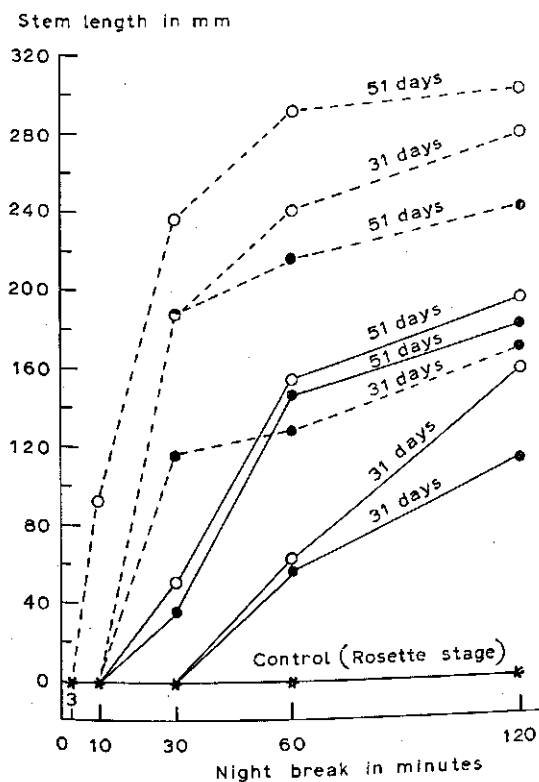
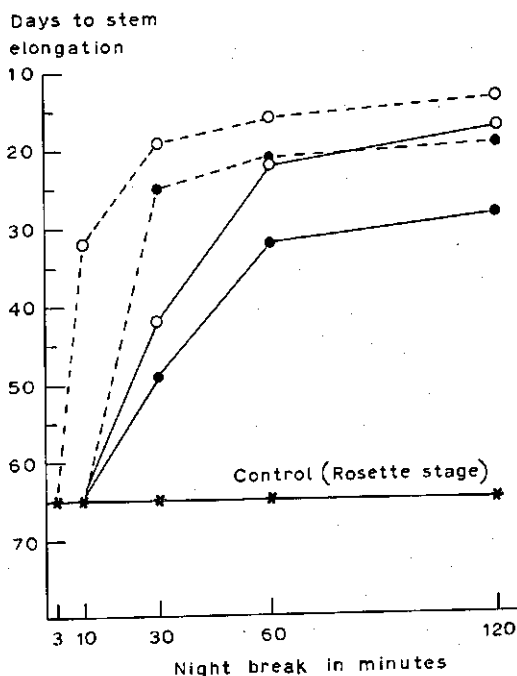


FIG. 25. Stem length in mm of *Hyoscyamus* after 31 and 51 days as affected by red (○) or far-red (●) night interruption (1000 ergs/cm²/sec) of varying durations given at the middle of the 14-hour dark period, combined with 10 hours of two types of basic light period: 1 (—) the new white fluorescent light, 2 (---) the same white fluorescent plus incandescent light, both at an intensity of ca. 25000 ergs/cm²/sec. The experiment started 8-3-67. Averages of 4 plants.

From the results obtained in the two preceding experiments, it is obvious that the presence of far-red in the main light in combination with a night interruption of red or far-red radiation leads to quick stem elongation. Moreover, the results suggest that a long duration of far-red light in the middle of the long dark period has a red action, but slower than the red itself, due to the overlap of the absorptionspectra of the red and far-red sensitive pigments.

The activity of red night interruptions may be explained on the basis of the phytochrome system, such that irradiation with red light transforms the red absorbing form (P_r) to the far-red absorbing form (P_{fr}) which is supposed to be active in inducing flowering in LDPs and is inhibitory in SDPs.

2. Effects of light of well defined spectral composition applied at the beginning or at the end, or at different points of the dark period

From the results obtained by the preceding experiments it seems that 2 hours of red or far-red radiation in the middle of the long dark period are sufficient to induce stem elongation and flower bud formation in *Hyoscyamus* plants.

Two questions remain. First, what is the effect of low intensities of well defined spectral regions not only in the middle but also after the close of the main light period and at various points during the long dark period on the photoperiodic reaction.

Hyoscyamus plants, selected for uniformity, were subjected daily to 10 hours of the same two light sources as used in the previous experiment as a basic irradiation at ca. 30 000 ergs/cm²/sec. The 14-hour dark period was interrupted by 2-hour exposures of blue, red or far-red radiation at an intensity of 1000 ergs/cm²/sec. The interruptions of the dark period were applied at various times 2 hours apart, resulting in 7 various light breaks; one is given immediately after the closing of the main white light period, the next one is applied 2 hours later and so on till the seventh one is given just before the next high light intensity period starts. One group of plants did not receive any night break at all. The experiment started 14-3-1966 and lasted 80 days. Average plants of this experiment are presented on plate 8. The scheme of treatments of this experiment is illustrated in fig. 26. Days to bolting are summarized in fig. 27.

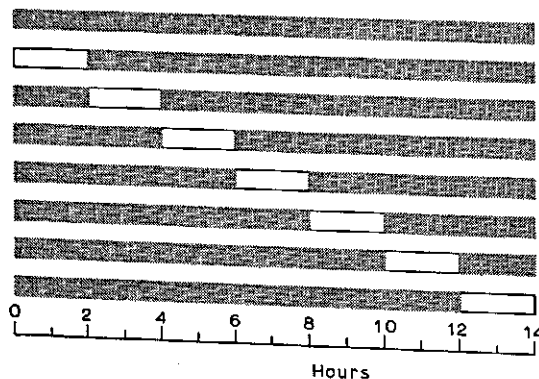
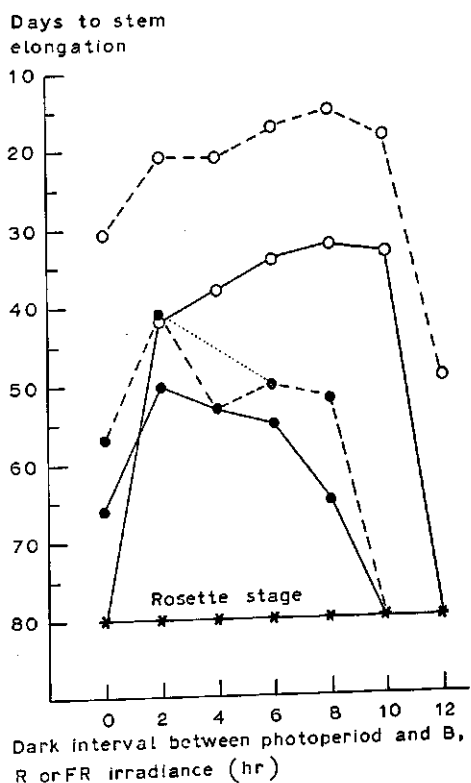


FIG. 26. Scheme of 2 hours blue, red, or far-red night interruption given at various times during the 14-hour dark period, combined with a 10 hour basic light period.

FIG. 27. Effects on stem elongation of *Hyoscyamus niger* caused by blue (*), red (○), or far-red (●) irradiations (1000 ergs/cm²/sec) of 2 hours at various times during 14 hour darkness after a basic photoperiod of 10 hours white fluorescent (—) or mixture of white fluorescent and incandescent light (---), both at the intensity of ca. 30000 ergs/cm²/sec. Control (no night interruption): rosette stage. The experiment started 14-3-'66, and lasted 80 days. Averages of 4 plants.



Again, short days of 10 hours at high light intensity are inhibitive for both light qualities. Generally, however, the mixed light source (TL/IL) causes slightly faster shooting than the other one (TL).

Blue night interruptions in combination with both of the main period qualities does not annihilate the short-day inhibition of shooting at none of the various irradiation times of the night.

Red night interruptions clearly cause elongation when combined with the mixed basic period in all 7 periods of application. There seems a gradual increase in shooting response from the beginning of the dark period till the 10th hour and thereafter a steep decrease. Two hours given directly after the main light period resulted in stem elongation after about 31 days, two hours given after 8 hours did so after about 15 days, and two hours just before the basic light period after only 49 days.

A similar picture was obtained from the plants in the fluorescent light. Shooting is slower in all treatments. It is even so that red night breaks given immediately after and before the high light intensity period are inactive. With the other points, increase in shooting is observed from the 2nd to the 10th hour.

Therefore, red radiation is effective as night break radiation with respect to stem elongation and flower bud formation.

Moreover, in this experiment the response to red radiation reaches a maximum

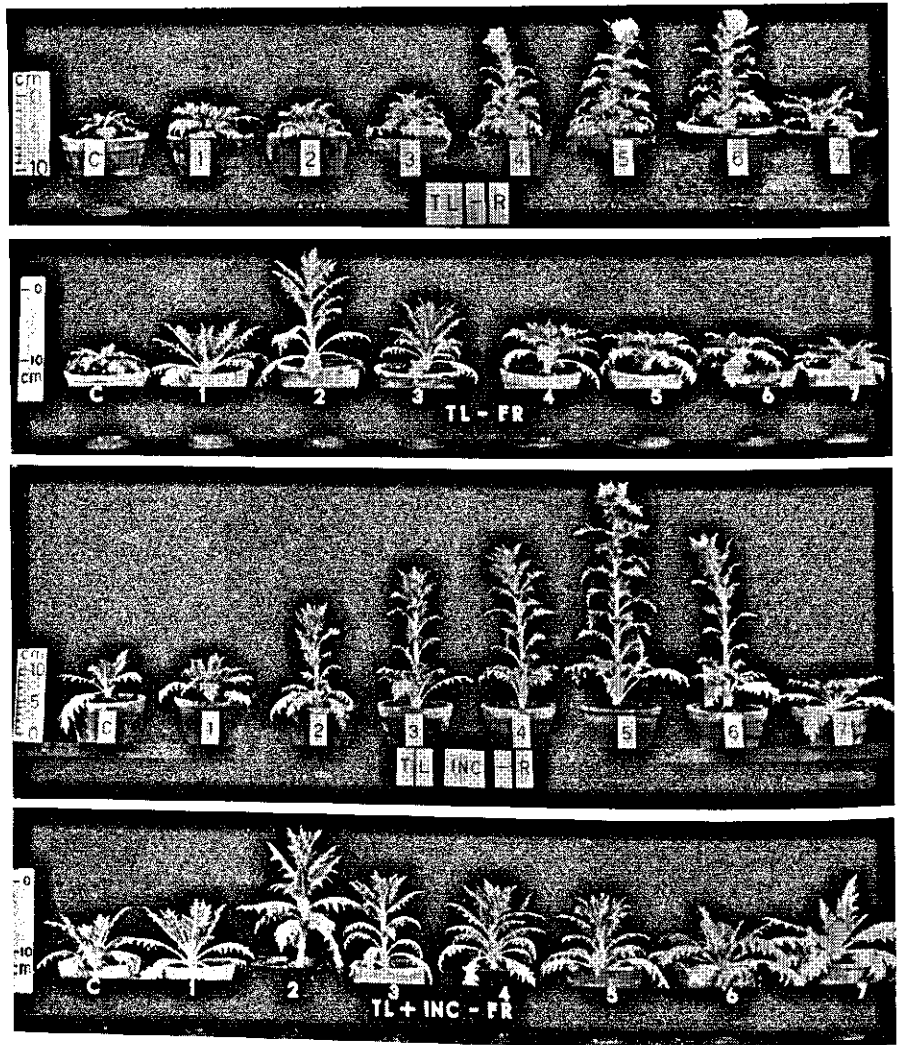


PLATE 8. Stem elongation and flowering as caused by red and far-red radiations of 2 hours, given at various times of 14 hour darkness, resulting in 7 points. One is given immediately after the closing of the main light period (1), the next one (2) is given 2 hours later and so on till the 7th one which is applied just before the next high light intensity. The basic daily light periods were 10 hours of either the new white fluorescent (TL) light or a mixture of the same white fluorescent plus incandescent light (TL + INC). See figure 26 and 27, p. 54 and 55. Photographed 3-5-1966.

of activity beyond the middle of the night period, while the available literature indicates that night breaks exactly in the middle of the night are most effective.

With regard to far-red interruptions, the moment of maximum activity, opposite to the red ones, is before the middle of the night. Moreover, the influence of the main light qualities is less obvious. Far-red radiation is maximally effective after 2 hours of darkness. Immediately after the photoperiod far-red is effective, but 2 hours later there is a clearer reaction, and a gradual decrease towards the end of the night, so that after 10 and 12 hour of darkness far-red is inactive.

3. *Effects of sequences of night breaks at various points of the dark period*

From the previous experiment, it was clear that red and far-red radiation had opposite response curves, red light being maximally active in the second part of the night, far-red radiation being maximally promotive in the first part, and less so in the second half.

The main aim of the following experiment is to confirm the previous phenomena and to determine the influence of a sequence of the two spectral regions red and far-red at several moments of the dark period.

Plants were subjected daily to 10 hours of the same two qualities of white light as used in the previous experiment, at an intensity of ca. 30 000 ergs/cm²/sec. The long dark period was interrupted at the 2-, 8-, or 10-hour points. The following light breaks at these 3 moments were given:

- a) 2 hours red;
- b) 2 hours far-red;
- c) 2 hours blue;
- d) 2 hours red, followed by 2 hours of far-red;
- e) 2 hours far-red, followed by 2 hours of red.

The intensity of red and far-red radiation was 1000 ergs/cm²/sec. Except for the night interruptions, the plants were kept in darkness, when not under the white light. The experiment started 14-6-1966 and was discontinued after 65 days.

Fig. 28 shows the same trends as indicated in the preceding experiment. Again, red light is more active in the second half of the night, with a maximum at the 10-hour point. Far-red radiation is promotive in the first half of the dark period showing a highest shooting effect at the 2-hour point.

The application of red night breaks at all three points caused stem lengthening progressively with age, and more rapidly so at the 10-hour point. The growth rate of stems in mm at the three moments of the night is plotted against the age of the plants in days in fig. 29.

It is clear, that additional admixture of far-red energy to the main light period increased stem lengthening.

The effects of adding 2 hours far-red radiation immediately after 2 hours red light, and the reverse, compared to the effects of red or far-red radiation only, which was illustrated previously in fig. 28, is summarized in fig. 30.

An addition of 2 hours far-red light as a supplement to a night interruption of red radiation increased the shooting response.

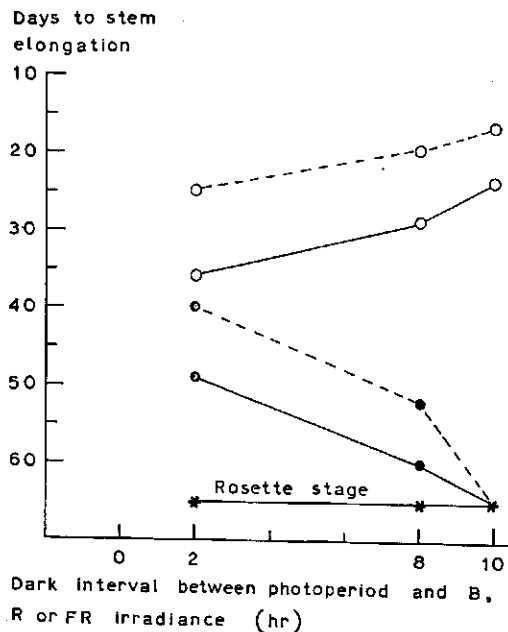


FIG. 28. Effects on stem elongation of *Hyoscyamus niger* caused by blue (★), red (○), or far-red (●) irradiations (1000 ergs/cm²/sec) of 2 hours at the 2nd, 8th, and 10th hour point of 14 hr darkness after a basic photoperiod of 10 hours of white fluorescent light (—) or of a mixture of white fluorescent plus incandescent light (---) both at the intensity of ca. 30000 ergs/cm²/sec. Control (no night interruption): rosette stage. The experiment started 14-6-'66, and lasted 65 days. Averages of 4 plants.

FIG. 29. Stem length in mm of *Hyoscyamus* at successive measurements as affected by 2 hours red night interruption (1000 ergs/cm²/sec) given at the 2nd (△), 8th (○), and 10th (□) hour point of 14 h darkness combined with 10 hours of two types of basic light period: 1 (—) white fluorescent light, 2 (---) the same white fluorescent plus incandescent light, both at the intensity of ca. 30000 ergs/cm²/sec. The experiment started 14-6-'66. Averages of 4 plants.

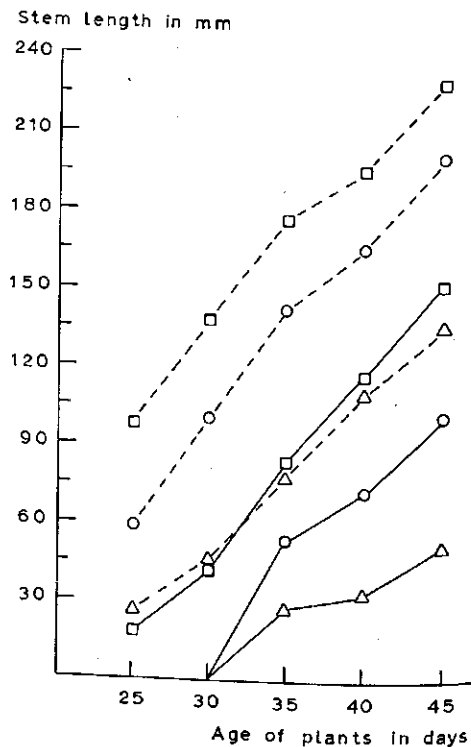
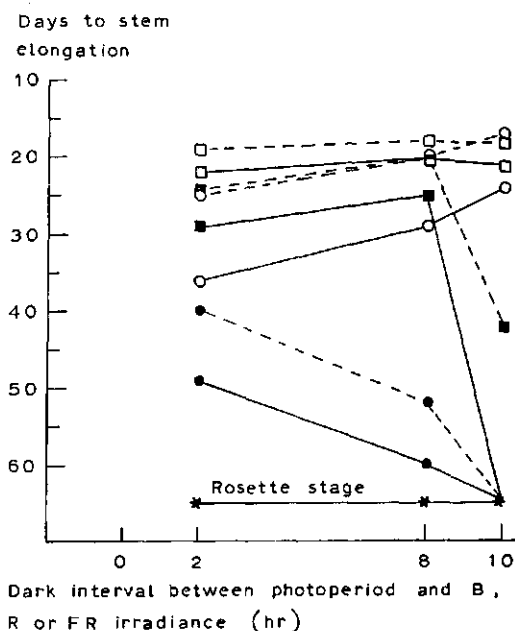


FIG. 30. Number of days to stem elongation of *Hyoscyamus* as affected by 2 hrs blue (*), red (○), far-red (●), 2 hrs red followed by 2 hrs far-red (□), or 2 hrs far-red followed by 2 hrs red (■) night interruption (1000 ergs/cm²/sec) given at the 2nd, 8th, and 10th hour point of 14 h darkness, and combined with 10 hours of two types of basic light period: 1 (—) white fluorescent light, 2 (---) the same white fluorescent plus incandescent light, both at an intensity of ca. 30000 ergs/cm²/sec. Control (no night interruption): rosette stage. The experiment started 14-6-'66, and lasted 65 days. Averages of 4 plants.



In combination with the mixed basic period, stem elongation started after 25, 20, and 17 days when red light was given at the 2-, 8-, and 10-hour points of the dark period respectively; while the application of far-red at these times gave stems only after 40, 52, and >65 days. However, application of 2 hours of far-red after 2 hours of red light, at the same three points, produced shoots already after 19, 18, and 18 days respectively. This specification shows that giving far-red after red light speeds up bolting particularly in the first half of the night.

The results involving the main light period in fluorescent light only, indicate that shooting is considerably slower, but the interference of red and far-red is very similar. Red light given at the 2-, 8-, or 10-hour points gave shoots after 36, 29, and 24 days and far-red radiation after 49, 60, and >65 days, respectively. Application of far-red light after red radiation, given at the same times, speeded up bolting, such that stems became visible after 22, 20 and 21 days, respectively.

It is concluded again that red light, given in the dark period, is essential for flower induction, and far-red radiation for elongation.

The influence on stem lengthening of far-red supplements to red night interruptions at the three points of the night is presented in fig. 31. Stem lengths increase constantly. The effect of the red/far-red sequence was stronger in combination with the fluorescent light than with the mixed light source.

After the previous series, it is necessary also to study the effect of adding 2 hours of red radiation as a supplement to far-red night breaks at the same three times in the night. The results are shown in fig. 30.

In the case of the basic fluorescent plus incandescent light bolting started

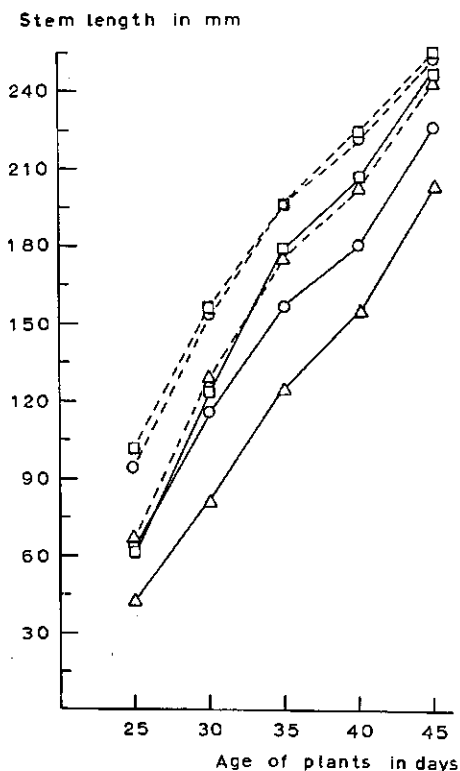


FIG. 31. Stem length in mm of *Hyoscyamus* at successive measurements as affected by 2 hrs red followed by 2 hrs far-red night interruption ($1000 \text{ ergs/cm}^2/\text{sec}$) given at the 2nd (Δ), 8th (\circ), and 10th (\square) hour point of 14 hrs darkness, combined with 10 hours of two types of basic light period: 1 (—) white fluorescent light, 2 (---) the same white fluorescent light plus incandescent light, both at the intensity of ca. $30000 \text{ ergs/cm}^2/\text{sec}$. The experiment started 14-6-'66. Averages of 4 plants.

after 24, 20, and 44 days, in case of the basic fluorescent light source after 29, 25, and > 65 days, at the 2-, 8-, and 10-hour points respectively.

There is a close similarity between these results and those obtained in the earlier series, presented in fig. 27. Shooting, after the application of red radiation at the 12-hour point was very slow under the mixed light source, and did not occur in the case of the fluorescent light.

The results indicate that the influence of far-red radiation given before red night interruptions is inactive or almost so.

It seems that there is a timing mechanism for the reaction upon red and far-red radiations during the dark period.

In these results of night break sequences, mentioned above, however, there is a difficulty of precisely what the application times have been of the sequences of red and far-red radiations. To clarify this complexity and to separate the effect of both red and far-red illumination times, the following experiment was designed.

Hyoscyamus plants were illuminated for 10 hours daily with the same two qualities of white light as used in the preceding experiment, at the intensity of ca. $30000 \text{ ergs/cm}^2/\text{sec}$. The 14-hour dark period was interrupted at the 2-, 4-, 10- or 12-hour points. The following treatments were applied:

a) 2 hours red;

- b) 2 hours far-red;
- c) 2 hours mixture of red and far-red.

Moreover, the following treatments were applied at the 2- or 10-hour points of the dark period:

- a) 4 hours red;
- b) 4 hours far-red;
- c) 4 hours mixture of red and far-red;
- d) 2 hours of red followed by 2 hours of far-red;
- e) 2 hours of far-red followed by 2 hours of red.

These treatments were applied at intensities of 1000 ergs/cm²/sec. The ratio of red to far-red energy in the mixture night break was approximately equal. The schedule of the treatments is presented in fig. 32. The experiment started on 20-10-1966 and was discontinued 65 days later. Representative plants are reproduced on plates 9, 10, and 11.

The results of the treatments with 2 hours red, far-red or the mixture, given at the 2nd, 4th, 10th or 12th hour of the dark period, are presented in fig. 33. The data confirm those obtained in the experiment of section 2, presented in fig. 27. They indicate that the maximum flower induction effect of red light is reached at the 10 hour point, with a steep activity drop to the 12th-hour. With the mixture of red and far-red radiations, the same trend as with red alone was observed, only somewhat faster. Far-red radiation produced fast shooting at the 2nd and also at the 4th hour and no stems appeared at the 10- or 12-hour points of the dark period, in combination with both white sources.

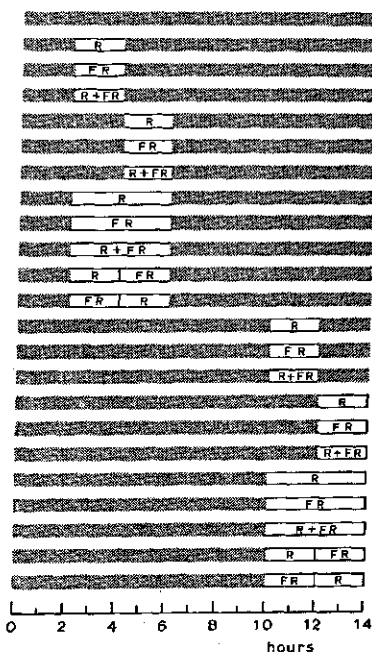


FIG. 32. Scheme of night break and extended day length, with red (R), far-red (FR), or a mixture of them (R + FR). Two sources for the basic light periods were used, v.z., 10 hrs TL or TL + IL.

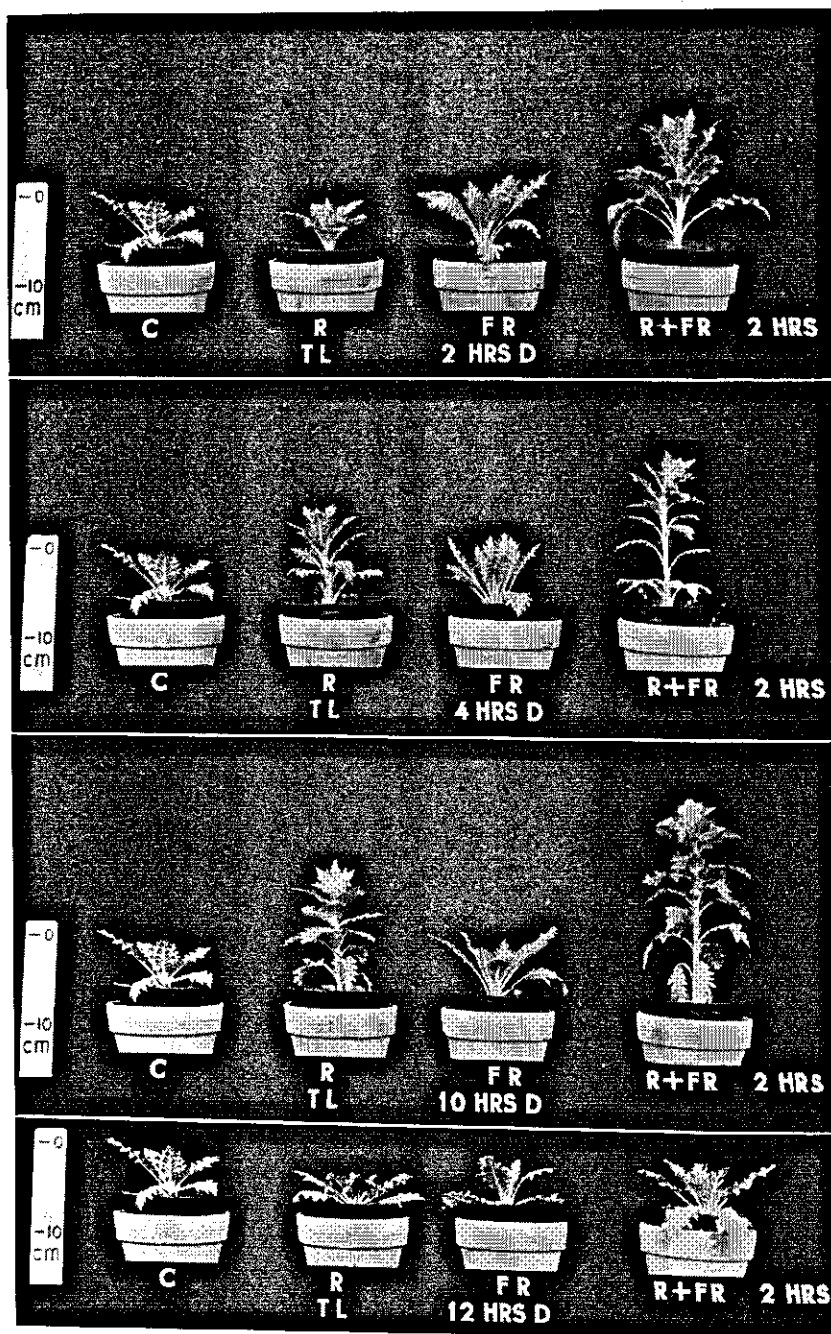
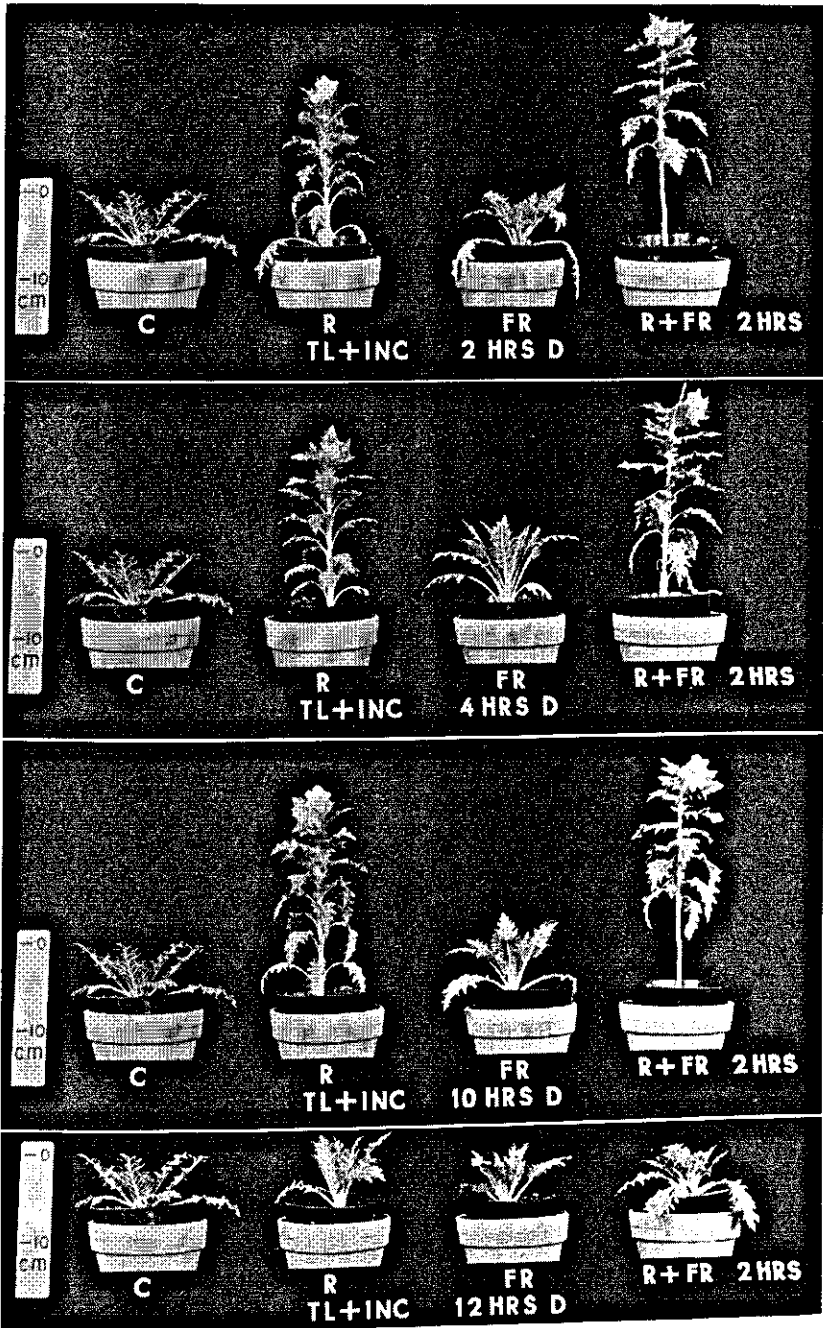


PLATE 9. Stem elongation and flowering as affected by red, far-red, or a mixture of red and far-red radiations of 2 hours at the 2nd, 4th, 10th and 12th hour point of 14 hours and 62) darkness, combined with daily 10 hours of two types of basic light periods. The



(Plate 9, continue)
 main light periods were either white fluorescent light (TL) or mixture of white fluorescent plus incandescent light (TL + INC). See figure 33, p. 64. Photographed 28-11-1966.

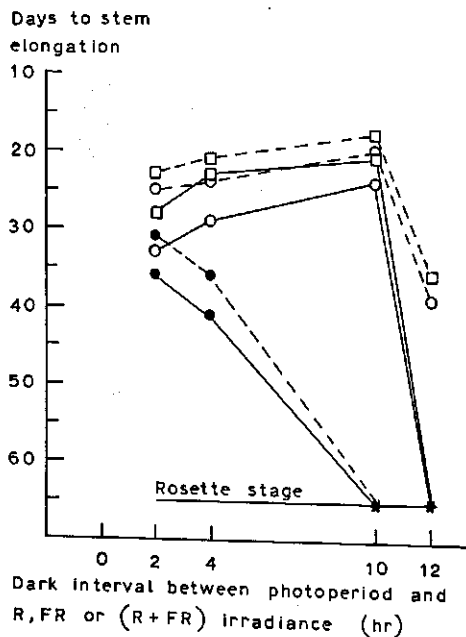


FIG. 33. Number of days to stem elongation of *Hyoscyamus* as affected by red (○), far-red (◐), or a mixture of red and far-red (□) irradiances (1000 ergs/cm²/sec) of 2 hrs at the 2nd, 4th, 10th, and 12th hour point of 14 hr darkness, combined with 10 hours of two types of basic light periods: 1 (—) white fluorescent light, 2 (---) the same white fluorescent plus incandescent light both at the intensity of ca. 30 000 ergs/cm²/sec. Control (no night interruption): rosette stage. The experiment started 20-10-'66, and lasted 65 days. Averages of 4 plants.

Table 3 shows the results of the various treatments of 2 and 4 hour night interruptions given after 2 or 10 hours of the dark period. Increasing the duration of red or far-red, or of the mixture to 4 hours, after 2 hours of darkness, causes earlier shooting, while shooting with the mixed basic light is fastest. However, when the interruptions are given after 10 hours of darkness, there is no clear speeding up when increasing from 2 to 4 hours under the mixed high light intensity source, while under the fluorescent main light source even some retardation is observed when 4 hours are given.

Concerning the data of the sequence of 2 hours red + 2 hours far-red, far-red enhances the red action, resulting in earlier shooting. This result is more or less equal to that obtained with 4 hours of red light. Plants bolted after 27, 22 or 24 days from the beginning of the treatments under the fluorescent high light intensity when they were treated at the 10-hour point with 4 hours red, the mixture of red and far-red, or a sequence of 2 hours red + 2 hours far-red, respectively. The mixture causes somewhat quicker action than the sequences.

With regard to the sequence of 2 hours far-red + 2 hours red, the effect of that sequence is due to the red reaction, as the following results may demonstrate.

In combination with the mixed light source, the mentioned sequence of 2 hrs far-red + 2 hours red, given after 2 hours of darkness, produces visible stems after 24 days which is similar to the effect of 2 hours red light after 4 hours of darkness. At the 10-hour point, 39 days are needed for stem elongation, with the same sequence, which is the same response as with 2 hours red given at the 12th hour of the dark period. The same holds for the fluorescent main light period. Thus far-red radiation given before red light is rather inactive.

TABLE 3. Days to stem elongation of *Hyoscyamus* as affected by 2 hours of red (R), far-red (FR), or a mixture of these (R + FR) (1000 ergs/cm²/sec) (See fig. 33) at the 2, 4, 10, and 12-hour point of darkness, or by 4 hours R, FR, (R + FR), 2 hours R + 2 hours FR, or 2 hours FR + 2 hours R at the 2nd and 10th hour of darkness, combined with 10 hours basic light periods. The basic light was either white fluorescent light (TL) or a mixture of the same white fluorescent plus incandescent light (TL/IL), both at the intensity of ca. 30 000 ergs/cm²/sec. The experiment started 20-10-1966 and lasted 65 days. Averages of 4 plants.

| Light quality of 10 hrs main light period | | TL + IL | | | | TL | | | |
|---|--|---------|----|----|----|----|----|----|----|
| Type of light break | Night break after ... hrs of darkness→ | 2 | 4 | 10 | 12 | 2 | 4 | 10 | 12 |
| 2 hrs R | | 25 | 24 | 20 | 39 | 33 | 29 | 24 | * |
| 2 hrs FR | | 31 | 36 | * | * | 36 | 41 | * | * |
| 2 hrs (R + FR) | | 23 | 21 | 18 | 36 | 28 | 23 | 21 | * |
| 4 hrs R | | 20 | | 19 | | 23 | | 27 | |
| 4 hrs FR | | 29 | | * | | 32 | | * | |
| 4 hrs (R + FR) | | 18 | | 19 | | 17 | | 22 | |
| 2 hrs R + 2 hrs FR | | 20 | | 18 | | 23 | | 24 | |
| 2 hrs FR + 2 hrs R | | 24 | | 39 | | 30 | | * | |

* = plants in rosette stage.

Besides days of shooting, stem lengths were observed. Fig. 34 indicates stem lengths in mm after 28 and 49 days of treatment under the two sources of high light intensity.

With the mixed high light intensity period, the following data have been observed after 28 days. When 2 hours red light was given at the 2nd, 4th, 10th, or 12th hour of darkness, average stem length is 42, 60, 107, and 0 mm, respectively, while 2 hours of far-red given at these times after the same treatment period showed no stems at all. Stem length after a treatment of 2 hours of the mixture of red and far-red radiations, applied at the same points, is 79, 107, 140, and 0 mm, respectively.

If 4 hours red, far-red or the mixture were given at the 2-hour point, stem length is 112, 0, and 155 mm respectively. In the case of a sequence of 2 hours red + 2 hours far-red, beginning after 2 or 10 hours of darkness, stem length is 109 and 127 mm respectively, while the other sequence of 2 hours far-red + 2 hours red induces stems of 48 and 0 mm length respectively.

With increasing age of the plant stem length increases. The average stem length was determined again after 49 days. It was 180, 213, 217, and 68 mm when 2 hours of red light was given after 2, 4, 10 or 12 hours of darkness respectively, it was 147, 147, 0, and 0 mm when far-red light was applied. Application of 2 hours of the mixture of red and far-red light induced stems of 232, 233, 240, and 130 mm length, respectively. Again, far-red radiation is elongative only at the beginning of the night. And red light does not work beyond the 10th hour. The application of 4 hours red, far-red, or the mixture after 2 hours of darkness

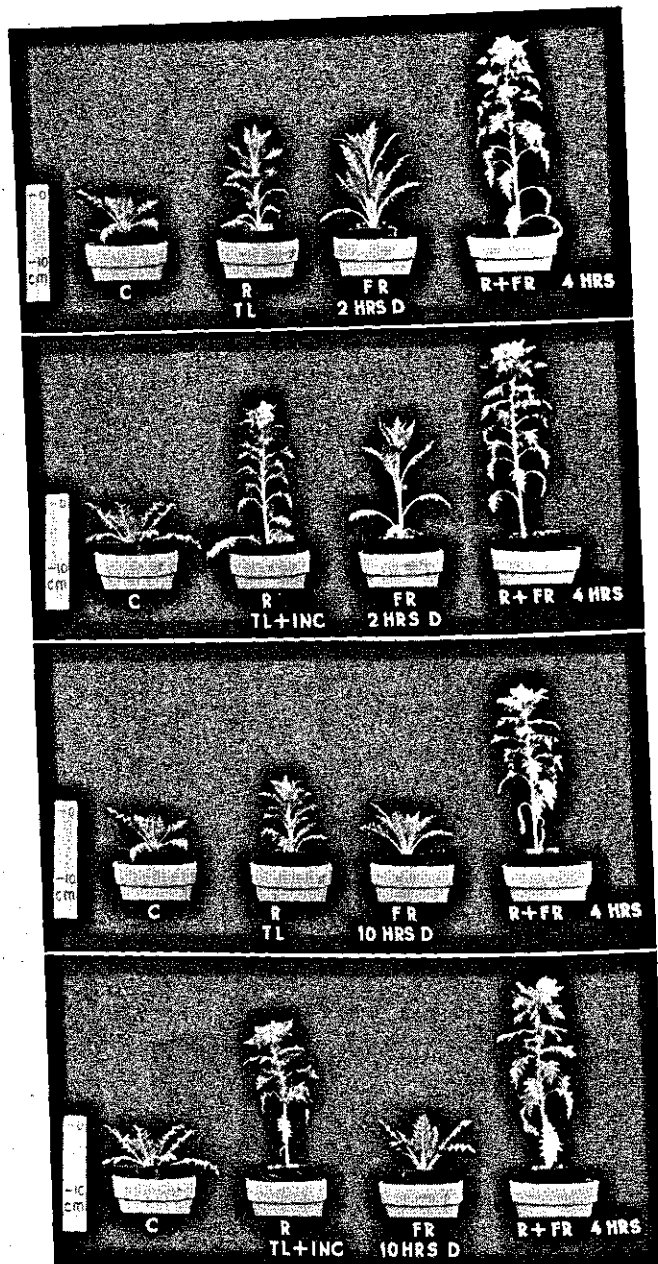


PLATE 10. Stem elongation and flowering as affected by 4 hours of red, far-red, or mixture of red and far-red radiations given at the 2nd and 10th hour-point of 14 hour darkness, combined with daily 10 hours of two types of basic light periods. The main photoperiods were either white fluorescent light (TL) or white fluorescent plus incandescent light (TL + INC). See table 3 p. 65. Photographed 28-11-1966.

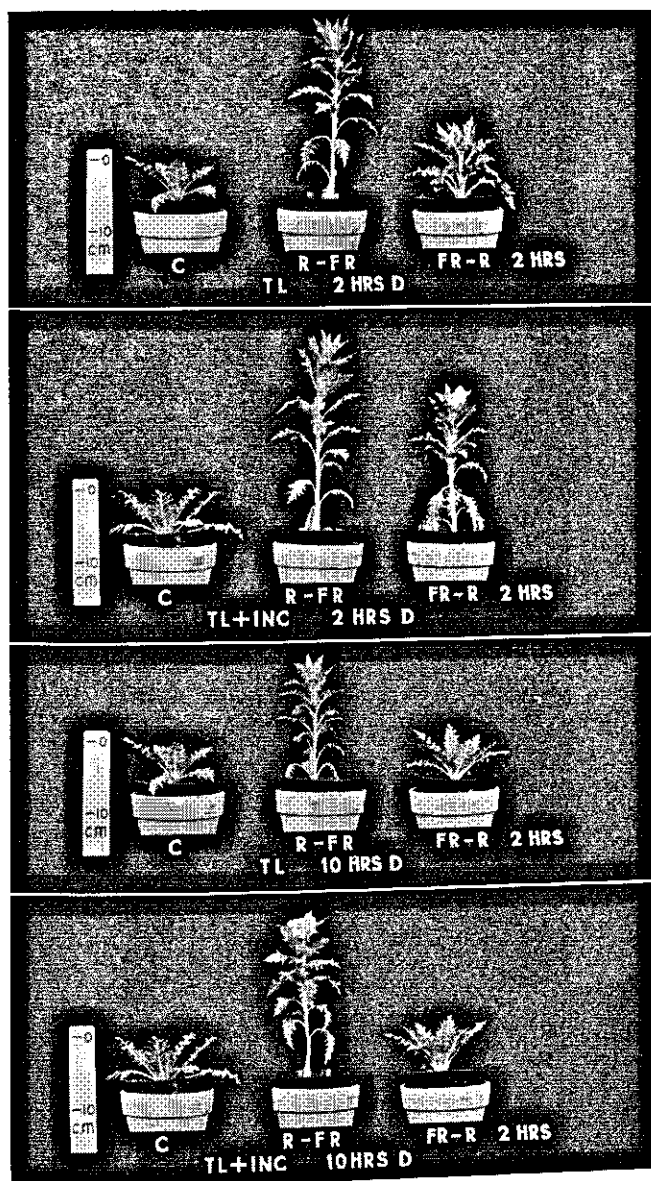


PLATE 11. Stem elongation and flowering as affected by 2 hours red followed by 2 hours far-red, or 2 hours far-red followed by 2 hours red given at the 2nd and 10th hour point of 14 hour darkness, combined with daily 10 hours of two types of basic light periods. The basic light periods were either white fluorescent light (TL) or a mixture of white fluorescent plus incandescent light (TL + INC). See table 3, p. 65. Photographed 28-11-1966.

induced stems of 212, 237, and 263 mm length, and after 10 hours of darkness stems of 200, 0, and 268 mm, respectively.

Increasing the duration of the night interruption somewhat enhances photoperiodic induction and subsequent stem lengthening, particularly in the beginning of the night. In the second part, no significant increase is obtained. Even 4 hours of far-red radiation at the 10-hour point of darkness, does not induce stem formation.

Due to the sequence of 2 hours red + 2 hours far-red, stem lengths after 2 and 10 hours of darkness are 272 and 223 mm, whereas the sequence of 2 hours far-red + 2 hours red induces stems of only 220 and 56 mm, respectively.

Comparing stem length of the first sequence (R + FR) given after 2 hours of darkness, with a treatment of 2 hours red at that time, stem length is 272 and 180 mm, respectively, while after 10 hours darkness it is 223 and 217 mm, respectively.

Thus, far-red pertinently enhances stem lengthening in the beginning of the night, while there was no enhancing effect at the 10th hour of darkness. This seems worthwhile to be emphasized in view of the absence of action of far-red at the 10th hour of the night if given alone. Apparently the possibility of action of far-red alone in the beginning of the night cannot be brought about or restored at the 10th hour by a preceding 2hr red illumination. Obviously, the possibility of reactions to far-red, for some reason has become zero at the 10th hour of the night, and stays so, also after a red illumination. The reason for this behaviour towards far-red still remains to be discovered.

Differences between night interruptions show up better with the fluorescent white light source only as compared with the mixed light period. This is shown below.

After 28 days from the beginning of the treatments, if red, far-red, or their mixture are given at the 2nd, 4th, 10th, or 12th hour of the dark period, no stems appeared with red or far-red interruptions in neither of the mentioned points, but stem lengths are 33, 62, 102 and 0 mm with the mixture of red and far-red radiations at these points, respectively. Therefore, the mixed light night break has a more rapid action, as compared with red or far-red radiation alone. With the mixture, shooting is fastest at the 10-hour point of the dark period.

The application of a treatment of 4 hours red, far-red or the mixture at the 2nd hour of darkness results in stem lengths of 53, 0, 169 mm, while at the 10th hour they are 112, 0 and 123 mm, respectively. Increasing the duration of a night break up to 4 hours increases its effect, particularly with red light.

The sequence of 2 hours red + 2 hours far-red gives stems of 112 and 57 mm at the 2nd and the 10th hour points, respectively. Far-red radiation obviously has an enhancing effect on the action of red light. Two hours of red or of far-red radiation after 2 hours darkness produce no visible shooting; the addition of 2 hours far-red after 2 hours red enhances bolting, and gives stems of 112 mm. Also, 2 hours red light, applied at the 10th hour, induce stems of 28 mm in average; this becomes 57 mm when the red light exposure is followed by 2 hours far-red. This is a confirmation of the previous data that indicated that far-red radiation is active photoperiodically only in the first half of the night.

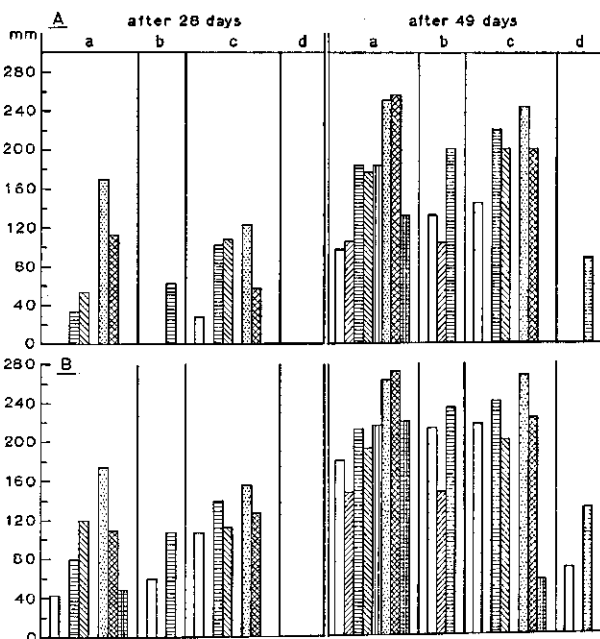


FIG. 34. Stem length in mm after 28 and 49 days as affected by various treatments of R and FR (see fig. 32 and table 3) at the 2nd (a), 4th (b), 10th (c), and 12th (d) hour of 14 hour darkness, combined with two basic light periods. The main light periods were either white fluorescent light (A) or mixture of the same white fluorescent plus incandescent light (B). The experiment started 20-10-'66. Averages of 4 plants.

□ 2 hrs R ▨ 4 hrs R ▩ 2 hrs R + 2 hrs FR
 ▤ 2 hrs FR ▧ 4 hrs FR ▪ 2 hrs FR + 2 hrs R
 ▦ 2 hrs (R + FR) ▫ 4 hrs (R + FR)

Comparing the sequence (R + FR) with 4 hours red light at the 2 hour point of darkness, stem length is 112 mm in the first case and only 53 mm in the last, whereas after 10 hours of darkness it is 57 and 112 mm, respectively. Again far-red radiation has a clear lengthening action in the beginning of the dark period, not in the second part. Red light, on the contrary, is more active in the latter part than in the former.

Comparing the same sequence (R + FR) with a treatment of 4 hours mixture of red and far-red at the second hour of darkness, stem length is 112 and 169 mm respectively; at the 10-hour point of darkness it is 57 and 123 mm, respectively.

Therefore, the application of red and far-red simultaneously has a stronger effect on stem lengthening than far-red as a supplement after red light.

On the other hand, the sequence of 2 hours far-red + 2 hours red light given after 2 and 10 hours of the dark period does not induce bolting after 28 days of the experiment.

The stem length observations described above concerning the fluorescent high light intensity period were repeated once more after 49 days of treatment.

Averages of stem lengths in mm when, at the 2nd, 4th, 10th and 12th hour of

darkness, 2 hours red radiation were given are 97, 132, 145, and 0 mm, respectively. With 2 hours far-red radiation at these times stems of 105, 105, 0, and 0 mm were produced respectively. However, the application of a treatment of 2 hours of a mixture of both radiations gives stems of 183, 200, 220 and 88 mm respectively.

Increasing duration of red, far-red or their mixture from 2 to 4 hours increases their effects. Stem length in mm is 177, 183, 250 when these treatments were given after 2 hours of darkness, whereas it is 200, 0, 245 mm respectively after 10 hours of darkness.

Concerning the sequence of 2 hours red + 2 hours far-red given after 2 or 10 hours of darkness shows stems of 255 and 200 mm in length, respectively. Whereas in the case of the reverse sequence (FR + R), stem length is 132 and 0 mm after the 2nd and 10th hour of darkness, respectively. Stem length at the 4th hour point when 2 hours red was given is equal to a stem length of the latter sequence; indicating that the action is primarily a red one.

Comparing stem length of the sequence R + FR with a treatment of 4 hours red light at the 2nd hour of darkness, shows that it is 255 mm in the former treatment while it is 177 mm in the latter. In this case we seem to have a clear effect of far-red radiation. However, stem length after 10 hours of darkness is equal (200 mm) in both treatments. Thus, it seems that there is no shooting-retarding effect for the last 2 hours of red in the treatment of 4 hours red, nor any antagonistic effect for the 2 hours of far-red radiation in the sequence red + far-red when the last 2 hours start at the 12-hour point of the dark period. This confirms our earlier observation (fig. 27) that 2 hours of red and of far-red are inactive at the 12th hour of darkness.

The data presented seem to indicate that the admixture of far-red energy to the main light period enhances the photoperiodic induction and increases stem lengthening. Obviously stem length increases with time. Red light and a mixture of red and far-red light has the same reaction curve in the night, however, the latter resulting in a more rapid induction. Increasing the duration of the night breaks increases stem length. A supplement of far-red after red night breaks increases stem lengths. This effect is stronger in the first half of the night than in the second. Moreover, the action of the sequence of far-red + red is a red action, as if far-red had not been given.

4. *The importance of the dark period before the basic light period*

From the data of the previous two sections of this chapter, it is apparent that 2 hours red light given in the dark period before the commencement of the high light intensity period are inhibitive in combination with the fluorescent basic period and only weakly promotive in the case of the mixed white light source. It was also evident that the maximum flowering response of red radiation applied at several points during the dark period was maximal, in both basic light sources, at the 10-hour point of the 14 hour night. Therefore it was of considerable interest to determine the function of the dark period between the red interruption applied at that point and the next high light intensity period.

Daily, after at least 10 hours of darkness, selected *Hyoscyamus* plants were subjected to the following treatments, before the next white light period was applied:

- 120 mins red light followed by 120 mins darkness;
- 120 mins red light followed by 90 mins darkness;
- 120 mins red light followed by 60 mins darkness;
- 120 mins red light followed by 30 mins darkness;
- 120 mins red light followed by 15 mins darkness;
- 120 mins red light directly followed by the main photoperiod;
- a control treatment, receiving 14-hour nights.

The light intensity of the red night interruption was $1000 \text{ ergs/cm}^2/\text{sec}$, during the high light intensity main periods it was $35\,000 \text{ ergs/cm}^2/\text{sec}$. Again, the two types of basic light as before were used; viz. white fluorescent light (TL/55/40 W) and a mixture of that type with incandescent light. The experiment started 6-7-1966 and was discontinued after 80 days.

Number of days to stem elongation of the various treatments in this experiment are shown in fig. 35. Control plants receiving 10 hours of light daily remained vegetative. Plants irradiated with 120 mins red light immediately before the start of the high light intensity period remained vegetative under the fluorescent light and became slightly reproductive in the case of the mixed white light. Plants given 2 hours of red radiation at the 10th hour of darkness, followed by 2 hours of darkness, are reproductive in combination with both white light

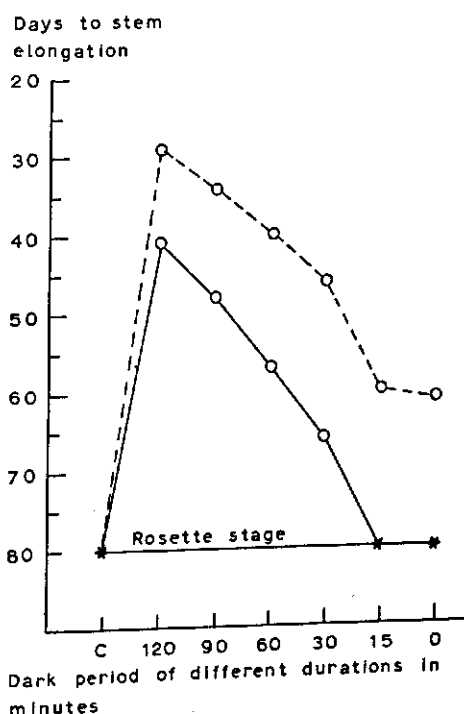


FIG. 35. Number of days to stem elongation of *Hyoscyamus* as affected by 120 mins red light ($1000 \text{ ergs/cm}^2/\text{sec}$) followed by dark periods of different durations before the commencement of the main light period. The basic photoperiods were either: 1 (—) white fluorescent light or 2 (---) white fluorescent plus incandescent light, both at the intensity of ca. $35\,000 \text{ ergs/cm}^2/\text{sec}$. Control (no night interruption): rosette stage. The experiment started 6-7-'66, and lasted 80 days. Averages of 4 plants.

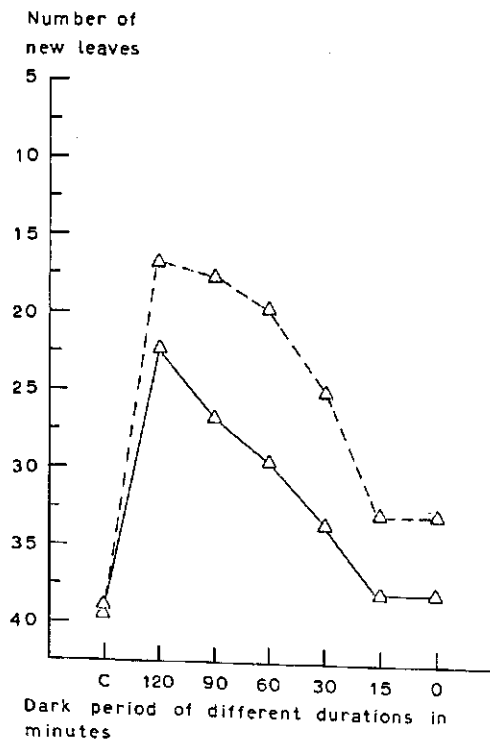


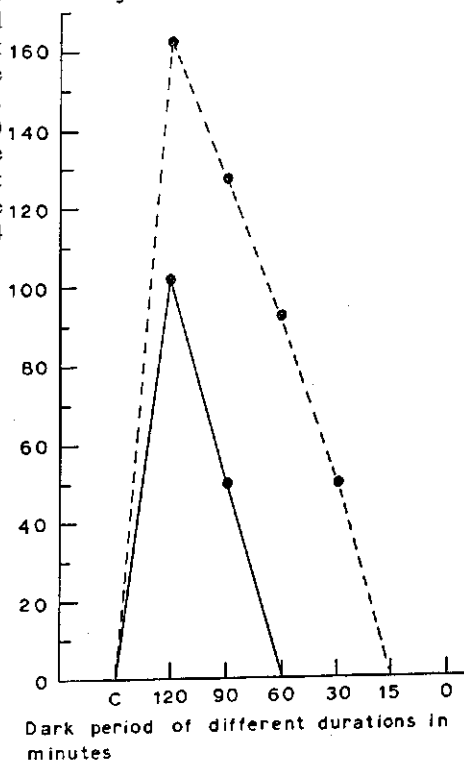
FIG. 36. Number of newly expanded leaves to bolting in *Hyoscyamus* as affected by 120 mins red light (1000 ergs/cm²/sec) followed by dark periods of different durations before the commencement of the main light period. The basic photoperiods were either: 1 (—) white fluorescent light or 2 (---) white fluorescent plus incandescent light, both at the intensity of ca. 35000 ergs/cm²/sec. The experiment started 6-7-'66, and lasted 80 days. Averages of 4 plants.

sources, however, the shooting response is quicker in case of the mixed source. Decreasing the dark period after 2 hours red light below 120 mins decreases the flowering response. In combination with the mixed white light source bolting is very slow and nearly equal when only 15 or zero mins of darkness were given after the red interruption. With the other source no shooting was observed for these treatments. Intercalation of minimally 30 mins of darkness after the red interruption is inductive also with the fluorescent photoperiod. In general more action was observed with a mixed basic period.

The data presented in fig. 36 compared to those in the previous one indicate that there is a correlation between the number of additionally produced leaves and the number of days until bolting. The sooner stem elongation begins, the lower the number of newly expanded leaves is. The vegetative plants form more leaves than any of the reproductive ones. It seems justified to conclude that, in *Hyoscyamus*, the data of days to shooting are a good measure for the rate of floral induction.

Stem lengths after 55 days treatment are shown in fig. 37. Again, extra far-red energy in the white light source, generally, causes more stem lengthening. With the mixed light source, treatments with 120, 90, 60, 30, 15 or 0 mins of darkness after the red interruption produced stem lengths of 163, 127, 92, 50, 0 and 0 mm respectively, whereas with the fluorescent basic light period this is 102, 50, 0, 0, 0, and 0 mm, respectively. Fifteen and 0 mins of darkness given after 2

FIG. 37. Stem length in mm of *Hyoscyamus* after 55 days as affected by 120 mins red light (1000 ergs/cm²/sec) followed by dark periods of different durations before the commencement of the main light period. The basic light periods were either: 1 (---) white fluorescent light or 2 (---) white fluorescent plus incandescent light, both at the intensity of ca. 35 000 ergs/cm²/sec. The experiment started 6-7-'66. Averages of 4 plants.



hrs red night break in the case of the mixed basic light period, and 60 and 30 mins of darkness applied after 2 hrs red night break combined with the fluorescent main photoperiod showed stem elongation at a later stage, see fig. 35.

Evidently, with longer duration of darkness after the red interruption at the 10-hour point of darkness, shooting is quicker and stems are longer, as could be expected from the fact that 2 hrs red light starting at the 10-hour point still are active in promoting shooting, whereas the same at the 12-hour point are much less active, and the present experiment provides data in between both.

C. EFFECTS OF COLOURED ILLUMINATION AS A BASIC PERIOD

The data described in chapter IV indicate that when *Hyoscyamus niger* was grown exclusively in light of different spectral regions, no long-day effect was obtained when the long-day treatment was given in green or red light, whereas the plants in blue light flowered very quickly. These observations are in harmony with those obtained by STOLWIJK and ZEEVAART (1955) and by CURRY and WASSINK (1956). From these results, it is not possible to determine whether the photoperiodic response is due to the main or the supplementary light period. In night break experiments with *Salvia occidentalis* (SDP) and *Hyoscyamus niger* (LDP), it was found that the long-day effect of an interruption of the long dark

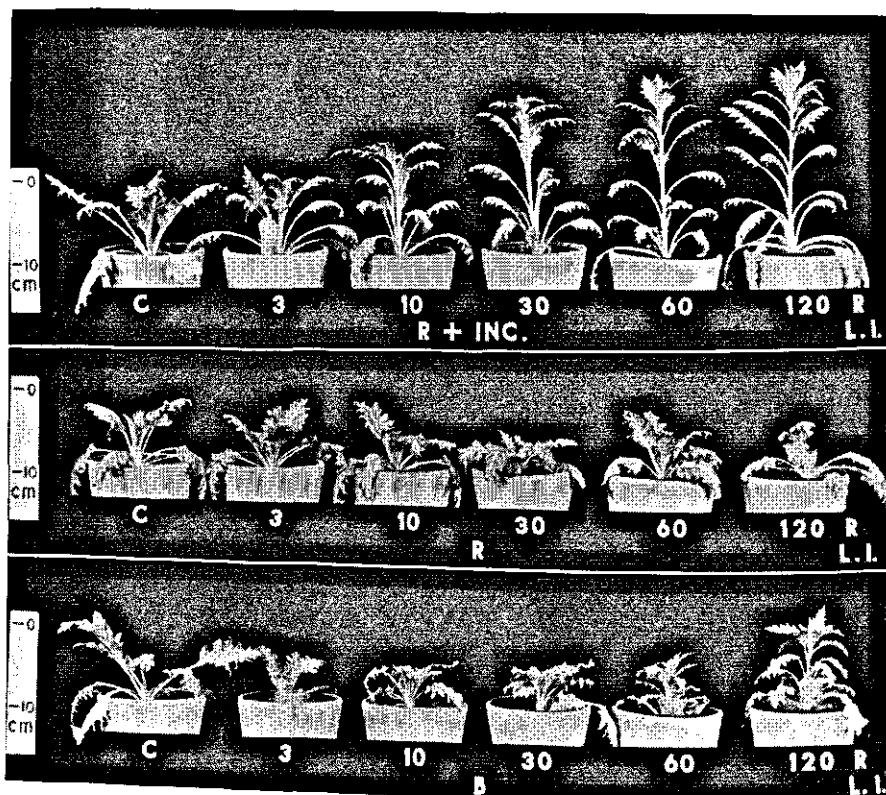


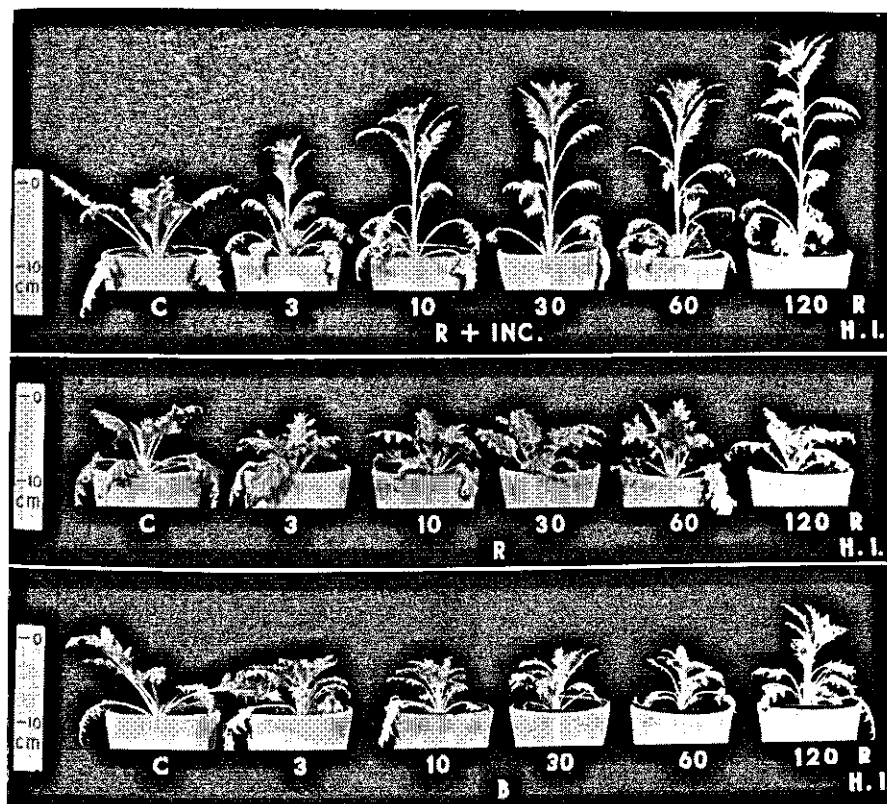
PLATE 12. Stem elongation and flowering as affected by red night interruption at two levels of light intensity: 1000 ergs/cm²/sec (L.I.), or 3800 ergs/cm²/sec (H.I.) of varying and 75) durations (3, 10, 30, 60 or 120 mins) given at the middle of a 14 hour dark period,

period with red light depends on the light quality of the main light period (MEIJER and VAN DER VEEN, 1957). These authors concluded that a night interruption was effective only after a main light period in blue light; after a main light period in red or green light a night break failed to induce flowering.

The object of the following experiments is to determine the photoperiodic and formative reactions of *Hyoscyamus* plants exclusively grown in short days of different colours combined with various treatments of night break, either in the middle, or at several points of the long dark period.

1. *Effects of different durations of spectral regions at two levels of light intensity applied around the middle of the dark period*

Selected *Hyoscyamus* plants were exposed daily to 10 hours of blue, red, or a mixture of red and far-red light at an intensity of 8000 ergs/cm²/sec. Around the middle of the long dark period, plants were irradiated for different times (0, 3, 10, 30, 60 or 120 mins) with red or far-red radiation, at two intensities (1000 or



(Plate 12, continue)

combined with daily 10 hours of three types of basic photoperiod. The main light periods were: mixture of red and far-red (R + INC), red (R), or blue (B). See figure 38 p. 76. Photographed 10-2-1967.

3800 ergs/cm²/sec). The experiment started 16-1-1967 and was ended after 75 days. Representative plants are shown on plates 12 and 13.

The data of fig. 38 show the effect of various durations of red illumination at the two intensities given in the middle of the long dark period combined with the main light periods of blue, red, or the mixture of red and far-red radiation on the photoperiodic effects.

Concerning the blue or mixed basic light periods, plants irradiated daily with 10 hours are in the rosette stage till the end of the experiment. There seems no clear difference photoperiodically between the series of durations. The photoperiodic response is saturated at 3 mins red night interruption. The saturation level is slightly higher with higher light intensities. Under the blue basic period, the photoperiodic induction is not fully saturated with the lower interruption intensity when only 3 mins red were applied. Finally, the photoperiodic reaction of all treatments under the mixed basic light quality is quicker than that in the parallel ones under the blue.

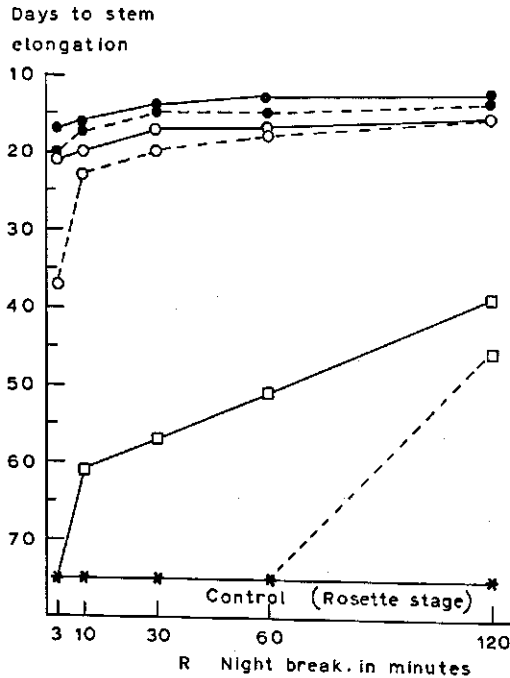


FIG. 38. Number of days to stem elongation of *Hyoscyamus* as affected by red night interruption at two levels of light intensity: 1 (---) 1000 ergs/cm²/sec, or 2 (—) 3800 ergs/cm²/sec of different durations given around the middle of the 14 hour dark period, combined with 10 hours of three types of basic photoperiod. The main light periods were: blue (○), red (□), or red + far-red (●), at an intensity of 8000 ergs/cm²/sec. The experiment started 16-1-67, and lasted 75 days. Averages of 4 plants.

In combination with the red main light period, increasing the duration of the high intensity red breaks above 3 mins, leads to faster reaction. Of the series of lower intensity of red breaks (1000 ergs/cm²/sec), only 120 mins are elongative. All plants of the control series remained vegetative also. These results seem to combine nicely with those obtained in the experiment of this paper, recorded in fig. 22. The original type of white fluorescent light, which emits only a small amount of far-red radiation, was used in the earlier experiment. Combination with low intensity red night breaks (600 ergs/cm²/sec) of a duration up to 100 mins did not produce a long-day effect with this white light. It may be concluded from these two series of data that the light intensity of red night interruptions is very important for the photoperiodic response under red photoperiods.

Observations on the rate of elongation of stems in the previous experiment are presented in fig. 39. From three weeks after the beginning of the experiment, stem lengths were determined weekly for the high intensity red night interruption series.

Generally, plants under the mixture of the red and far-red main light period show the highest stems, the red ones the shortest stems under the blue basic period elongate at an intermediate rate. With longer night interruptions, stem length increases for both the mixture and blue. From the present data, it cannot be established whether it is reduction in the red/far-red ratio or the total increase in far-red energy (HELSON, 1965) that has increased the rate of development.

The influence on the photoperiodic reaction of the various durations of far-

FIG. 39. Stem length in mm of *Hyoscyamus* at successive measurements as affected by red night interruption (3800 ergs/cm²/sec) of different durations in mins: 3 (—), 10 (---), 30 (-.-.-), 60 (-....) or 120 (---) given at the middle of the 14 hour dark period, combined with 10 hours of three types of basic photoperiod. The main light periods were: blue (□), red (○), or red + far-red (●) at the intensity of 8000 ergs/cm²/sec. Control (no night interruption): rosette stage. The experiment started 16-1-'67. Averages of 4 plants.

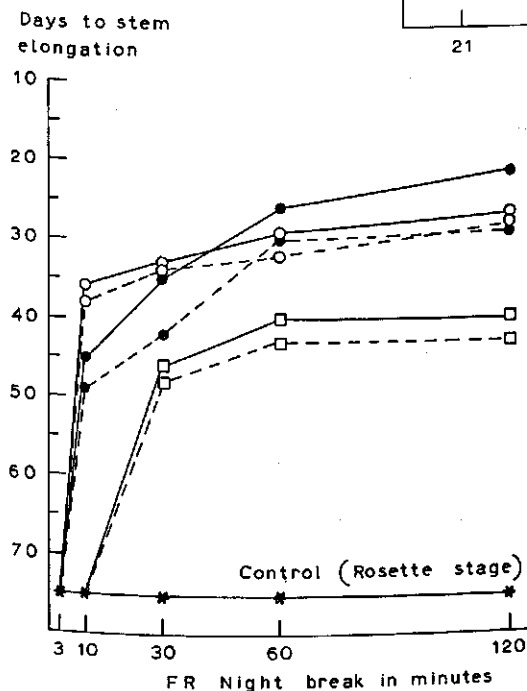
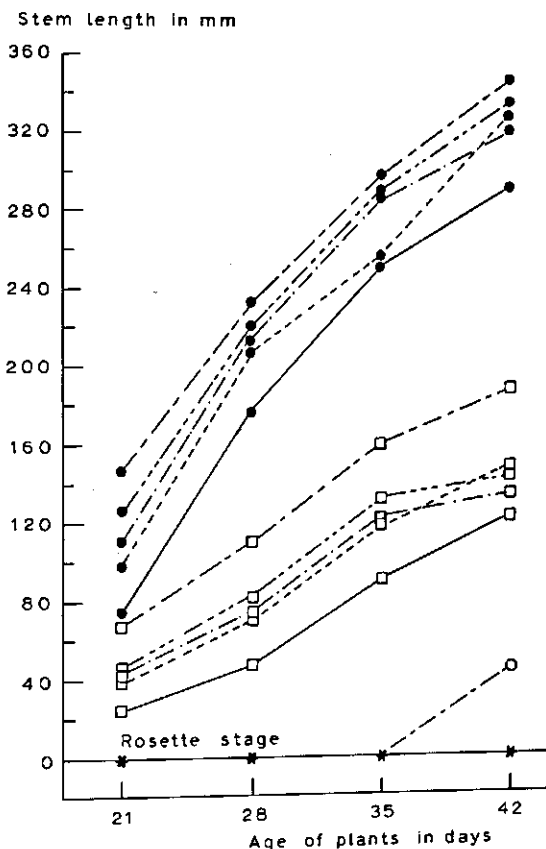


FIG. 40. Number of days to stem elongation of *Hyoscyamus* as affected by far-red night interruption at two levels of light intensity: 1 (---) 1000 ergs/cm²/sec, (—) 3800 ergs/cm²/sec of different durations given at the middle of the 14 hour dark period, combined with 10 hours of three types of basic photoperiod. The main light periods were: blue (□), red (○), or red + far-red (●) at an intensity of 8000 ergs/cm²/sec. The experiment started 16-1-'67, and lasted 75 days. Averages of 4 plants.

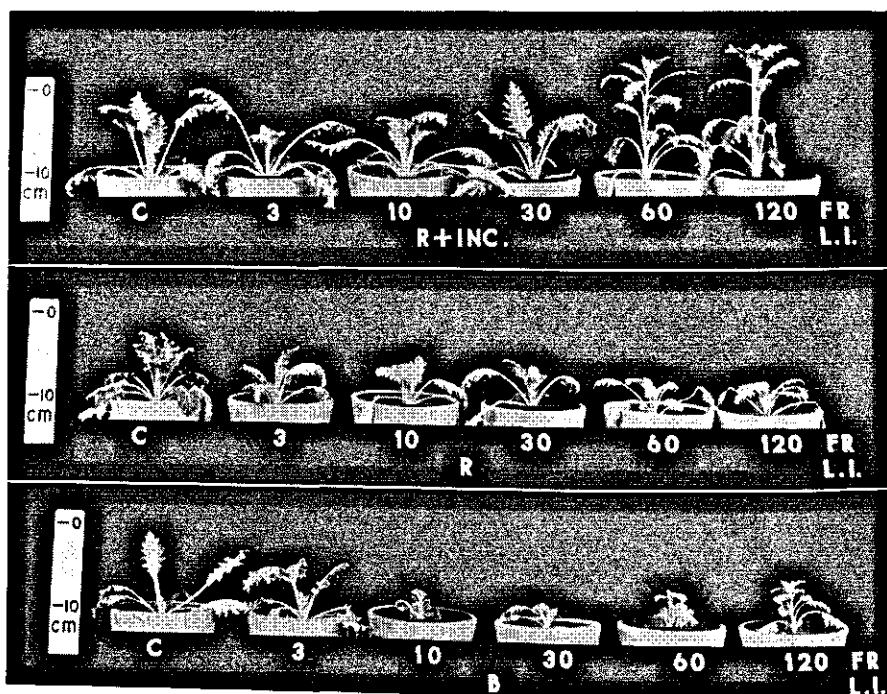
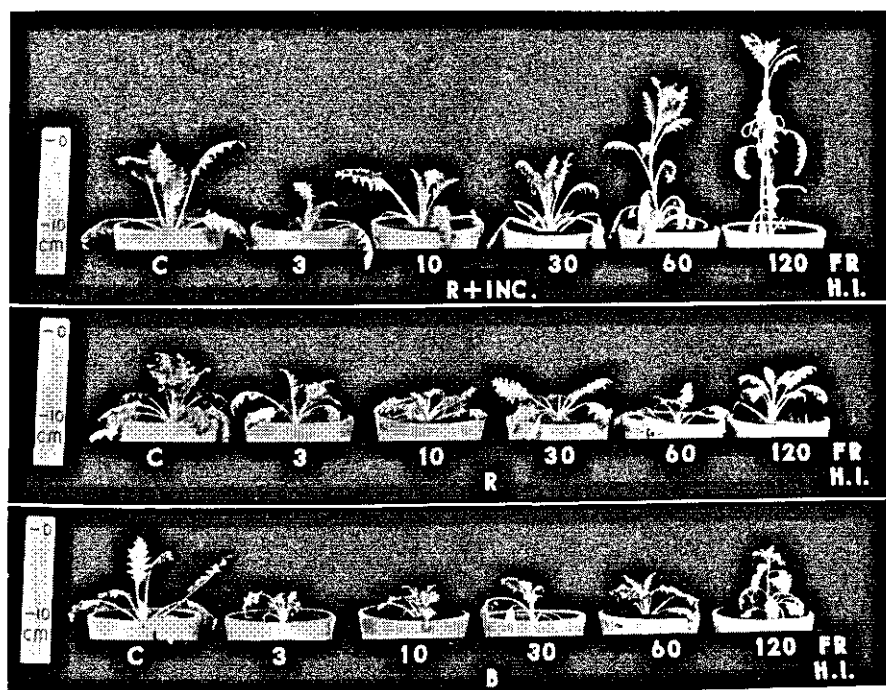


PLATE 13. Stem elongation and flowering as affected by far-red night interruption at two (pp. 78 levels of light intensity: 1000 ergs/cm²/sec (L.I.), or 3800 ergs/cm²/sec (H.I.) of and 79). varying durations (3, 10, 30, 60 or 120 mins) given at the middle of a 14 hour dark

red night interruptions at two levels of intensity given in the middle of 14-hour night of a blue, red or red + far-red mixed short day regime is graphically presented in fig. 40. The data indicate that far-red radiation near the middle of the long dark period is elongative in combination with all main light period colours. In general, increasing the duration of the far-red night break enhances the photoperiodic reaction, however, 3 mins were too short and failed to induce flowering under all three colours applied in the basic light period. The form of the reaction curves for the blue and red basic light colours is similar, but the red reaction is slower at all durations of the interruption. With the red basic light even 10 mins far-red do not cause the long-day effect, not even when given at high intensity. The last observation is in accordance with the data presented in fig. 15 chapter IV, where 10 mins of far-red immediately after a blue short day caused reproductive development, whereas the same amount after a red short day was inactive. The response curve of the mixed basic light group shows a faster response with 120 and 60 mins of far-red radiation as compared with blue; however, with 30 or 10 mins it is slower. Concerning the far-red intensity, it does not seem to be very important at this level.

Comparing these results with those obtained in fig. 38, obviously red night breaks cause quicker shooting than far-red ones in case of blue and mixed (R + FR) short days, whereas far-red interruptions, in general, cause faster



(Plate 13, continue)

period, combined with three types of basic photoperiod. The main light periods were: mixture of red and far-red (R + INC), red (R), or blue (B). See figure 40 p. 77. Photographed 28-2-1967.

shooting in combination with a red short day.

The plants of the preceding experiment showed etiolation of the leaves in blue and mixed light. After 10-hour irradiations with blue, red or mixed light for 30 days, petiole lengths were measured. They were longest with blue, followed by the mixed light, and shortest with red. Although the plants remained vegetative under these short day conditions, distinct formative effects occurred as a result of exposure to blue and the mixed light source. Petiole lengths as a result of the night interruptions of the preceding experiment are presented in fig. 41 and 42.

Concerning red night breaks, no distinct differences occur as a result of the various durations as compared with their controls, in combination with red or mixture of red and far-red basic periods. The only difference is that petioles are longer with the mixed basic periods than with the red.

With regard to the blue main light period, red night breaks produce a sharp reduction in petiole length in comparison with the control treatment.

It can be seen that the average of petiole length is 70 mm when the plants are exposed to a 10-hour blue day, whereas night interruptions of 3 or 10 mins red shift the lengths to 51 and 40 mm, respectively. Thereafter, this reaction seems saturated so that increasing duration of night break does not further increase the effect. The calculated relative petiole lengths presented in fig. 41 serve to further illustrate the described trends.

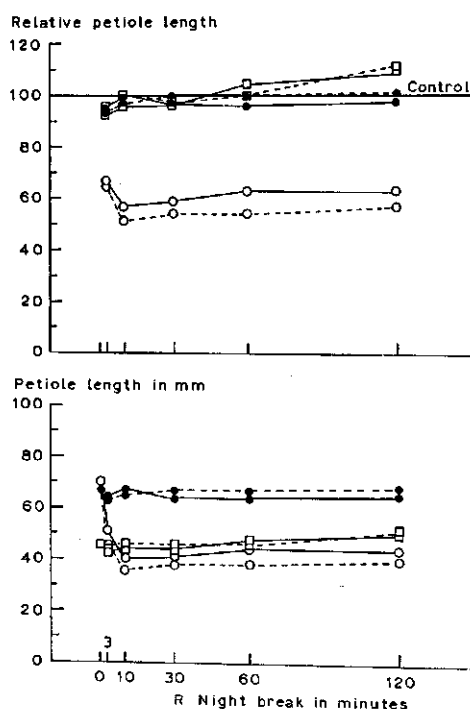


FIG. 41. The average leaf petiole length in mm and its relative length in *Hyoscyamus* as affected by red night interruption at two levels of light intensity: 1 (---) 1000 ergs/cm²/sec, 2 (—) 3800 ergs/cm²/sec of varying durations given at the middle of the 14 hour dark period, combined with 10 hours of three types of basic photoperiod. The main light periods were: blue (○), red (□), or red + far-red (●) at an intensity of 8000 ergs/cm²/sec. The experiment started 16-1-'67. Averages of 4 plants. Measurement after 30 days.

The average petiole length in mm as affected by varying times of far-red night breaks, in combination with short days of the three colours previously mentioned, is illustrated in fig. 42. Petiole lengths increase markedly due to far-red night breaks as compared with their controls under the basic periods of red and the mixture of red and far-red light, and with the mixture much more so than with red light. The effect increases with the increase of the duration up to 60 mins with the mixed basic period and only up to 30 mins with the red.

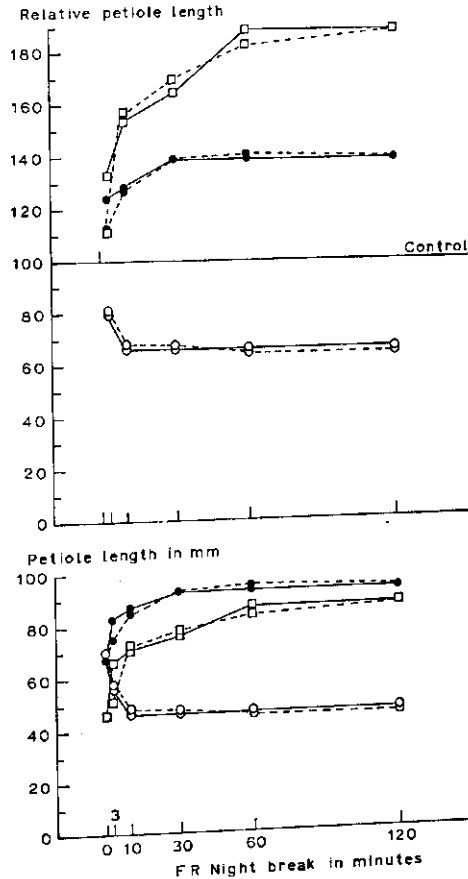
On the other hand, petiole lengths decreased as a result of exposure to far-red night interruptions in combination with short days in blue light.

As with shooting, no distinct differences in petiole length were observed between the light intensities of night break applied. Relative petiole lengths clearly show the described effects.

2. Effects of night breaks of well defined spectral composition applied at the beginning or at the end, or at different points of the long dark period

From the data of the foregoing experiment (figs. 38, 40), it was concluded that blue light as a basic period has more or less the same effect as the mixed source (R+FR) in combination with the red or far-red night breaks in the middle of the night, while a main light period in red light has a far weaker effect. Moreover, night interruption intensities seem to have only minor effects, when applied for 2 hours. Thus, it seems justified for the next experiment to use 2 hrs of the higher

FIG. 42. The average leaf petiole length in mm and its relative length in *Hyoscyamus* as affected by far-red night interruption at two levels of light intensity: 1 (---) 1000 ergs/cm²/sec, 2 (—) 3800 ergs/cm²/sec of varying durations given at the middle of the 14 hour dark period, combined with 10 hours of three types of basic photo-period. The main light periods were: blue (○), red (□), or red + far-red (●) at an intensity of 8000 ergs/cm²/sec. The experiment started 16-1-'67. Measurement after 30 days. Averages of 4 plants.



intensity of red and far-red as night breaks in combination with the red and mixed basic light periods.

Of course, it is of interest to see what happens when the same irradiations are applied at different moments of the night.

Selected *Hyoscyamus* plants were illuminated daily for 10 hours with red light or with a mixture of red and far-red radiation at an intensity of 8000 ergs/cm²/sec. The 14 hour dark period was interrupted with 2-hour exposures of red or far-red radiation. The night breaks were applied at 7 points; one is given immediately adjoining the day period, the other six begin successively 2 hours later, so that the last one is applied directly before the next day light period begins. Additional treatments with blue are given at the 2nd, 6th, and 10th hour of darkness and are of 10 mins or 2 hours duration. The light intensity of blue, red, or far-red radiation is 3800 ergs/cm²/sec. The experiment started 17-4-1967 and was ended after 65 days.

Days to bolting are summarized in fig. 43. Control plants irradiated with short days of 10 hours both in red light and in the red plus far-red mixture, remained vegetative till the termination of the experiment.

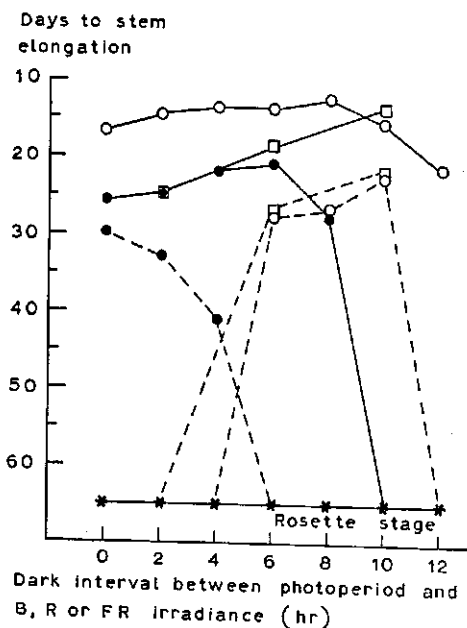


FIG. 43. Effects on stem elongation of *Hyoscyamus* caused by blue (□), red (○), or far-red (●) irradiation (3800 ergs/cm²/sec) during 2 hours at various times of the 14 hour dark period after a basic photoperiod of 10 hours red (---) or a mixture of red and far-red irradiation (—), both at the intensity of 8000 ergs/cm²/sec. The experiment started 17-4-'67. Controls (basic light only) remain in the rosette stage, and lasted 65 days. Averages of 4 plants.

The red night interruption is promotive, in combination with the mixed light source, when it is given at each of the 7 periods during the night. It is only somewhat less promotive at the end of the night (2 hours red directly before switching on the high light intensity). In combination with the red basic period, red night breaks are inhibitive during the first 6 hours of the night, thereafter they are promotive, with the maximum response at the 10th hour of darkness, and they fail again at the 12th hour of darkness.

Far-red night breaks are elongative in the first part of the dark period, until the 8th hour with the red plus far-red mixed basic light period, and only until the 4th hour with the red light day quality.

With the 10 mins blue night breaks at the 2nd, 6th and 10th hour point of darkness, plants remained vegetative in combination with both basic light periods. However, 2-hour blue night interruptions at these points in combination with the mixed photoperiod are as active as far-red night breaks at the 2nd and 6th hour points (though less active than red night breaks) and also as active as a red night break at the 10th hour point. With the red basic period, blue light is active only at the 6th and 10th hour points; the same as red night interruptions.

Though it is tempting to conclude, in the case of the R + FR basic light period, from the course of the line representing the effect of blue night interruptions of long duration, that they are active as red or far-red depending on where in the night they are applied; this does not appear certain as yet in view of the situation of the point representing the effect of a red night break at the 2nd hour point under these conditions.

Short periods at high intensity blue light in the present experiment or long durations (2 hours) at low intensity (fig. 27) failed to induce flowering.

3. Effects of sequences of night breaks at different points of the dark period

It was previously demonstrated in figs. 38 and 40 that 10 mins of red radiation given in the middle of the 14-hour dark period were promotive with either mixed (R + FR) or red basic light periods, while 10 mins far-red were not. Fig. 43 showed that red night interruptions are promotive at all points of the long dark period, in combination with the mixed basic period, whereas with the red day, they were active only at the 6th, 8th, and 10th hour of the dark period.

Therefore, an experiment was designed to analyse these data further. The effects of 10 mins far-red radiation applied after either 10 mins or 2 hours red light given at several points during the dark period were studied.

Hyoscyamus plants were irradiated daily with 10 hours of red light or with a mixture of red and far-red irradiation at the intensity of 8000 ergs/cm²/sec. The 14-hour night was interrupted by the following treatments:

- a) 2-hour periods of red light; the first one beginning immediately after the day period and the others always 2 hours later, resulting in 7 points, the same as used before in the experiment of fig. 43;
- b) 2 hours red followed immediately by 10 mins of far-red radiation, only for the first six points of a;
- c) 10 mins red;
- d. 10 mins far-red;
- e) 10 mins blue;
- f) 10 mins red followed immediately by 10 mins far-red.

The last 4 treatment series were applied also at each of several points, 2 hours apart, starting after 2 hours of darkness. The light intensity of these night break illuminations was 3800 ergs/cm²/sec. The experiment started 28-6-1967 and was discontinued after 65 days.

The results of the first two treatments (a and b) are presented in fig. 44. First of all, the trends obtained previously in fig. 43 are confirmed in showing that 2-hour red night breaks cause elongation at all points studied when combined with the mixed main light period, and are active only at the 6th, 8th and 10th hours of darkness with the red main light period. Ten mins far-red radiation given at the various points are inactive (fig. 45). However, the application of 10 mins of far-red radiation immediately after 2 hours of red light applied at the first 5 points, in combination with the red basic period markedly enhance the action of the red interruptions. The ineffectiveness of the two hours of red light at 0, 2 and 4 hours of darkness, is changed into a fast shooting effect by the subsequent application of only 10 mins of far-red radiation. Moreover, the elongative reaction of red interruptions at the 6th, 8th and 10th hours of darkness is markedly enhanced by the supplementation of 10 mins far-red.

With the mixed main light period 10 mins far-red radiation supplementary to the 2-hour red interruptions at the various points of the night cause almost no change of the shooting response.

As also shown in fig. 45, 10 mins blue or far-red night interruptions applied at the various points of the night are inactive with both main light periods used. However, 10 mins red night breaks at the same points are promotive with the

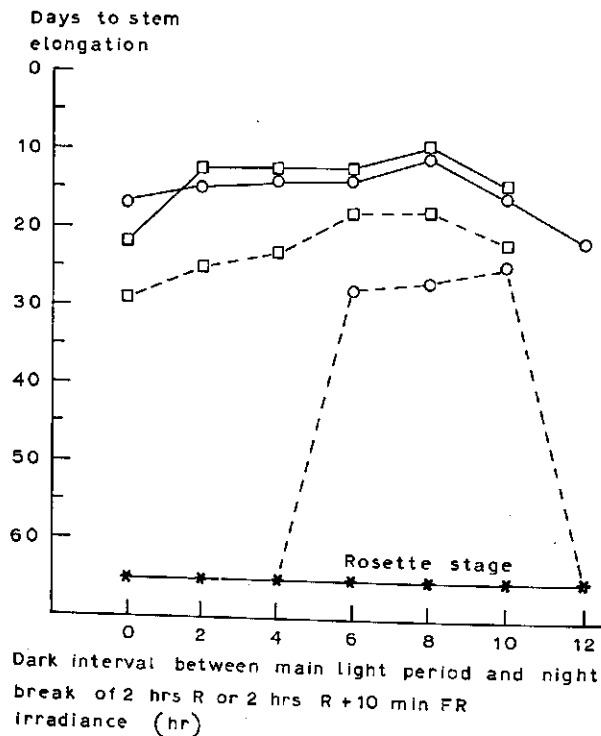
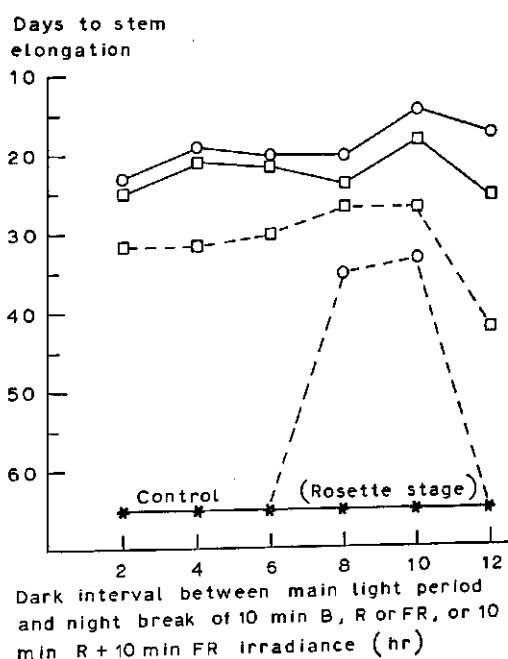


FIG. 44. Effects on stem elongation of *Hyoscyamus* caused by 2 hrs red (○), or 2 hrs red followed by 10 mins far-red (◻) night interruption ($3800 \text{ ergs/cm}^2/\text{sec}$) given at various times during 14 hr darkness, combined with a basic photoperiod of 10 hours either red (---) or a mixture of red and far-red radiation (—); both at the intensity of $8000 \text{ ergs/cm}^2/\text{sec}$. Control (no night interruption): rosette stage. The experiment started 28-6-'67, and lasted 65 days. Averages of 4 plants.

mixed basic light period showing slightly earlier shooting at the 10th hour of darkness; in case of the red basic period, red night breaks are only active at the 8th and the 10th point of the dark period. Short durations of either blue or far-red night breaks are inactive (fig. 45), while long durations are elongative (fig. 43). Moreover, either short or long durations of red radiation are promotive at the various points in case of the mixed photoperiod, while with the red basic period, brief (10 mins) red interruptions are only active at two points (the 8th and 10th hour) and long durations (2 hours) at three points (the 6th, 8th and 10th hour).

When the plants were irradiated successively with 10 mins red and 10 mins far-red radiation at the various points (fig. 45), it seems that far-red radiation reduces the effect of red night interruption under the mixed basic period. However, in spite of this inhibitory action of far-red radiation, the promoting action of red light upon shooting was not much reduced. Concerning the red is inactive (fig. 45), and red interruption at some points of the dark period also is inactive (fig. 45), the succession of 10 mins red and 10 mins far-red leads to

FIG. 45. Effects on stem elongation of *Hyoscyamus* caused by 10 mins blue (★), red (○), far-red (★), or 10 mins red followed by 10 mins far-red (□) irradiations (3800 ergs/cm²/sec) given at various times during 14 hr darkness, combined with a basic photoperiod of 10 hours either red (---) or a mixture of red and far-red radiation (—); both at the intensity of 8000 ergs/cm²/sec. The experiment started 28-6-'67, and lasted 65 days. Averages of 4 plants.



flowering (fig.45) at all points of the dark period. Moreover, the addition of far-red immediately after the promotive red radiation at the 8th and 10th hour of darkness increases the flowering response.

GENERAL DISCUSSION

1. Introduction

Before entering into the discussion of the subject, it is desirable to recall that our strain of *Hyoscyamus* reacts as a typical long day plant. Under artificial light, increasing the duration of the light period shooting is speeded up, and the approximate critical day length lies between 10 and 12 hours (fig. 3 and table 1). This result is in accordance with those obtained by LANG and MELCHERS (1943) (from whom the seeds originally were obtained,) and HSU and HAMNER (1967). However, stem formation in *Hyoscyamus* can occur in short days; when 30 or even 10 minutes far-red radiation is given after short basic photoperiods in various colours of light (figs. 10, 13). This observation is another example to emphasize that it is senseless to use the term 'critical daylength' (STOLWIJK and WASSINK, 1956; DE LINT, 1958).

In *Hyoscyamus*, stem elongation and flower bud initiation are closely linked, so that elongated plants normally have flower buds and rosette plants have not.

Available evidence indicates that the light absorbing pigment system in bolting is the red-far-red 'phytochrome' system (STOLWIJK and ZEEVAART, 1955; VAN DER VEEN and MEIJER, 1959). However, the reaction system seems rather complicated, so that interpretation of data on the basis of phytochrome alone probably is insufficient.

Results from experiments with supplementary irradiation may deviate from night break results (STOLWIJK and ZEEVAART, 1955; CATHEY and BORTHWICK, 1957; DOWNS et al., 1958; PIRINGER and STUART, 1958).

MEIJER (1959) made extensive experiments to study the difference in results between night break and supplementary irradiation. His publication is concerned with *Salvia occidentalis* (SDP), but as far as comparable data are available, *Hyoscyamus* behaves similarly (STOLWIJK and ZEEVAART, 1955; VAN DER VEEN and MEIJER, 1959).

In the following discussion, phytochrome in the P_{fr} -form is regarded as the active principle in biological control of various phenomena, including flowering (HENDRICKS and BORTHWICK, 1963, 1965). The P_{fr} -form was suggested to act on a product of the so-called 'high energy reaction' (EVANS, HENDRICKS and BORTHWICK, 1965; HENDRICKS and BORTHWICK, 1965; LANE and KASPERBAUER, 1965), so that the phytochrome controlled step should follow the high energy reaction in one sequence. The relation between the high energy reaction and phytochrome, however, is uncertain. MOHR (1959) suggested that they act independently. However, recently, HARTMANN (1966) proposed that the high energy reaction could be a consequence of the characteristics of phytochrome itself. The high energy system becomes operative in plants when P_{fr} is present for an

extended period in concentrations above some minimum, and has a high $P_{fr} \rightleftharpoons P_r$ turnover rate.

Earlier, LANE, CATHEY and EVANS (1965) had also suggested that flower induction in LDPs is fully controlled by phytochrome. They proposed that flower induction requires optimum daily P_{fr} -action over a long period. The optimum level of P_{fr} -action is assumed to vary between species and probably also with light intensity and colour. They postulated that at the end of each day, when the product of the high energy reaction is at its highest concentration, the optimum P_{fr} -level is low. If this product is metabolically consumed, the optimum P_{fr} -level rises. They assumed further that the changes in optimum P_{fr} -level could be controlled by an endogenous rhythm rather than by the supply of substrate for P_{fr} -action.

Recently, it has been shown indeed, that light control of flowering and stem elongation of *Hyoscyamus* can be a direct function of phytochrome as an indirect high energy reaction, with an action maximum at 710–720 nm (SCHNEIDER, BORTHWICK and HENDRICKS, 1967). Evidence for a high energy type reaction was previously obtained by STOLWIJK and ZEEVAART (1955) and by VAN DER VEEN and MEIJER (1959). These authors applied radiation of limited spectral regions during the main photoperiods for *Hyoscyamus niger* and found that flowering occurred in blue and far-red.

2. Morphogenetic effects of broad band coloured irradiation used throughout the photoperiods

The experiments described in chapter IV show that *Hyoscyamus* plants grown in long days of blue light (16 hrs) produced stems while plants in green and red long days remained in the rosette stage (table 2). Blue light is more elongative also than white light. This observation confirms the conclusion drawn by WASSINK et al. (1951) as to antagonism exists between certain spectral regions, since white light is a mixture of blue and the (inhibitive) red and green wavelength regions. In addition, plants in blue and white light have higher top/root ratios than plants exposed to green or red light (table 2). It is clear that a large portion of the top/root differences in the various light qualities is due to differences in root growth, blue and white light reducing it and the other colours stimulating it. Furthermore, strong elongation of petioles occurred as a result of exposure to blue, whereas excessive elongation is absent in the other wavelength bands, and in white light. Thus, there seems a correlation in wavelength dependence between elongation of petioles of the basal leaves and stem elongation (CURRY and WASSINK, 1956).

Moreover, plants receiving long days in blue light (table 2) or 10 hr-basic blue days followed by 6 hrs blue light at lower intensity (fig. 7) bolted. It is uncertain whether this observation can be understood from the assumption that both forms of phytochrome have some absorption in the blue (BORTHWICK and HENDRICKS, 1960; BUTLER et al., 1964; ODA, 1962; FURUYA and HILLMAN, 1964; HARTMANN, 1966; PRATT and BRIGGS, 1966) or that we must assume participation of another pigment.

3. Morphogenetic effects of supplementary light of various wavelengths

Hyoscyamus reacts to supplementary irradiation in the blue and far-red regions with a marked elongation of stem (fig. 4) and petioles (fig. 6). This indicates that in *Hyoscyamus* the high energy mechanism of the B-FR reaction exists MOHR (1959); MOHR and NOBLE (1960).

Plants grown in red light are in the rosette stage, while additional far-red annihilates the inhibitive effects of red light (fig. 4). We may assume that in the first case P_{fr} is present in a high concentration, inhibiting flowering, but the additional far-red decreases the P_{fr}/P_r ratio, thus permitting flowering. Moreover, 2 hrs blue light given after 14 hr-basic days in red light cause bolting, but obviously more slowly than a far-red supplement. Thus, it is evident that blue light resembles far-red in its effect.

Red supplementary light, after a short day in white light which contains a higher fraction of far-red than our original type of white light (fig. 2), is more active than far-red irradiation (figs. 7, 18). This is, however, in contrast to the available literature which reports that red extensions mostly are ineffective (WASSINK et al., 1950, 1951; STOLWIJK and ZEEVAART, 1955; DE LINT, 1958, 1960; VINCE et al., 1964; EVANS, BORTHWICK and HENDRICKS, 1965; VINCE, 1965), inhibiting or delaying floral induction due to high concentrations of P_{fr} which is maintained during the entire supplementary period (VINCE, 1965). In our opinion, this result, and the long day effect observed when 6 hrs of red light were given after a short day in blue light (fig. 7) may be interpreted on the basis of the phytochrome system, assuming that the red supplementary radiation acts as the main light period. Then, the phytochrome at the end of the red light period is in the P_{fr} -form which is gradually reversed to P_r in the following dark period. After the decrease to lower level the concentration of the P_{fr} -form is raised again by the subsequent periods of either blue or white light, which then act as supplementary light, leading to stem elongation and flower bud formation. This result suggests that induction in *Hyoscyamus* requires intermediate P_{fr} -levels, while higher concentrations are inhibitive. This assumption is similar to that proposed by CUMMING (1963) for *Chenopodium rubrum* (SDP) and by LANE et al. (1965) for LDPs.

Distinct stem elongation results from far-red supplementary irradiation after short day treatments in various colours (figs. 7, 8). This observation suggests that far-red somehow promotes the action of P_{fr} (EVANS, 1964). Far-red light, due to its high transmission through the leaves, might be able to maintain a fair proportion of P_{fr} (EVANS, BORTHWICK and HENDRICKS, 1965). From these indications, *Hyoscyamus* plants may require a specific balance in the amounts of the two pigment forms for the photoperiodic and morphogenetic effects observed.

An antagonism between red and far-red was not observed under the experimental conditions (figs 18, 19, 20). Generally, in far-red followed by red extensions and in red followed by far-red extensions, the two colours increase each other, when they are given after a short photoperiod in white light. This result is in accordance with those of STOLWIJK and ZEEVAART (1955) and EVANS, BORTHWICK and HENDRICKS (1965). The latter concluded that induction in

Lolium temulentum requires the presence of P_{fr} over a prolonged period and also, independent of the first requirement, over some period, the presence of P_r .

From our data, it could be concluded that red light is essential for flower induction and far-red for elongation. But likewise, the activity of our various sequences may be explained by the maintenance of an intermediate proportion of P_{fr} -phytochrome over a long period leading to rapid stem elongation and flowering.

On the other hand, daylength extensions of blue light followed by red cause a long day effect more or less equal to that of extensions by red light alone (figs. 18, 19). This suggests that blue irradiation acts as darkness, and red light after blue performs as a night break.

4. *Morphogenetic effects of various patterns of night breaks given in the middle of the dark period by light of various wavelengths*

The relation between the various durations of red and far-red radiation, given in the middle of the 14-hour darkness, as well as the precise effect of the colour of the main light period on the photoperiodic response has been studied in chapter V (figs. 22, 24, 25, 38, 39, 40). The data show that the colour of the basic photoperiod in conjunction with red night interruption plays an important role in the photoperiodic and formative responses. Our results demonstrate that short photoperiods rich in far-red (figs. 22, 24, 25, 38) or blue irradiation (fig. 38), combined with red night interruptions yield quick bolting. This confirms the conclusion of VAN DER VEEN and MEIJER (1960). The data illustrated in fig. 38 show that a somewhat higher level of saturation of the photoperiodic response is reached with higher light intensities applied as night interruption.

In contrast, in combination with a red main light period, increasing the light intensity of red breaks up to 3800 ergs/cm²/sec, even a 3 mins exposure leads to flowering. At the lower intensity of red breaks (1000 ergs/cm²/sec), only 120 mins were found elongative. This observation seems to combine nicely with those recorded in fig. 22, obtained with the original type of white fluorescent light which contains a small amount of far-red radiation. Combined with low intensity red night breaks (600 ergs/cm²/sec) this did not produce a long-day effect. It may be concluded from these two series of data that the light intensity of red night interruptions is very important for the photoperiodic response in combination with main photoperiods in red light. These results oppose the conclusion of MEIJER and VAN DER VEEN (1957); MEIJER (1959); MEIJER and VAN DER VEEN (1960) that a red night break fails to induce flowering after a main light period in red light.

Concerning the durations of night breaks it is clear that a 10 mins red night break combined with white light, rich in far-red light (figs. 22, 24, 25), is sufficient to induce elongation and flower bud formation. The longer the duration of the red irradiation, the sooner stem elongation occurs. This confirms the demonstration of VINCE (1965) for *Lolium temulentum* that increased duration of red night breaks increased their effect. However, in *Hyoscyamus*, the photoperiodic

response is saturated at 3 mins red night break in combination with blue or mixed (R + FR) basic light periods (figs. 38, 39), and no clear-cut photoperiodic differences exist in the series of red night break durations or intensities.

Generally, upon red night interruption, plants under a main light period consisting of a mixture of red and far-red radiation show the highest stems, the red ones the shortest, stems under the blue basic period are intermediate (fig. 39).

These data suggest that with regard to the reaction; upon a red night interruption, there is a similarity between the blue and far-red irradiations when used as basic short periods, the former, however, is slower than the latter. These data might be explained by assuming that the concentration of P_{fr} in the case of red short days is high which delays flowering response under red night interruptions. However, the intermediate concentration of P_{fr} under the mixed (R + FR) and the blue short photoperiods permits the brief red night interruption to act soon.

Certainly, the activity of the red night interruption in our data is due to a phytochrome control of flowering in *Hyoscyamus niger*, since short red night breaks are given. Phytochrome control of flowering attained by short irradiations was also noted by MEIJER (1959), and MEIJER and VAN DER VEEN (1960). Effectiveness of red-light control of flowering of *Hyoscyamus niger* was reported by PARKER et al. (1950). However, 15 mins fluorescent light breaks, given in the middle of a 16-hour dark period, failed to induce flower initiation in *Anethum graveolens* (dill), *Beta vulgaris* (sugar beet), *Hyoscyamus niger* (henbane), or *Lolium temulentum* (darnel), and did so in *Hordium vulgare* (barley) only after a few weeks (LANE et al., 1965). Moreover, NAYLOR (1941) found that incandescent light breaks of 100 ft-c intensity for up to 30 mins in the middle of each night had no effect on the flowering of dill.

We may agree with VINCE (1965) that it is not very easy to relate the results obtained under artificial light to those from experiments where plants received daylight.

Concerning far-red night interruption (figs. 22, 24, 25, 40), it is clear that far-red radiation is promotive in most cases in durations of at least 10 mins; increase in duration enhances the photoperiodic reaction, but its action is slower than that of red light. The activity of far-red night breaks is less pronounced than that of red ones when the basic photoperiod is rich in far-red or blue energy. However, far-red interruptions cause faster shooting than red night breaks in combination with red short days (figs. 38, 40). Thus, the quality of the main light period has an effect on the photoperiodic reaction of night interruptions with coloured light. Furthermore, the results suggest that blue or far-red energy in the main light period in conjunction with far-red in the middle of the dark period is essential for stem elongation and flower bud formation. This suggestion may be related to the effect of far-red night interruption long of duration which has a red action but slower than the red itself, which may be due to the overlap of absorptionspectra of the red and far-red sensitive pigments. Additionally, it seems that the difference in far-red intensity as applied for night interruptions has no important effect (fig. 40).

It is of interest to mention that with the red basic light period a short night

break, e.g. of 10 mins far-red does not cause the long day effect (fig. 40). This observation is in accordance with the data presented in fig. 15, where a 10 mins far-red supplement, given after a red short day, was inactive. However, the same amount given either after the close of a blue short day (fig. 15) or in the middle of the 14-hour darkness period in this experiment (fig. 40) was elongative. These observations may suggest that in the case of the red basic period the antagonism of red and far-red is mainly responsible for the result, whereas with blue, the co-operative effect of blue and far-red (in HARTMANN's sense (1966)) plays the primary role.

With increased duration of the night breaks, stem elongation occurs more rapidly, and the numbers of leaves indeed are inversely correlated herewith (figs. 22, 23). This guarantees that the growth rates of the plants under the various light treatments were very similar.

Although *Hyoscyamus* plants remained vegetative under blue, red or mixed (R + FR) short days, strong etiolation of the leaves in the blue and mixed light occurred. Petiole lengths as a result of the night interruptions under the preceding light regimes were studied (figs. 41, 42).

Concerning red night breaks, it is observed that no distinct differences occur as a result of the various durations as compared with their controls, in combination with red or mixed basic periods. The only difference is that petiole lengths are greater with the mixed basic periods than with the red one. However, petiole lengths increase markedly owing to far-red night breaks as compared with their controls under the basic periods of red and the mixture of red and far-red light, and much more so with the mixture than with red light. With regard to the blue main light period, it is found that either red or far-red night breaks produce a sharp reduction. These data, in general, confirm the demonstration of WASSINK and SYTSEMA (1958), using *Hyoscyamus niger*, that low intensity red light interruption in the middle of the dark period decreased the ultimate petiole length, whereas with a brief supply of far-red alone, petioles grew longer than in uninterrupted darkness.

The striking result of petiole length reduction as a result of red or far-red night breaks in combination with blue short days depends on so far unknown factors. However, it seems that this phenomenon requires further analysis.

In addition, it seems that the etiolation phenomenon is independent of the flowering response. In other words, it is not easy to combine the photoperiodic behaviour and the formative effects on petiole length in a clear-cut tentative explanation.

5. Photoperiodic effects of night breaks of various spectral regions applied at various points during the long dark period

The effect on the photoperiodic response of night breaks of well defined spectral regions at various points during the long dark period has been studied (figs. 27, 28, 30, 33, 43, 44, table 3). Basic photoperiods rich in far-red or blue energy in conjunction with various spectral regions at several moments during

the long dark period have a more pronounced influence on the photoperiodic reaction than basic periods rich in red light.

In general, red and far-red night breaks showed opposite response curves, red light being most active in the second part of the night, far-red in the first. Mixture of these two radiations has the same trend of red action, but is more active than the red itself (table 3). Not only the colour of the main photoperiod and the moment of application of the night break play an important role in the photoperiodic response, but also the colour, the duration and the intensity of the night break.

Like already preliminary remarked, our results correspond with the phenomenon observed with the SDP *Xanthium pensylvanicum* (REID et al., 1967). Their results exactly resemble ours with *Hyoscyamus niger* (LDP), but in the opposite sense. The sensitivity towards red and far-red at various points during the long dark period changes more or less in opposite direction. The results of REID et al occurred with very long dark periods (48 and 722 hrs), but not in dark periods of normal lengths, e.g. 12 hrs, which ours do.

It is interesting that a short-day plant shows more or less the same phenomenon we found in a long-day plant.

Long durations of blue light as a night break at high intensity ($3800 \text{ ergs/cm}^2/\text{sec}$), are active as red or far-red (fig. 43) depending on where in the night they are applied, while short periods (10 mins) at high intensity (fig. 45) or long durations (2 hrs) at low intensity ($1000 \text{ ergs/cm}^2/\text{sec}$) fail to induce flowering (fig. 37).

It is uncertain whether the photoreceptor of blue light is the same as that of the red, far-red system, or another pigment.

The data illustrated in figs. 43, 44, 45 demonstrate that, in combination with R + FR main light periods, red night interruptions cause rapid shooting when applied at each point of darkness even immediately after or before the basic light period without the intercalation of any darkness. In contrast, with red basic light periods a sufficiently long dark period must either precede or follow the night interruption, the preceding one being at least 6 hours, the following one being at least 2 hours. Perhaps we may suggest that the red basic light period contains a high ratio of red/far-red and thus end up with a high concentration of P_{fr} , which requires a sufficiently long dark period (at least 6 hrs of the 14-hours darkness) for conversion to P_r . By the red night break this P_r could then be converted back to P_{fr} which should lead to the flowering response.

This suggestion seems also applicable to the data in fig. 27 where two white light sources were used as short basic light periods; one of them is more rich in far-red radiation (TL/IL) than the other (TL). In the latter case, 2 hrs of red light given either directly after or before the main photoperiod were ineffective, while red light given later at several points during the dark period was active. However, in combination with the mixed white light source, red light was active at each point during the long dark period, even directly after or before the basic light period.

For bolting in *Hyoscyamus*, it seems that darkness is necessary at least for

30 mins, between a red night interruption and the main photoperiod, particularly if the latter is rich in red (fig. 35).

Therefore, it may be suggested that there is an interaction between the availability of photosynthetic products of the preceding day and the presence of P_{fr} with respect to the effectiveness with time of night breaks (HENDRICKS, 1963).

Altogether, these observations suggest, again, that optimum induction in *Hyoscyamus niger* occurs at intermediate phytochrome P_{fr} -levels, higher concentrations inhibiting induction. This interpretation may also answer the question why long or continuous days in red light are inhibitive (table 2, and see plate 2). This assumption is in harmony with the suggestion of CUMMING (1963); LANE et al. (1965).

The promotive effect of far-red radiation during the first part of darkness and its ineffectiveness in the second half, may be due not only to the requirement of a proper P_{fr}/P_r ratio, but also to an endogenous rhythm in the *Hyoscyamus* plant changing sensitivity to either P_{fr} or P_r , or to the ratio of these two.

6. Photoperiodic effects caused by sequences of night breaks in the dark period

Evidence for the red, far-red reversibility system in photoperiodism was presented by BORTHWICK et al. (1952); STOLWIJK and ZEEVAART (1955); DOWNS (1956); CATHEY and BORTHWICK (1957); BORTHWICK (1959); MEIJER (1959).

It appears that far-red reversal of red night break action is not complete, especially when the duration of far-red given after red is extended or when a dark period is intercalated between the red and far-red, or when the temperature during the intervening dark period is lowered (DOWNS, 1956; CATHEY and BORTHWICK, 1957; BORTHWICK, 1959).

Thus, the role of phytochrome in photoperiodic control of flowering is demonstrated also in the effects of interruption of the dark period with red light and reversal by subsequent far-red irradiation. Exceptions, however, have been found (NAKAYAMA, 1958; NAKAYAMA et al., 1960; PURVES, 1961; BORTHWICK and DOWNS, 1964; EVANS, BORTHWICK and HENDRICKS, 1965; ESASHI, 1966; HILLMAN, 1966; CLELAND and BRIGGS, 1968).

Our data (figs. 30, 31, 34, table 3) did not demonstrate antagonism between red and far-red irradiation during the night in combination with white light sources. Far-red irradiation given after a red night break enhanced the promotive action of the red light. However, the application of red and far-red simultaneously during the long night had more effect on stem elongation than far-red supplied after a red night break.

Certainly, the durations of both red and far-red were long (2 hrs); thus, far-red radiation given after a red night break could not overcome the flowering promotive action of the red light. The failure of reversibility might be attributed not only to the long duration of red light, but also to the long duration of far-red which converts P_{fr} , particularly in the first part of the night.

The data illustrated in fig. 45 show that 10 mins far-red night interruption applied at various points of the night were inactive with a main light period rich in far-red ($R + FR$) or not (R). However, 10 mins red night breaks at the same

points were promotive in combination with the mixed basic light period (R + FR); in case of the red basic period, red night breaks were only active at the 8th and 10th hour points of the 14 hr dark period. When *Hyoscyamus* plants were irradiated successively with 10 mins red and 10 mins far-red radiation at various points of the dark period, far-red radiation reduced the effect of the red night interruption in combination with the mixed (R + FR) basic period. Thus, in spite of the antagonistic action of far-red against red with respect to the phytochrome system, the elongative action of red light observed in this type of experiment was not much reduced.

In connection with the red basic period, far-red radiation given at the various times of darkness was inhibitory, as well as red interruption at some points. However, especially in the first half of the dark period, the succession of 10 mins red and 10 mins far-red led to flowering. Thus, also here, reversibility of red action by subsequent far-red radiation was not found.

This observation is in agreement with the work of NAKAYAMA (1958); HILLMAN (1959); NAKAYAAM et al. (1960); ESASHI (1966); HILLMAN (1966); REID et al. (1967); CLELAND and BRIGGS (1968).

From the fact that 10 mins red or far-red were inactive in promoting flowering at several points of the night and from the promotive action of their succession in combination with the basic photoperiod in red light, we may conclude that stem elongation in *Hyoscyamus niger* requires a mixture or a balance of P_{fr} and P_r during the dark period.

7. General review

Altogether, it seems that the roles of irradiation in growth and flowering in *Hyoscyamus niger* are complicated.

Hyoscyamus seems to require the phytochrome system, since the high energy reaction is engaged in the plant, and P_{fr} at intermediate level (CUMMING, 1963; LANE et al., 1965) should be present for a prolonged period each day. Thus, we may accept that the high energy reaction (MOHR, 1959; MOHR and NOBLE, 1960; MOHR, 1964) is manipulating in an indirect way through the phytochrome system (HARTMANN, 1966, 1967). However, the phytochrome system is not absolutely identified by its reversibility during the dark period alone. But phytochrome could be identified by programs of simultaneous irradiation with the two physiologically ineffective wavelengths 660 and 770 nm which are mainly absorbed in P_r and P_{fr} .*

Moreover, there is evidence that changes in sensitivity to red and far-red during the night are connected with some sort of endogenous rhythm in *Hyoscyamus*.

This is in agreement with VINCE (1965) who found in *Lolium temulentum* a pronounced change in the relative response to red or far-red light during a photoperiod extension and suggested that an endogenous rhythm might be at the basis of this phenomenon.

* I owe this remark to DR. K. M. HARTMANN.

Generally speaking, we conclude that *Hyoscyamus* plants require P_{fr} at intermediate levels or specific ratios between P_{fr} and P_r for optimal induction, higher levels being inhibitive.

The promotive action of blue light during the photoperiod or the dark period can be understood from the fact that both forms of phytochrome have another (small) absorption maximum in the blue.

The reactivity of *Hyoscyamus* plants towards red and far-red is different throughout the night. This shows the existence of some 'endogenous rhythm' which might also be understood as causing different changes in P_r - and P_{fr} -levels at various points of a long dark period, or bringing about changes in the sensitivity of the plant towards primary effects brought about by the phytochrome system.

SUMMARY

The present paper is concerned with bolting and morphogenesis of *Hyoscyamus niger* L. as reactions upon radiation in the visible spectrum.

Experiments are described in which *Hyoscyamus* plants were exposed to light of various well defined spectral regions. The light of these spectral regions was applied at low intensity, supplementary to a short day in various colours, or at high intensity, as exclusive source of light. Special attention was paid to study the influence of these spectral regions given in the middle or at various other points of the 14-hour dark period, in combination with short days in various colours. Moreover, morphogenetic responses caused by sequences of night breaks of different spectral regions during the dark period were tested.

In *Hyoscyamus*, stem elongation and flower bud initiation are linked, so that elongated plants normally have flower buds and rosette plants have not.

A-MORPHOGENETIC EFFECTS OF BROAD BAND COLOURED IRRADIATION AT HIGH INTENSITY USED THROUGHOUT THE MAIN PHOTOPERIOD OR AT LOW INTENSITY, SUPPLEMENTARY TO SHORT PHOTOPERIODS IN VARIOUS COLOURS

1. *Hyoscyamus* reacts as a typical long day plant. Artificial white light, increasing the duration of the light period, speeded up shooting, at an approximate critical day length between 10 and 12 hours (fig. 3 and table 1). However, stem formation in *Hyoscyamus* occurred in short days when 30 or even 10 mins far-red radiation was given after short basic photoperiods in various colours of light (figs. 10, 13).

2. *Hyoscyamus* plants, grown in long days of blue light produced stems while plants in green and red long days remained in the rosette stage (table 2). Blue light was also more elongative than white light. Plants in blue and white light had higher top/root ratios than plants exposed to green and red light (table 2). A large portion of the top/root differences in the various light qualities was due to differences in root growth, blue and white light reducing it, and the other colours stimulating it. Moreover, strong elongation of petioles occurred as a result of exposure to blue light, whereas this elongation was absent in green and red wavelength bands, and in white light.

3. *Hyoscyamus* reacted to supplementary irradiation in the blue and far-red regions with a marked elongation of stems (fig. 4) and petioles (fig. 6). This result indicated that in *Hyoscyamus* the high energy mechanism of the B-FR reaction exist. Plants grown in red light remained in the rosette stage, while additional far-red annihilated this inhibitive effect of red light (fig. 4). Moreover, 2 hrs blue light given after long days in red light (14 hrs) caused bolting, but obviously more slowly than a far-red supplement. Therefore, blue light resembled far-red in its influence but appeared less effective.

4. Red supplementary light, after a short day in white light was more active

than far-red under similar conditions (figs. 7, 18). This observation, however, is in contrast to the available literature which indicates that red extensions mostly are inactive. This contradiction may be due to the fact that the white light used for the main photoperiod contains a relatively high fraction of far-red. This result, and also the long-day effect observed when 6 hrs of red light were given after a short day in blue light (fig. 7) suggest that induction in *Hyoscyamus* requires intermediate P_{fr} -levels. Additionally the fact that plants in long days red light remain vegetative, supports the idea that high concentrations of P_{fr} are inhibitive.

5. Antagonism between red and far-red was not observed under the experimental conditions (figs. 18, 19, 20). Generally, in far-red followed by red extensions and in red followed by far-red extensions, the two colours increased each other in producing the effect, when they were given after a short photoperiod in white light. On the other hand, daylength extensions of blue light followed by red caused a long day effect more or less equal to that of extensions by red light alone (figs. 18, 19). In this case, blue irradiation acts as darkness and red light after blue acts as a night break.

B-MORPHOGENETIC EFFECTS OF VARIOUS PATTERNS OF NIGHT BREAKS GIVEN IN THE MIDDLE OR AT VARIOUS OTHER POINTS OF THE DARK PERIOD

1. The colour of the basic photoperiod in conjunction with a red night interruption exerts an important influence on the photoperiodic and formative responses. Short photoperiods, rich in far-red (figs. 22, 24, 25, 38) or blue irradiation (fig. 38), combined with red night interruptions yielded quick bolting. Increase of the light intensities applied as night interruption resulted in still earlier flowering. At the intensity of red light of 1000 ergs/cm²/sec, only 120 mins night breaks were found elongative, in combination with a main photoperiod in red light. With the light intensity of the red night breaks increased to 3800 ergs/cm²/sec, even a 3 mins exposure led to flowering.

2. A 10 mins red night break combined with white light rich in far-red (figs. 22, 24, 25) was sufficient to induce elongation and flower bud formation. The longer the duration of the red irradiation, the sooner stem elongation occurred. However, the photoperiodic response was saturated at 3 mins red night breaks in combination with the blue or mixed (R + FR) basic light periods (figs. 38, 39), and, therefore no clear-cut photoperiodic differences existed in the series of red night break duration or intensities. Generally, upon red night interruption, plants under a main light period consisting of a mixture of red and far-red radiation showed the highest stems, the red ones the shortest, while the blue ones were intermediate (fig. 39). Upon a red night interruption, there was a similarity between the blue and red + far-red irradiations when used as basic short periods, the former, however, was slower than the latter.

3. Far-red night interruptions (figs. 22, 24, 25, 40), were promotive in most cases in durations of at least 10 mins, while increasing the duration enhanced the flowering reaction, but the reaction was slower than that upon red night breaks. However, far-red interruptions caused faster shooting than red night

breaks in combination with short days in red light. The difference in far-red intensity applied for night interruptions had no important effect.

4. It was observed that with increased duration of the night breaks, stem elongation occurred more rapidly, and consequently the number of leaves also were nicely correlated herewith (figs. 22, 23).

5. No distinct differences in petiole length occurred as a result of the various durations of red night breaks as compared with their controls, when these night breaks were combined with red or $R + FR$ basic periods. The only difference was that petiole length was greater with the mixed ($R + FR$) basic periods than with the red one. However, petiole lengths increased markedly owing to far-red night breaks as compared with their controls under the basic periods of red light and of the mixture of red and far-red light, and stronger so with the mixture than with red light. With the blue main light period, it was found that either red or far-red night breaks produced a sharp reduction of the petiole length.

6. Red and far-red night breaks showed opposite photoperiodic response curves when applied at various moments during the long dark period (figs. 27, 28, 30, 33, 43, 44, table 3), red light being most active in the second part of the night, far-red in the first part. A mixture of these two radiations showed the red action curve, but on a higher level than the red itself (table 3).

7. Long durations of blue light as a night break at high intensity (3800 ergs/cm²/sec) were elongative mainly as red (fig. 43) when applied at various moments of the night, while the time curve of the action as spread over the night does not exclude the possibility of some far-red activity in the first half of the night. Short periods (10 mins) at high intensity (fig. 45) or long durations (2 hrs) at low intensity (1000 ergs/cm²/sec) blue light failed to induce shooting (fig. 27).

8. It was demonstrated that in combination with mixed light periods, red night interruptions caused rapid shooting when applied at each point of darkness even immediately after or before the basic light period without the requirement of a dark intercalation (figs. 43, 44, 45). In contrast, with red basic light periods sufficiently long dark periods must precede (at least 6 hrs of the 14-hour darkness) or follow (at least 2 hrs) the night interruption. This observation seems in agreement with the data in fig. 27, where two white light sources were used as a short basic light period; one of them contains more far-red (TL/IL) than the other (TL). In the latter case, 2 hrs of red light given either immediately after or before the main photoperiod were ineffective, only red light given later at several points during the dark period was effective for stem elongation and flower bud formation. However, in combination with the mixed white light source, red light was active at each point during the long dark period, even immediately after or before the basic light period. Additional experiments with variable short lengths of dark periods immediately before the main light period, showed that, for bolting in *Hyoscyamus*, it was necessary to intercalate at least some 30 mins darkness between a red night interruption and the main photoperiod in case the latter is rich in red (the TL-source) (fig. 35).

C-PHOTOPERIODIC RESPONSES CAUSED BY SEQUENCES OF NIGHT BREAKS IN THE DARK PERIOD

1. The data did not demonstrate antagonism between red and far-red irradiation during the night in combination with artificial white light sources (figs. 30, 31, 34, table 3). Far-red irradiation given after red night breaks enhanced the promotive action of red light, speeding up bolting particularly in the first half of the night. However, the application of red and far-red simultaneously during breaks in the long night had more effect on stem lengthening than far-red after a red night break. The explanation seems to be that, since the durations of either red or far-red were long (2 hrs), far-red radiation given after a red night break could no more annihilate the flowering promotion of red light.

2. Ten minutes far-red night interruption applied at various points of the night were inactive (fig. 45) with main light periods rich in far-red (R + FR) or not (R). However, 10 mins red night breaks at the same points were promotive for stem elongation in combination with the mixed basic light period (R + FR); in case of the red basic period, red night breaks were only active at the 8th and 10th hour point of the 14-hour dark period.

3. When *Hyoscyamus* plants were irradiated successively with 10 mins red and 10 mins far-red radiation at various points of the dark period, it seemed that far-red radiation reduced the elongative effect of the red night interruption in combination with the mixed (R + FR) basic period. This seems a partial manifestation of the antagonistic action of far-red against red with respect to the phytochrome system, however, the elongative action of red light observed was not much reduced (fig. 45). That some reduction of the red light effect was observed here, in contradiction to section 1 above, may be due to the fact that light periods were much shorter (viz., 10 mins v. 2 hrs).

In connection with the red light basic period, far-red radiation given after various times of darkness was inactive, and red interruptions at some points also. However, especially in the first half of the dark period, the succession of 10 mins red and 10 mins far-red led to flowering at all points of the dark period (fig. 45).

Thus, reversibility of red action by subsequent far-red radiation in general, viz., except in first part of this section was not found in our experiments.

Generally, the whole of our experiments with Hyoscyamus niger L. leads to the following conclusions:

1. *In general, applications of red and far-red reinforce each other, and the application of their mixture is particularly effective.*
2. *The observed promotive action of blue light throughout the photoperiod or during the night appears to fit well together with the above results.*
3. *The activity of Hyoscyamus plants to red and far-red is different throughout the night viz., greater to far-red in the first half and to red in the second half.*
4. Speculating about the internal mechanism by which the observed reactions can be provisionally understood, it seems that those mentioned under item 1 may be explained in terms of phytochrome reactions by assuming the require-

ment of intermediate P_{fr} -levels or specific ratios between P_{fr} and P_r for optimum induction.

The blue light effects (item 2) fit well in this suggestion as both forms of phytochrome have a definite absorption in the blue region.

The observation of the changes in sensitivity during the night (item 3) reveals the existence of some 'endogenous rhythm' which possibly might be understood as causing different changes in P_r - and P_{fr} -levels at various points of a long dark period, or bringing about changes in the plant's sensitivity towards phytochrome stimulation.

Direct evidence as to phytochrome reactions under the conditions of our experiments so far is not available but appears to form an extremely important object for future research.

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SAMENVATTING

Het hiervoor beschreven onderzoek betreft het schieten en de morfogenese van *Hyoscyamus niger* L. als reactie op straling in het zichtbare spectrum.

Er worden proeven beschreven, waarin *Hyoscyamus* planten werden blootgesteld aan licht van verschillende goed gedefinieerde spektrale gebieden. Het licht van deze spektrale gebieden werd in lage lichtintensiteit toegediend als aanvulling op een korte dag in verschillende kleuren, of in hoge lichtintensiteit als enige lichtbron. Er werd bijzondere aandacht besteed aan het onderzoek van de invloed van de belichting in deze spektrale gebieden, toegediend in het midden of op diverse andere tijdstippen van de 14-urige donkerperiode, in combinatie met korte dagen in verschillende kleuren. Bovendien werden de morfogenetische reacties veroorzaakt door opeenvolgende nachtonderbrekingen van verschillende spektrale gebieden gedurende de donkerperiode nagegaan. Bij *Hyoscyamus* zijn stengelstrekking en bloemknopaanleg gekoppeld, zodat gestrekte planten normaliter bloemknoppen hebben en rozetplanten niet.

A-MORFOGENETISCHE EFFEKTEN VAN GEKLEURD LICHT VAN EEN BREED SPEKTRAAAL GEBIED, BIJ HOGE INTENSITEIT TOEGEPAST GEDURENDE DE GEHELE HOOFDBELICHTING, OF, BIJ LAGE INTENSITEIT TOEGEPAST ALS AANVULLING OP KORTE BELICHTINGSTIJDEN BIJ DIVERSE KLEUREN

1. *Hyoscyamus* reageert als een typische langedag-plant. Onder wit kunstlicht dat de duur van de lichtperiode verlengde, werd het schieten verhaast, bij een kritische daglengte van bij benadering tussen 10 en 12 uur (fig. 3 en tabel 1). Evenwel had bij *Hyoscyamus* in korte dag stengelvorming plaats wanneer 30 of zelfs 10 minuten langgolvig-rode bestraling werd gegeven na korte basis belichtingsperiodes bij diverse lichtkleuren (fig. 10 en 13).

2. *Hyoscyamus* planten, opgekweekt in lange dagen met blauw licht, vormden stengels, terwijl planten in groene en rode lange dag in het rozetstadium bleven (tabel 2). Blauw licht werkte ook meer strekkend dan wit licht. Planten in blauw en wit licht hadden hoger spruit/wortel verhoudingen dan planten blootgesteld aan groen en aan rood licht (tabel 2). Voor een groot deel waren de verschillen in de spruit/wortel verhoudingen bij de diverse lichtkwaliteiten te wijten aan verschillen in wortelgroei, waar blauw en wit licht deze verminderden en de andere kleuren deze bevorderden. Bovendien vond als gevolg van blauwe belichting sterke strekking van de bladstelen plaats, terwijl deze strekking ontbrak bij toepassing van de andere golflengte gebieden en van wit licht.

3. *Hyoscyamus* reageerde op aanvullende belichting in de blauwe en langgolvig-rode gebieden met een opvallende strekking van de stengels (fig. 4) en de bladstelen (fig. 6). Dit resultaat wees op het bestaan van het hoge-energie mechanisme van de blauw-langgolvig rood reactie. In rood licht bleven de planten in het rozetstadium, doch met een aanvullende belichting met langgolvig-

rood werd de remmende invloed van rood opgeheven (fig. 4). Twee uur aanvullend blauw licht, na korte dagen in rood, bracht de planten tot doorschieten, doch duidelijk trager dan het geval was bij een aanvullende belichting met langgolvig-rood. Blauw licht heeft dus weliswaar dezelfde uitwerking als langgolvig-rood doch duidelijk minder effectief.

4. Na een korte dag in wit licht bleek een aanvullende belichting met rood licht meer effect te hebben dan een aanvullende belichting met langgolvig-rood (fig. 7, 18). Deze waarneming blijkt in tegenspraak met gegevens uit de literatuur volgens welke dagverlengingen met rood gewoonlijk geen effect hebben. Deze tegenspraak kan het gevolg zijn van de omstandigheid dat het voor de hoofdbelichting gebruikte witte licht relatief een belangrijke fraktie langgolvig-rode straling bevatte. Samen met de omstandigheid dat 6 uur rood in aansluiting op een korte dag in blauw (fig. 7) een lange dag effect oplevert, doet dit vermoeden dat voor de inductie van *Hyoscyamus* een intermediair P_r niveau vereist is, terwijl van hogere concentraties een remmende werking uitgaat.

5. Bij de gegeven proef omstandigheden werd tussen rood en langgolvig-rood geen antagonistische werking waargenomen (fig. 18, 19, 20). In het algemeen versterkten de beide kleuren elkaars werking, wanneer in aansluiting op een korte dag in wit licht langgolvig-rood volgde op een rode aanvullende belichting, of rood licht volgde op een aanvullende belichting met langgolvig-rood. Aan de andere kant werkte een dagverlenging met blauw licht gevolgd door rood licht als een lange dag, min of meer overeenkomend met een dagverlenging met uitsluitend rood licht (fig. 18, 19). In dit geval werkt blauw licht als donker en rood na blauw als een nachtonderbreking.

B-MORFOGENETISCHE EFFEKTEN VAN VERSCHILLENDE WIJZEN VAN NACHTONDERBREKINGEN, IN HET MIDDEN OF OP ANDERE TIJDSTIPPEN VAN DE DONKERPERIODE

1. De kleur van de hoofdbelichting is in verband met nachtonderbrekingen met rood licht van groot belang voor de fotoperiodieke en formatieve effecten. Korte fotoperioden rijk aan langgolvig-rode straling (fig. 22, 24, 25, 38), of een blauwe belichting (fig. 38) gekombineerd met rode nachtonderbrekingen, hebben een snel doorschieten tot gevolg. Bloei werd wat eerder bereikt wanneer voor de nachtonderbrekingen hogere lichtintensiteiten werden gebruikt. In combinatie met een rode hoofdbelichting, en een intensiteit van de rode nachtonderbrekingen van 3800 ergs/cm²/sec bleek 3 minuten licht reeds tot bloei te leiden, terwijl 120 minuten nachtonderbreking nodig waren indien de intensiteit van het nachtonderbrekingslicht 1000 ergs/cm²/sec was.

2. In combinatie met een witte hoofdbelichting rijk aan langgolvig-rode straling bleek een rode nachtonderbreking van 10 minuten (fig. 22, 24, 25) voldoende voor het verkrijgen van stengelstrekking en bloemknopvorming. Stengelstrekking geschiedde des te sneller naarmate de duur van de rode nachtonderbreking toenam. In combinatie echter met een hoofdbelichting in blauw of in een mengsel van rood en langgolvig-rood (fig. 38, 39) bleek dat de fotoperiodische reactie reeds met een nachtonderbreking van 3 minuten rood licht verza-

digd was. Om deze reden zijn er geen duidelijke verschillen in bloeireactie in de series met verschillende duur en intensiteit van de rode nachtonderbreking. In het algemeen geldt dat bij een nachtonderbreking met rood licht de planten met een hoofdbelichting bestaande uit een mengsel van rood en langgolvig-rood de langste stengels bezaten, die met een rode hoofdbelichting de kortste, terwijl in blauw de stengellengte tussenliggende waarden bereikte (fig.39). De resultaten voor een rode nachtonderbreking in combinatie met een korte hoofdbelichting waren voor blauw en mengsels rood + langgolvig-rood gelijk, hoewel met blauw de reactie trager verliep.

3. Nachtonderbrekingen met langgolvig-rood (fign 22, 24, 25, 40) werkten bij een belichtingsduur van tenminste 10 minuten in de meeste gevallen bloeibevorderend. Met langere belichtingstijden werd de bloeireactie weliswaar bevorderd, maar verliep toch steeds trager dan bij rode nachtonderbrekingen. Daarentegen werd in combinatie met een korte hoofdbelichting in rood een sneller doorschieten bereikt met nachtonderbrekingen in langgolvig-rood dan in rood. Er werden geen belangrijke verschillen gevonden bij gebruik van verschillende intensiteiten langgolvig-rood licht.

4. Waargenomen werd dat bij een toenemende duur van de nachtonderbreking stengelstrekking eerder optrad, als gevolg waarvan ook het aantal bladen een duidelijk verband vertoont met de duur van de nachtonderbreking. (fign 22, 23).

5. Geen duidelijke verschillen in bladsteel lengte werden gevonden ten opzichte van de controles, wanneer verschillende tijden van een rode nachtonderbreking werden gegeven in combinatie met een basis belichting in rood of in een mengsel van rood en langgolvig-rood. Het enige verschil was dat de bladstelen langer waren in het geval van de basisbelichting in rood + langgolvig-rood.

Bij een nachtonderbreking met langgolvig-rood daarentegen, waren de bladstelen aanmerkelijk langer dan de controles. Dat gold voor een basis belichting in rood, maar nog in sterkere mate voor een basis belichting in rood + langgolvig-rood. Met een hoofdbelichting in blauw bleek dat zowel met een onderbreking met rood als met langgolvig-rood een sterke verkorting van de bladsteellengte optrad.

6. Een onderbreking met rood en langgolvig-rood licht gegeven op verschillende tijdstippen van de lange donkerperiode, gaf een tegengestelde fotoperiodische reactie te zien (fign 27, 28, 30, 33, 43, 44, tabel 3). Rood licht bleek het meest werkzaam in de tweede helft van de nacht, langgolvig-rood in de eerste helft. Gekombineerd vertoonden ze het beeld van een rode onderbreking alleen, doch versterkt (tabel 3).

7. Lange onderbrekingen met een hoge intensiteit blauw ($3800 \text{ ergs/cm}^2/\text{sec}$) op verschillende tijdstippen van de nacht, bevorderden evenals rood de stengelstrekking (fig. 43).

De resultaten sluiten echter de mogelijkheid niet uit dat gedurende de eerste helft van de donkerperiode de werking van blauw meer gelijk is aan die van langgolvig-rood.

Met korte perioden blauw licht (10 minuten) van hoge intensiteit (fig. 45) of

lange perioden (2 uur) met een lage intensiteit ($1000 \text{ ergs/cm}^2/\text{sec}$) kon geen doorschieten geïnduceerd worden (fig. 27).

8. Aangetoond werd dat in combinatie met basisbelichtingen in menglicht, rode nachtonderbrekingen op ieder moment van de donkerperiode een snelle 'schiet' reactie opleverden. Ook wanneer ze gegeven worden direct na of voor de hoofdbelichting (fign 43, 44, 45). In tegenstelling hiermee moet bij een basisbelichting in rood licht van de 14 uur donker minstens 6 uur aan de nachtonderbreking voorafgaan of twee uur hierop volgen.

Het bovenstaande blijkt in overeenstemming met de gegevens uit fig. 27, waar voor een korte basisbelichting twee verschillende bronnen van wit licht gebruikt zijn. De ene meer langgolvig-rode straling bevattend (TL/IL) dan de ander (TL). In het laatste geval bleek twee uur rood licht direct voor en na de hoofdbelichting geen effect te hebben. Alleen rood licht op tussenliggende momenten van de donkerperiode bleek wat betreft stengelstrekking en bloemknopvorming werkzaam te zijn. Daarentegen was in combinatie met wit licht dat langgolvig-rood bevatte (TL/IL) rood licht gedurende elk moment van de donkerperiode werkzaam, ook direct na of voor de hoofdbelichting. Aanvullende proeven waarbij direct vóór de hoofdbelichting donkerperiodes van verschillende duur werden gegeven toonden aan dat wanneer een hoofdbelichting gebruikt wordt die rijk is aan rood licht (TL, fig. 35) minstens 30 minuten donker de rode nachtonderbreking van de opvolgende hoofdbelichting dienen te scheiden voor het verkrijgen van bloei bij *Hyoscyamus*.

C-FOTOPERIODISCHE REAKTIES ALS GEVOLG VAN ACHTEREENVOLGENDE ONDERBREKINGEN IN DE DONKERPERIODE

1. Er zijn geen aanwijzingen dat nachtonderbrekingen met rood en langgolvig-rood in combinatie met wit kunstlicht antagonistisch werken (fign 30, 31, 34, tabel 3). Langgolvig-rood na een nachtonderbreking met rood vergroot de werking van het rode licht, in het bijzonder de bloeibevorderende invloed van rood licht tijdens de eerste helft van de donkerperiode. Een gelijktijdige toediening van rood en langgolvig-rood tijdens nachtonderbrekingen blijkt de stengelstrekking echter meer te bevorderen dan langgolvig-rood in aansluiting op een rode nachtonderbreking. Een verklaring hiervoor lijkt te zijn de omstandigheid dat zowel rood als langgolvig-rood gedurende een lange tijd (2 uur) gegeven werden. Zodat het langgolvig-rood na de rode nachtonderbreking de bloeibevorderende werking van het rode licht niet meer te niet kon doen.

2. Onderbrekingen met 10 minuten langgolvig-rood licht gedurende verschillende momenten van de nacht, hadden noch bij een basisbelichting met als zonder langgolvig-rood een effect (fig. 45) Met een rode basisbelichting bleken rode nachtonderbrekingen alleen effectief indien gegeven tijdens het achtste en negende uur van de veertienurige donkerperiode.

3. Wanneer *Hyoscyamus* planten op verschillende momenten van de donkerperiode achtereenvolgens 10 minuten rood en 10 minuten langgolvig-rood ontvingen, dan leek in het geval van een gemengde basisbelichting (rood + lang-

golvig-rood) langgolvig-rood de strekkende invloed van de rode belichting te reduceren. Dit zou een aanwijzing kunnen zijn voor een gedeeltelijk antagonistische werking van rood en langgolvig-rood licht via het fytochroom systeem. Dit schijnt in tegenspraak met wat eerder onder 1 werd opgemerkt (fig. 45,) hetgeen wellicht verklaard wordt door de veel kortere belichtingstijden (10 minuten in plaats van 2 uur) in het laatste geval. Samen met een rode basisbelichting bleek langgolvig-rood op verschillende momenten van de donkerperiodes niet werkzaam, evenmin als onderbrekingen met rood licht op bepaalde momenten. Daarentegen leidde een opeenvolging van 10 minuten rood en 10 minuten langgolvig-rood op alle momenten van de donkerperiode tot bloei, in het bijzonder duidelijk gedurende de eerste helft van de donkerperiode (fig. 45).

Aldus werd uitgezonderd in het eerste gedeelte van dit onderdeel in het algemeen geen reversibiliteit van de werking van rood door opvolgend langgolvig-rood waargenomen.

In hun geheel leiden onze experimenten met Hyoscyamus tot de volgende algemene conclusies.

1. *In het algemeen versterken rood en langgolvig-rood licht elkaars werking; een gelijktijdige toediening is in het bijzonder effectief.*

2. *De waargenomen bevorderende werking van blauw licht gedurende de fotoperiode of tijdens de nacht, blijkt zeer wel hiermee overeen te komen.*

3. *De reactie van Hyoscyamus planten op rood en langgolvig rood-licht is gerekend over de duur van de nacht verschillend, met een grote gevoeligheid voor langgolvig-rood tijdens de eerste helft, en voor rood tijdens de tweede helft van de nacht.*

4. Bij een overweging van het mechanisme dat aan de waargenomen verschijnselen ten grondslag zou kunnen liggen, lijkt het onder punt 1 vermelde, verklaard te kunnen worden in het kader van het fytochroom systeem, er vanuit gaande dat voor een optimale bloeireactie intermediaire P_{fr} niveaus of specifieke verhoudingen aan P_{fr} en P_r vereist worden.

Wanneer we aannemen dat beide vormen van het fytochroom in het blauw absorberen dan passen ook de effecten van blauw licht (punt 2) in dit beeld.

De waarneming dat de gevoeligheid tijdens de nacht verandert (punt 3) duidt op het bestaan van een of ander 'endogeen ritme', dat de oorzaak kan zijn voor het ontstaan van veranderingen in de P_r en P_{fr} niveaus tijdens de lange donkerperiode, of veranderingen in de fysiologische gevoeligheid t.o.v. het fytochroom systeem kan bewerkstelligen.

Directe aanwijzingen dat fytochroom reacties bij onze proef omstandigheden betrokken zijn, ontbreken voorsnog. Dit lijkt ons een zeer belangrijk onderwerp voor verder onderzoek.

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