AN ATTEMPT TO ANALYSIS OF THE EFFECT OF LIGHT ON STEM ELONGATION AND FLOWERING IN *HYOSCYAMUS NIGER* L.

P. J. A. L. DE LINT

AN ATTEMPT TO ANALYSIS OF THE EFFECT OF LIGHT ON STEM ELONGATION AND FLOWERING IN *HYOSCYAMUS NIGER* L.

(MET EEN SAMENVATTING IN HET NEDERLANDS)

Dit proefschrift met stellingen van

PIETER JOHANNES ADRIANUS LEENDERT DE LINT,

landbouwkundig ingenieur, geboren te Willemstad, 20 juli 1926, is goedgekeurd door de promotor, Dr. E. C. WASSINK, hoogleraar in het Plantenfysiologisch onderzoek en de Fysiologie der planten.

De Rector der Landbouwhogeschool,

W. F. EIJSVOOGEL

Wageningen, 14 november 1960

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(MET EEN SAMENVATTING IN HET NEDERLANDS)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS IR. W. F. EIJSVOOGEL,
HOOGLERAAR IN DE HYDRAULICA, DE BEVLOEIING,
DE WEG- EN WATERBOUWKUNDE EN DE BOSBOUWARCHITECTUUR,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP WOENSDAG 7 DECEMBER 1960 TE 16 UUR

DOOR

P. J. A. L. DE LINT

H. VEENMAN EN ZONEN N.V. – WAGENINGEN – 1960

Aan mijn Ouders

Aan mijn Vrouw

"... all this tumult of miracle,
and our perceptive participance in it,
is not ours by any *right*. It is a gift".

ALAN DEVOE

STELLINGEN

I

Daglengte-gevoelige planten kunnen geacht worden preferent te bloeien in continu donker indien ze van voldoende suiker worden voorzien.

Dit proefschrift.

II

Een nachtonderbreking dient gezien te worden als de fotoperiode van een cyclus welke duurt van het begin der onderbreking tot aan het einde der donkerperiode, zodat de donkerperiode van de oorspronkelijke cyclus verkort wordt met deze nieuwe cyclus.

Dit proefschrift.

III

De fotoperiodische werking van licht berust waarschijnlijk niet op een verminderde gevoeligheid van de plant voor de lichtinvloed tijdens de belichting, maar op het feit dat deze invloed voornamelijk eerst in het donker effect krijgt.

A. KADMAN ZAHAVI, Bull. Res. Counc. Israel **9D**, 1960: 1–20. Dit proefschrift.

IV

De hypothesen, dat bloei optreedt o.i.v. een bloeihormoon en dat bloei geremd wordt in ongunstige daglengten, zijn goed te verenigen.

D. VON DENFFER, Naturw. **37**, 1950: 296–301; 317–321.
A. LANG, Ann. Rev. Pl. Physiol. **3**, 1952: p. 269.

V

In een intensiteitenreeks is de relatie tussen de strekking van een stengel en elk zijner afzonderlijke internodiën in overeenstemming met de theorie dat de fysiologische activiteit door licht verhoogd wordt.

B. F. THOMSON, Amer. J. Botany **46**, 1959: 740–742.

VI

De waarneming van een „negatieve temperatuur coëfficient" bij een fysiologisch verschijnsel duidt erop, dat dit verschijnsel op een samenspel van verschillende fysiologische processen berust.

F. W. WENT, Experimental control of plant growth, 1957, pp. 343.
S. D. RICHARDSON, Proc. Kon. Ned. Akad. Wet. Amsterdam **C 59**, 1956: 428–438.

Dissertatie P. J. A. L. de Lint,
Wageningen, december 1960.

VII

Veel meer de duur der dagelijkse belichting dan de lichtintensiteit lijkt van belang voor de productie van landbouwhuisdieren, wat bij beoordeling van het stalklimaat tot uitdrukking dient te komen.

Photoperiodism and related phenomena in plants and animals (R. B. WITHROW, Ed.), 1959.

VIII

Bloeivervroeging bij fruitbomen na uitbuigen berust op veranderingen in de groeistofhuishouding door de werking van de zwaartekracht.

J. C. VENDRIG, Proefschrift, Utrecht 1959.

IX

De „steady state uptake" is een van de „initial phase uptake" onafhankelijk proces.

G. G. LATIES, Ann. Rev. Pl. Physiol. **10**, 1959: 87–112.

X

De beschrijving van de bodemvruchtbaarheid op basis van uitwisselingseigenschappen van de grond is onvoldoende voor veldomstandigheden.

A. C. SCHUFFELEN en G. H. BOLT, Vortrag 2. und 4. Kommission der IBG, Hamburg, 1958: 131–146.

XI

Het is onjuist te veronderstellen, zoals in de Memorie van Toelichting op de Rijksbegroting 1961 geschiedt, dat de hoeveelheid kosten een goede maatstaf vormt voor de prestatie van de bedrijfsleider.

Rijksbegroting 1961, Hoofdstuk XI; M.v.T. 2:18.

XII

Het honoreren van de beloning voor de bedrijfsleidersarbeid in de garantieprijzen van landbouwproducten in de vorm van een procentuele opslag van enige kosten-posten zal intensivering aanmoedigen.

Rijksbegroting 1961, Hoofdstuk XI; M.v.T. 2:18.

XIII

Het is noodzakelijk, in de studie aan de Landbouwhogeschool, meer gelegenheid te scheppen voor een vruchtbare verdieping in de eenheid der wetenschappen.

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De inhoud van dit proefschrift verschijnt tevens in de Mededelingen der Landbouwhogeschool te Wageningen/Nederland **60** (14) 1960.

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(met een samenvatting in het Nederlands)

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CHAPTER I

INTRODUCTION

I.1. PHOTOPERIODISM

The reaction of plants to the length of the photoperiod (83) is called: photoperiodism (26). Plants may grow vegetatively for a long time under definite light-dark regimes. A variety of reaction types with regard to the photoperiodic response was demonstrated to exist. Moreover, the response can be qualitative or quantitative (11, 43, 100), and there are plants that are totally insensitive to any known light-dark combination (25, 29, 35, 68). This diversity of reactions renders it difficult to fit them into a general scheme.

The present paper is concerned with observations on flowering and stem elongation in relation to photoperiod in an annual strain of *Hyoscyamus niger*, known as a long-day plant.

The author feels that the steadily growing pile of results on flowering in continuous darkness suggests that vegetative growth is the light-regime produced feature, while darkness generally favours flowering (27). Flowering in continuous darkness has since long been observed, also in *Hyoscyamus niger* (44). In a series of other plants, flowering in darkness has been observed since (2, 16, 65, 79), mostly with embryo cultures on sugar media. *Kalanchoë blossfeldiana* also flowers in darkness if kept at temperatures below 15°C (65), contrary to what was reported earlier (32).

If it is true that plants in darkness flower either earlier than or as early as those in light, it appears not very appropriate to use the term "photoinduction" to characterize the reaction under light regimes allowing flowering. When light is promotive, it probably will be so *via* non-specific effects, *e.g.*, photosynthesis. It will be assumed that the plant autonomously generates the "flowering state", *i.e.*, the internal condition which is a prerequisite for flowering. Light, or light in combination with darkness, may inhibit the production of the floral stimulus *(Xanthium)*, or the capacity to produce it *(Perilla)* (52, 102). Plants under non-inhibitive conditions are gradually induced to flower (8, 30, 73). Such induction, sufficient for flower bud initiation, may require a few hours up to several weeks.

BEST (2), on the basis of extensive observations in rice, has presented a general view on the daylength dependence of inhibition of flowering in short-day and long-day plants (Fig. 1).

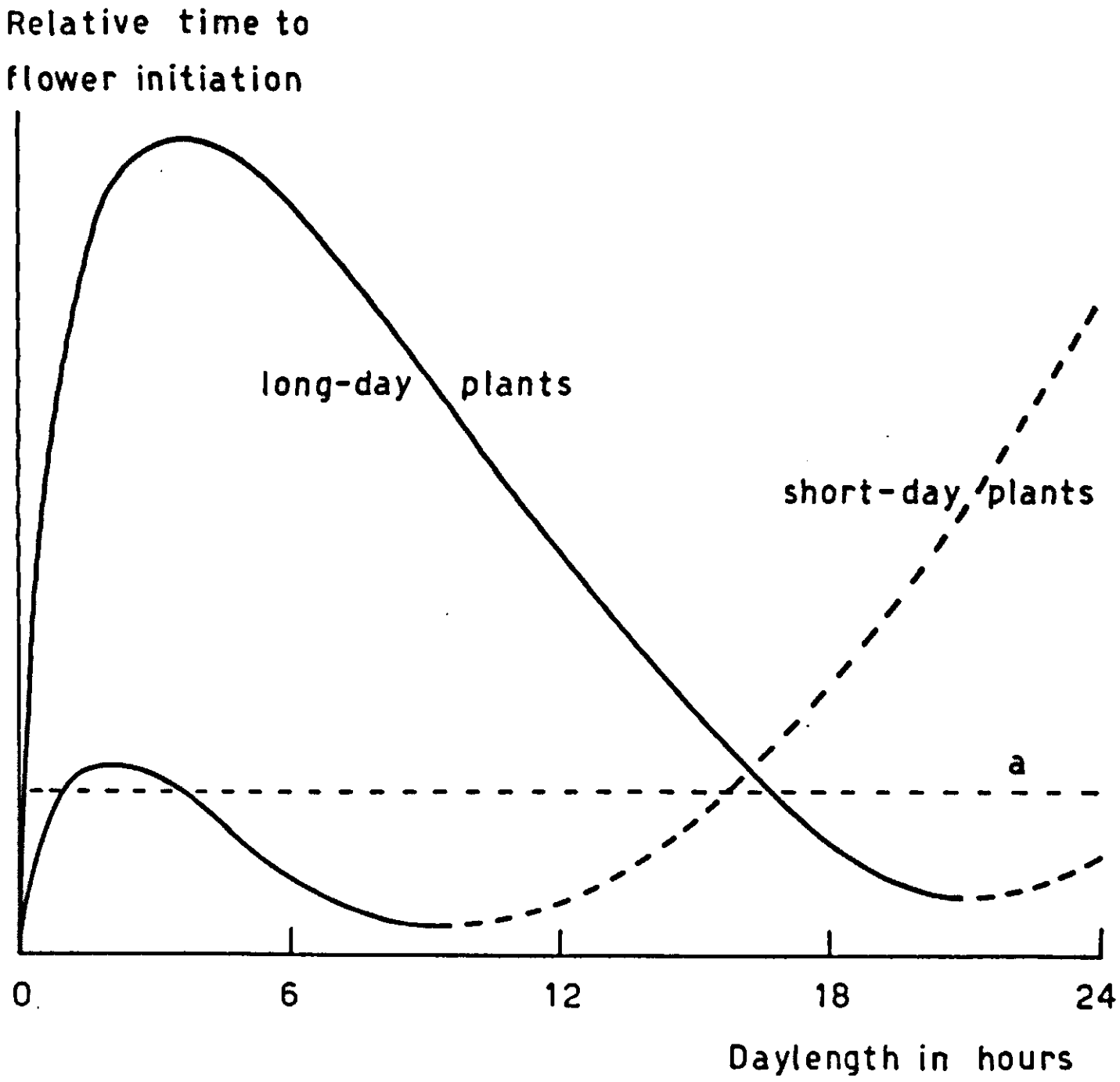

FIG. 1. Schematic daylength-dependence curves of flowering in long-day and short-day plants, as proposed by BEST (2). (Line *a* added, see text).

The line representing inhibition of flowering for long-day plants has a maximum at a photoperiod of about 4 hours, and minima at 0 and at about 20 hours; whether for periods over 20 hours some inhibition comes in again is uncertain as yet. The curve for short-day plants reaches a minimum after about 8 hours (instead of 20), and rises to high values at longer daylengths. In principle, these curves are similar, differing only in quantitative aspects.

The critical daylengths between vegetative growth and flowering are characterized by a level *a* (Fig. 1) the position of which depends upon experimental conditions and duration of treatment. It will shift, *e.g.*, to a relatively higher position if the treatment is continued for more days, or if the plants are in a condition of lower sensitivity to the light inhibition. Daylengths at which the reaction-curves rise above *a* result in vegetative growth.

The curve for long-day plants (Fig. 1) becomes understandable if we assume either that an inhibitor-precursor, generated during illumination, is transformed into an inhibitor only during darkness or that the inhibitive action of light in some way or other is compensated during the light period, *e.g.*, because the sensitivity of the plant to the action of the inhibitor is reduced as a result of illumination; the latter is suggested by KADMAN-ZAHAVI (39).

It will be evident that such an assumption leads to a plausible description of

the curve for long-day plants. In these plants, neither darkness nor long daily irradiation, but only alternation of light with dark periods of appreciable duration are inhibitive. The inhibition rises to a maximum in the region in which the amount of light is the limiting factor (from 0 to about 4 hours). We will assume that, in the maximum position (4 hours of light), there is just enough inhibitor-precursor to be active during the following 20 hours of darkness (with shorter days, the last dark hours will not be inhibitive, because the small amount of inhibitor-precursor has disappeared before then). In the region between 4 and 20 hours of light the dark-period becomes increasingly limiting for the inhibition; the light produces a sufficient amount of inhibitor-precursor which, however, cannot function at full capacity because the following light-treatment starts before the inhibition has become completely effective. In connection herewith, it may be remarked that experiments with near-infrared supplementary irradiation also allow the conclusion that light influences mainly become effective in the dark period following the irradiation (Section I.2.).

It is obvious that the reaction of short-day plants upto light-periods of 10 hours can be explained in the way as is developed above for the curve of long-day plants. However, with light periods over 10 hours, a third factor seems to become increasingly inhibitive for flowering. Experiments with *Hyoscyamus*, to be discussed in chapter V, are suggestive of the possibility that something similar can be produced also in the case of long-day plants by adequate combination of specific wavelength regions.

The curves constructed by BEST (2, *cf.* Fig. 1) do not consider a difference in principle between quantitative and qualitative dependence on photoperiod. Effects of treatments including specific narrow spectral regions are in favour of this view. DE LINT (47) has described an experiment in which a short-day treatment including 2 hours of low intensity near-infrared produced shooting in *Hyoscyamus niger* (a qualitative long-day plant), while WASSINK, STOLWIJK and BEEMSTER (95) reported an equal result, years ago, for *Brassica rapa* (a quantitative long-day plant). This indicates that no fixed critical daylength exists and that this is not less valid for plants denoted as "qualitatively sensitive" than for those denoted as only "quantitatively sensitive".

It may also be remarked that, in general, inhibition processes hardly reach the 100% level. This may explain why older plants can become less sensitive to an inhibitive treatment or, ultimately, even come to flower in normally inhibitive cycles (31). This is of importance for experiments on "summation" of induction (9), because the effect of favourable cycles is modified by intercalated inhibitive cycles: The latter cycles add some further induction at a rate depending on their position on the BEST-curve (see Fig. 1).

Before entering into a more detailed discussion of these effects (*cf.* I.3.), we will consider some coloured light results.

I.2. FORMATIVE INHIBITION OF ELONGATION WITH SPECIAL REFERENCE TO SPECTRAL COMPOSITION

A series of extensive reviews on coloured light effects have been published recently (50, 56, 76, 94). It, therefore, seems sufficient to mention only the main hypotheses and data as far as important for our discussion.

Light inhibits elongation, as was recognized already by RAY (70), and confirmed by BONNET (4). Recently, THOMSON (81, 82) has presented evidence for

the view that light inhibition results from an acceleration of the elongation rate and a shortening of the growth period, as was defended long ago by SIERP (74). The combination of the two changes is such as to result in decreased length. According to this view, the general principle for the light action upon growth appears to be that each cell physiologically is at a higher level of activity: it grows more quickly, but ages in a shorter time.

The inhibition percentage of the length of an organ, or part of it, is a linear function of the logarithm of light energy and also of light intensity and of duration of illumination (46, 67, 97). The specific activities of narrow wavelength regions can be calculated from the relative positions of the parallel straight lines obtained in inhibition/log. energy graphs (67). In fact, the curves are not strictly linear, but S-shaped. Thus, plants grown in darkness are the longest obtainable, unless elongation is terminated for other reasons.

The action spectrum for the light function, as generally obtained, has a high maximum in the red region with a sharp drop to near-infrared and a more gradual decrease over orange and yellow to green (24, 66, 101). In the blue region a second weak maximum has been demonstrated, the nature of which is still under investigation (*e.g.*, 19, 20, 22, 58, 72, 88).

In addition, the near-infrared region has the peculiar effect of annihilating the inhibition of a preceding irradiation. This "red–near-infrared antagonistic system" (21, 37, 41) can only function if the inhibitive action of an irradiation is realized mainly afterwards, in the following dark period. Blue light can produce a similar effect as near-infrared, only much weaker (Chapter III, p. 12).

With higher energy irradiations, however, some of the results reported are not in accordance with the spectral sensitivity discussed above (28, 36, 56, 59). These results suggest a spectrum for lower energies and another one for more intense irradiation.

In experiments of prolonged duration at high light intensities, plants in blue light are often shorter than those in red light (*cf.* 64 and 94). It is tempting to suppose this to be connected with differences in the maximum inhibition levels reached by the log-lines in the different colours. Such level differences could be caused by an interaction between cell division and cell elongation. An alternative assumption might be that, in prolonged experiments, the concentrations of the pigments responsible for the photomorphogenetic reactions become unequal in different wavelength regions. The plants in red light could become less sensitive to irradiation after some time. May be, a first demonstration of this was given by BLAAUW-JANSEN in her detection of a "red light factor" (3). Differences in plant species sometimes can be referred to as differences in sensitivity to light, which might be based on pigment concentration (56).

I.3. PHOTOPERIODIC REACTION IN *HYOSCYAMUS NIGER*

I.3.1. *General*

In white light, the annual strain of *Hyoscyamus niger* flowers in days with more than 12 hours of light, and is vegetative (rosette growth) in shorter days. However, in continuous darkness flowering is also possible, provided the plants are defoliated (44). About six non-inhibitive days are sufficient to enable the plants to come into flower in subsequent inhibitive days. The necessary number of days can be given in fractions (9). However, some extra days must be added, probably due to back reactions, or disappearance of older leaves. This suggests

that a stable transportable stimulus is accumulated in favourable days so that its production in part of a plant is sufficient for flowering in the entire plant.

The night length appears of primary importance for the inhibitive function of short days, since short light periods in combination with short dark periods do not retard flower initiation (51). At higher night temperatures, shorter nights are sufficient to obtain inhibition of flowering (44). However, dark periods can also be too long, since the critical day is longer in 24-hour cycles than in 48-hour ones (*cf.* 43).

Flowering of defoliated plants occurs irrespective of daylength; induction is mainly independent of irradiation of the growing points, stems and roots. The leaves mainly convey the inhibitive impulse into the other mentioned organs (6).

The above facts are in agreement with the suggestions given in section I.1. (p. 2), *viz.*, that the autonomous production of the "flowering state" is inhibited in dark hours adjacent to light, due to the fact that an inhibitor-precursor is produced by light but rendered active only in subsequent darkness. It is not our aim to qualify this working hypothesis as the sole possibility but we will demonstrate that it opens a way for understanding a large variety of experimental results. SCHWABE (73) has presented a scheme of the daylength dependence in short-day plants which seems quite similar. Both are a combination of floral stimulus and floral inhibition hypotheses.

I.3.2. *Coloured light effects*

In *Hyoscyamus*, stem elongation and flower bud initiation always appear together. Nevertheless, it seems justified to visualize both phenomena as due to virtually independent mechanisms, because in other plants stem elongation and photoperiodic induction of flower bud initiation were demonstrated to be actually manifest as independent reactions. A few cases in which this seems apparent, may be quoted. In lettuce, elongation of the vegetative stem could be induced in several ways, independent of flower bud initiation (1, 95). STROUN (78) presented evidence to distinguish a "photostade" preferably connected with floral induction, and a "spectrostade" affecting elongation. A similar demand for two reactions, *viz.*, one mediated preferably by red light, another by near-infrared, had already been put forward by STOLWIJK and ZEEVAART (77), and later by MEYER (56), for *Hyoscyamus*. In several cases, gibberellic acid promotes stem elongation without simultaneous induction of flowering (13, 80, 99). This type of evidence leads TSCHAILACHJAN (80) to postulate a requirement for "anthesin" for floral induction, and for "gibberellin" for stem elongation. CURRY and WASSINK (15) observed that, in *Hyoscyamus*, administering suitable doses of gibberellin tended to annihilate spectral differences in stem elongation (coupled with flower bud initiation), which may be initiated by a gibberellin effect as postulated by TSCHAILACHJAN.

For these various reasons, it seems justified to suggest that certain light regimes preferably affect a "periodic" reaction of the plant, primarily definable as "daylength effect" and primarily connected with floral induction, while others preferably affect stem elongation by way of a "formative" reaction, primarily definable as "wavelength effect".

The coupling of the two reactions in shooting of *Hyoscyamus*, evidently, is such that floral induction renders a plant less sensitive for formative stem inhibition, and contrary, that a strong elongation impuls renders it less sensitive for the inhibition of floral induction. This can be understood with the additional

assumption that floral induction not only is an impuls for flower bud initiation but to some extent also for stem elongation. Or in other words that flower bud initiation is a "periodic" function only, whereas elongation depends on the "periodic" and the "formative" reaction.

In the following chapters, we will encounter an additional number of observations supporting the justification of the distinction made (p. 17 and p. 31), also in *Hyoscyamus*.

Available evidence indicates that the light absorbing pigment system for both types of reactions as a rule is the "red – near-infrared system", acting antagonistically in both reactions, in a number of situations.

Hyoscyamus flowers in short days upon a night interruption; red light is highly active which action can be antagonized by near-infrared (17, 77). The necessary nightbreak energy depends upon the length of the basic day and on the spectral composition of the basic daylight (56, 77). Red night interruptions produce stem elongation if combined with short days in the blue region, or with short days in green with a near-infrared admixture, but not in combination with red or green short days (56). These facts, together with the earlier observation of STOLWIJK and ZEEVAART (77) that blue, violet, and a red – near-infrared mixture applied as long-day irradiation lead to rapid stem elongation, whereas red and green do not or only slowly, suggest that red night interruptions are effective only in weakly inhibited plants. (See also 15).

Results from experiments with supplementary irradiation seem to deviate from night-break results since the red – near-infrared antagonism is either absent (10, 18, 69, 77) or effective in opposite direction (61).

Nevertheless, it seems evident that these effects must have basic reactions in common. This seems compatible with the above suggested distinction of formative and periodic reactions which together determine the degree of inhibition. Day extension with red light, *e.g.*, is favourable for shooting because it promotes the floral induction reaction. Supplementary near-infrared, however, is promotive for shooting by reducing the inhibitive effect of the basic day with respect to the formative reaction. Any additional irradiation favourable for shooting does so by reducing the effect of an inhibitive impulse, as the sum of its effects on the formative and the periodicity reaction chains.

The observation of STOLWIJK and ZEEVAART that plants in red light form stems if, each day, 9 hours of blue light are intercalated appears elucidative in this respect. It has to be mentioned, however, that in this case the blue light contained some near-infrared admixture which, according to additional evidence, was mainly responsible for the effect (*cf.* Chapter III, p. 12).

MEYER (56) made extensive experiments on the difference in the results between night-break and supplementary irradiation. His publication contains results on *Salvia*, but as far as comparable data are available, these results hold for *Hyoscyamus* as well. Below, his results are summarized from his colour plates 1 and 2.

The first important fact demonstrated by MEYER was that the red – near-infrared antagonism occurs with supplementary irradiation; it is, however, the reverse from the normal. In a long day in green light the plants are vegetative, with a short near-infrared supplement they flower, and a red supplement following as a third treatment again keeps them vegetative; near-infrared could be replaced by blue (*cf.* 56, Plate 1c). Such facts have erroneously led to the assumption that the reaction of *Hyoscyamus* and cruciferous plants, in general,

is opposite to that of other plants. Red supplementary light produces the long-day effect only with a green + near-infrared short day (56, Plate 1d).

MEYER further supplemented a 10-hour day in blue light (B) by red light extensions of different duration (r) which, however, all ended 6 hours before the end of the night. The shortest of these irradiations with red light was $\frac{1}{2}$ hour (10h. B + $7\frac{1}{2}$h. D + $\frac{1}{2}$h. r + 6h. D), the longest one 8 hours, filling up the first part of the night (10h. B + 8h. r + 6h. D); other periods were 4, 2, or 1 hours of red light, respectively, (10h. B + 4h. D + 4h. r + 6h. D), (10h. B + 6h. D + 2h. r + 6h. D) and (10h. B + 7h. D + 1h. r + 6h. D). All treatments result in a long-day effect, except the 8-hours supplement and the dark control (10h. B + 14h. D) (*cf.* 56, Plate 2e). MEYER concludes that the first hours following the blue are essential for the blue effect; red light supplied during these hours will antagonize the blue function. This conclusion seems to be premature, since two controls are lacking, *viz.*, (10h. B + 4h. D + 8h. r + 2h. D) and (10h. B + 4h. r + 10h. D); as long as the results of these two are unknown, other explanations seem to be possible.

Red night breaks (15 minutes high intensity) only effectively elongated a short day if the short day is either in blue, or in one of the other colours if mixed with near-infrared (*cf.* 56, Plate 2g). The red – near-infrared antagonism was demonstrated in such experiments (*cf.* 56, Plate 2h).

A last series (*cf.* 56, Plate 2i) dealt with the question, why in experiments with a basic irradiation in blue light the red is active as night interruption (series h), while with a day in red light the near-infrared is active as night interruption (series i). As soon as near-infrared followed a red light period, the night-break action was "normal" again, *i.e.*, red produced the long-day effect and near-infrared antagonized this (56). However, DE LINT demonstrated (48) this reaction to be still more complicated, at least in *Hyoscyamus*. The effect of a near-infrared supplement or admixture to a day in red light leads to the question of exactly at which moment near-infrared should be given. According to KÖNITZ (42) on *Chenopodium*, this seems best about the middle of the light period. MEYER obtained a somewhat more complicated result with a basic green light period (*cf.* 56, Plate 1b).

More detailed information about literature data will be presented at the beginning of each experimental chapter, as a further introduction to specific problems. Moreover, additional results will be presented.

In the following pages it will become evident that most of the available data on stem elongation and flowering of *Hyoscyamus* are understandable accepting the validity of BEST's suggestions, and taking into account the properties of the red – near-infrared antagonistic system.

CHAPTER II

TECHNIQUE

II.1. GENERAL

The plants were grown, and the experiments were performed either in a greenhouse under natural daylight, or in darkened basement rooms under artificial illumination. If necessary, the natural day was shortened by covering the plants with board panels from 17 to 7h. Artificial light was supplied in

well-defined spectral composition, obtained from special lamp types, mostly in combination with filters of specific transmission. The filters were glass, plexiglass, copper sulphate solution, and water. When two or more wavelength regions were compared in their effects on plant growth, they usually were given in equal intensities on energy basis. When light was given from above only, the growing plants were lowered or the lamps lifted so that the region around $^1/_3$ of total stem length from the top of the plants was kept at the same distance from the lamps throughout an experiment. An irradiation was ended either by automatically switching off the lights, or by moving the plants into darkness or into another light treatment. Transport was in darkness, over short distances only.

Air humidity was not under control; it mostly was around 65%. The temperature was about 20°C, except on hot summer days (which usually are scarce in The Netherlands). Equal night and day temperatures were aimed at. The irradiation equipments are mainly those described earlier (91, 93) and also used by STOLWIJK (76). Because a few improvements have been made, a brief characteristic of the set-up will be given again, with a short description of the changes (II.3.1.).

II.2. *HYOSCYAMUS NIGER*

The plant species used throughout this investigation is *Hyoscyamus niger*, annual, yellow flowering strain $\frac{(ann\ pall)}{(ann\ pall)}$. Seeds were originally obtained from Dr. A. LANG, Los Angeles (77). It is known as a qualitative long-day plant. The "critical daylength" is between 13 and 14 hours at 20°C for white light (PHILIPS *TL55*, "daylight"). The same species was used in all earlier work on *Hyoscyamus* from this laboratory (15, 47, 48, 77, 90, 96).

Plants were grown in short days, either in the greenhouse under 10-hour days, or indoors under 8 or 10 hours of white fluorescent light per day at high intensity (*ca.* 25,000 ergs/cm² sec) until the plants were ready for the experiments.

II.3. IRRADIATION

II.3.1. *Equipment*

White light is supplied by sets of eight 40 Watt, PHILIPS *TL55*, "daylight" fluorescent lamps mounted in horizontal panels of 120 × 70 cm. Each panel with the plants underneath is enclosed by some layers of black cloth, to form separate units. The panels hang on chains to be lifted for intensity corrections. The light intensity obtained is between 20,000 and 30,000 ergs/cm² sec on plant level. Five cm below the lamps a sheet of colourless glass is mounted to secure more equal ventilation over the plants during day and night hours.

Irradiation in narrow wavelength bands is given in separate cabinets for each colour. There is a series for low intensity irradiation (intensities up to 6000 ergs/cm² sec), each cabinet of which has four special, "monophosphor" fluorescent lamps on top (except the near-infrared cabinet which has six 100 Watt incandescent lamps), and one for high intensity irradiation, the cabinets of which have monophosphor fluorescent lamps on top and along the two long side walls (yielding intensities up to 12,000 ergs/cm² sec, or about 50,000 ergs/sec cm² ⌀ sphere; *cf.*, *e.g.*, 76). The inner dimensions of the low intensity cabinets

are: 60 cm wide, 120 cm long, 100 cm high; of the high intensity ones they are: 35, 110, 85.

Plants in the high intensity cabinets are placed at the bottom, whereas in the others they can be placed at various positions of height. There are doors on both short sides of the cabinets. Diagrams of a cabinet of each type are presented in figure 2.

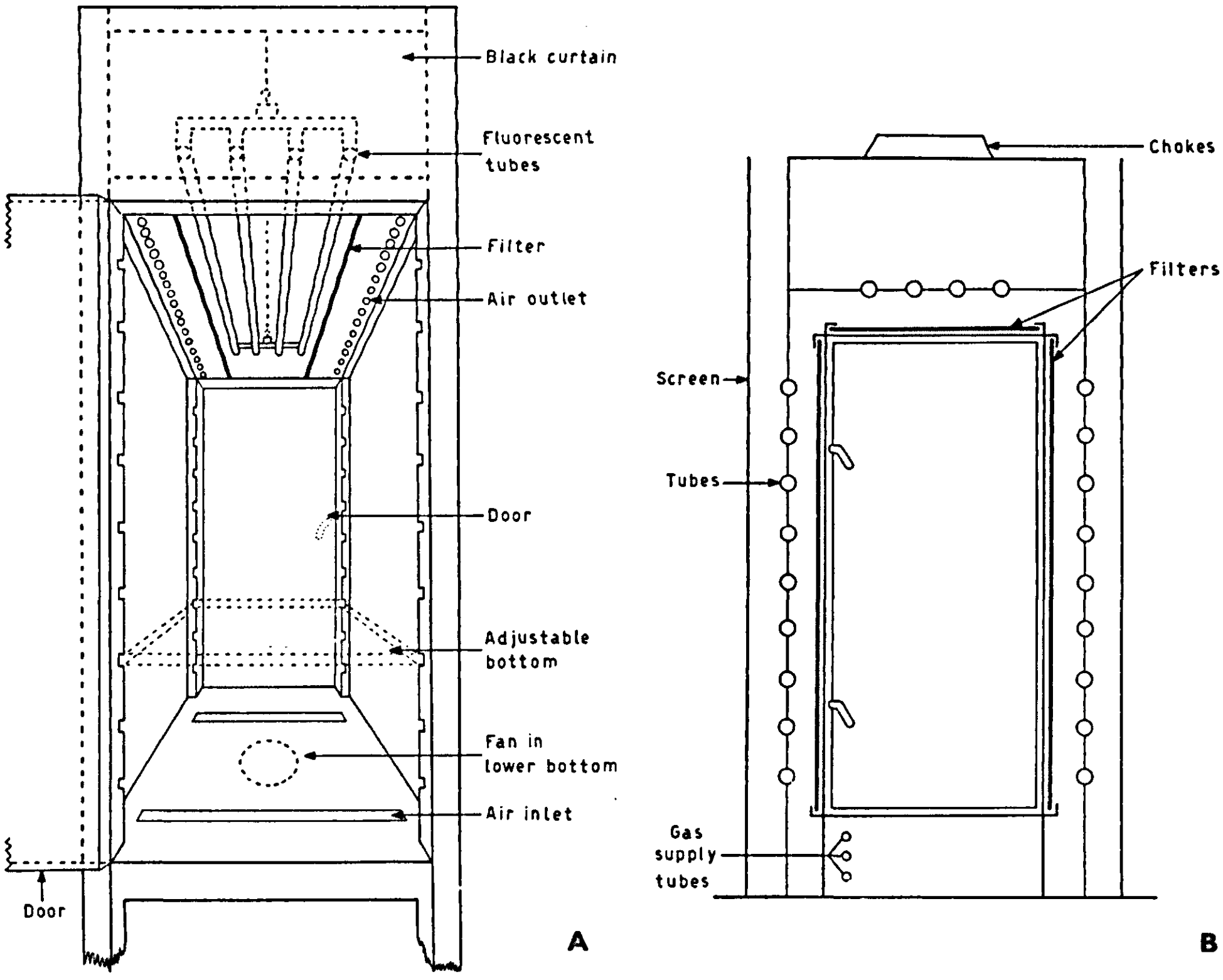

FIG. 2. Simplified diagrams of cabinets for irradiation in narrow spectral regions: A. Low intensity cabinet. B. High intensity cabinet.

In the rooms in which the series of cabinets are placed, temperature is controlled by a central heating system in combination with a fan supplying cool air from outdoors. In the high intensity cabinets the door positions are used to correct for temperature differences resulting from the presence of various numbers of lamps on the different cabinets; entrance of light through the door opening is avoided. Each of the low intensity compartments has a small fan in the lower panel of the double bottom, introducing air from below. This fan is operated by a mercury regulator in the cabinet, hanging on plant level, in combination with a relay switch.

Figure 3 presents the spectral characteristics of the incident irradiations in the compartments. In general, the irradiation in the low and high intensity cabinets is of the same spectral composition. However, red in low intensity is filtered through copper sulphate solution (Fig. 3, d), in the high intensity

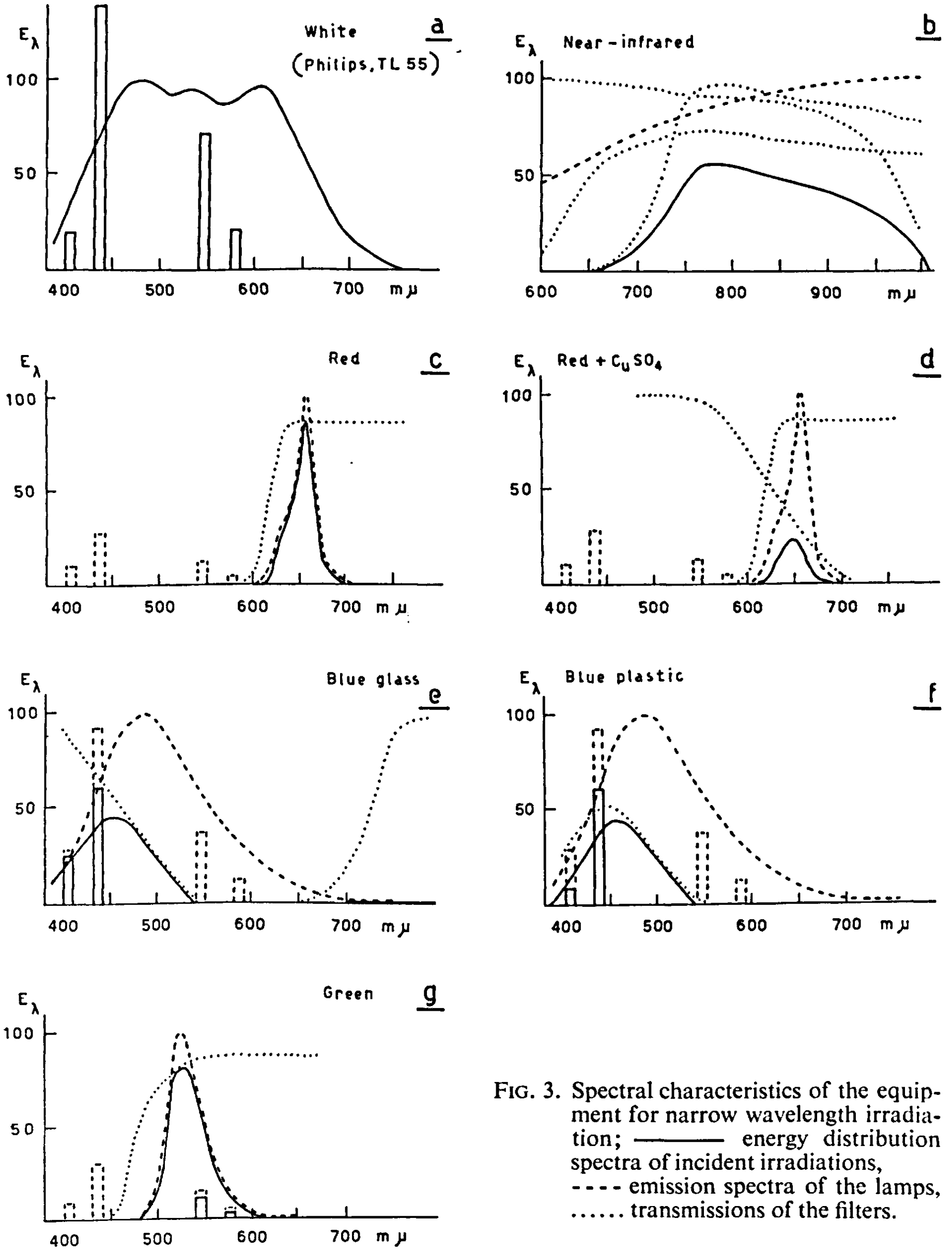

FIG. 3. Spectral characteristics of the equipment for narrow wavelength irradiation; ——— energy distribution spectra of incident irradiations, - - - - emission spectra of the lamps, ... transmissions of the filters.

cabinet it is not (Fig. 3, c). Two qualities of blue light are available, the one used earlier, containing some near-infrared (Fig. 3, e) and the new quality without any detectable near-infrared admixture (Fig. 3, f). The last one was used already for experiments described in a few previous papers (15, 49, 96, 103). The different effects on plant growth of low intensity irradiations obtained with these two filters have especially been worked out by DE LINT (49, see also 89), and by MEYER (54, 55).

II.3.2. *Measurement*

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 (34). 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 correspondence to their angle of incidence. The intensities of mixed irradiations were determined by separate measurement of the two components.

CHAPTER III

RELATION BETWEEN BLUE LIGHT AND OTHER SPECTRAL REGIONS IN STEM ELONGATION AND FLOWERING

III.1. Introduction

During recent years, highly confusing results have been obtained from photomorphogenesis and photoperiodism experiments with blue irradiation in different modes of application.

Wassink, Stolwijk *et al.* (see, *e.g.*, 94) observed elongation on daily irradiation with blue light following white, an effect comparable to that of near-infrared irradiation. However, as subsequently established, spectrographically obtained blue light produced no elongation, or only a small one *(cf.* 5). The blue light used earlier in our laboratory contained an admixture of about 3% of near-infrared, while a new blue plastic filter introduced by this laboratory (15) made it possible to obtain blue light without near-infrared contamination. Supplementary low intensity irradiation obtained with this new filter did not produce any or only a weak elongation (49, 89). Moreover, the 3% near-infrared contamination alone caused the full original effect (49, 89). Recently, Meyer (54) again reported elongation upon irradiation with blue light obtained with the new filter, *viz.*, in light-grown seedlings of gherkin, and so did others (58).

Since the elongation in blue light of low intensity, supplementary to a day in white light for the greater part appeared to be due to admixture of near-infrared, it became necessary to study the effect of pure blue light in high intensity. Especially the reaction of *Hyoscyamus* was of importance, because in blue light this plant formed stems far more rapidly then in other colours, except red with a large admixture of near-infrared.

It was repeatedly observed that stem elongation in *Hyoscyamus* takes place with the same speed in blue light free from near-infrared and in blue with about 3% of this irradiation. So, it was concluded that, in higher intensities, the elongation was a real blue light effect (15).

The question of what exactly is the nature of the blue light activity is not yet answered. A series of possibilities remains open, three of which were put forward already earlier (90). Firstly, the observed effect is a true effect of blue irradiation. Secondly, even the plastic filter used might not be absolutely opaque to near-infrared irradiation. This is highly improbable, since not the slightest transmission could be detected with the most sensitive methods of detection (15). Thirdly, the plant pigments transform part of the absorbed light into fluorescence in the near-infrared region, which may be re-absorbed by the pigment system, and initiate elongation (86).

In view of the introductory chapter, a fourth possibility exists in the case of

stem elongation in *Hyoscyamus*. If blue light were completely inactive (*cf.* 89), the plants in blue light would be photoperiodically at the zero-daylenght position (Fig. 1, p. 3), and blue would represent a non-inhibitive treatment, analogous to, *e.g.*, defoliation.

III.2. High intensity blue irradiation

The results presented in this section mainly are of methodical importance, especially with respect to the near-infrared in the blue irradiation, and are an extension of III.1.; the more important ones were already briefly published (89).

III.2.1. *Various filter combinations for blue irradiation*

Four filter combinations for blue light were used, *viz.*,

1. Blue glass, originally used, transmitting some near-infrared.
2. Blue plastic, yielding our new blue light quality, free from near-infrared.
3. Blue glass plus copper sulphate solution.
4. Blue plastic plus copper sulphate solution.

If the blue plastic filter should transmit any trace of near-infrared irradiation, not detectable by normal methods (15), we may expect that by additional filtering of the light with copper sulphate solution ($1\frac{1}{2}$ cm, $\frac{3}{4}$ saturated) the near-infrared contamination of the blue light is removed.

Plants placed in our high light intensity cabinets in either of these four blue light qualities during 14 hours each day started elongation of stems approximately at the same day (Table 1).

Table 1. Days to stem elongation in *Hyoscyamus* as influenced by blue light quality given during 14 hours per day in intensities of about 9500 ergs/cm²sec, from blue fluorescent lamps. Further description of light qualities, see text. The experiment started 15-1-'58. Averages of 4 plants.

Light filter	Days to stem elongation
Blue glass	18
Blue glass + $CuSO_4$	18
Blue plastic	19
Blue plastic + $CuSO_4$	20

It must be concluded that the elongating effect of blue light cannot be taken away by strict removal of the near-infrared.

III.2.2. *Blue – red antagonism in simultaneous irradiation*

In a communication at the Second Photobiological Congress in Turin, 1957, some conclusions based on the results to be described in this section were preliminarily communicated *(cf.* 89, Figure 10). A more detailed description of the experiments will be given here.

Plants of *Hyoscyamus*, grown in the greenhouse under short days, were irradiated during 15 days in our high intensity coloured light cabinets during 16 hours per day. Thereafter, they were kept at 10-hour days in white light. This experiment was mainly performed to collect information on gibberellic

acid effects under coloured light irradiations. Only the relative stem lengths of the control group without gibberellic acid treatment are presented here (Fig. 4). Representative plants are shown on plate 1a.

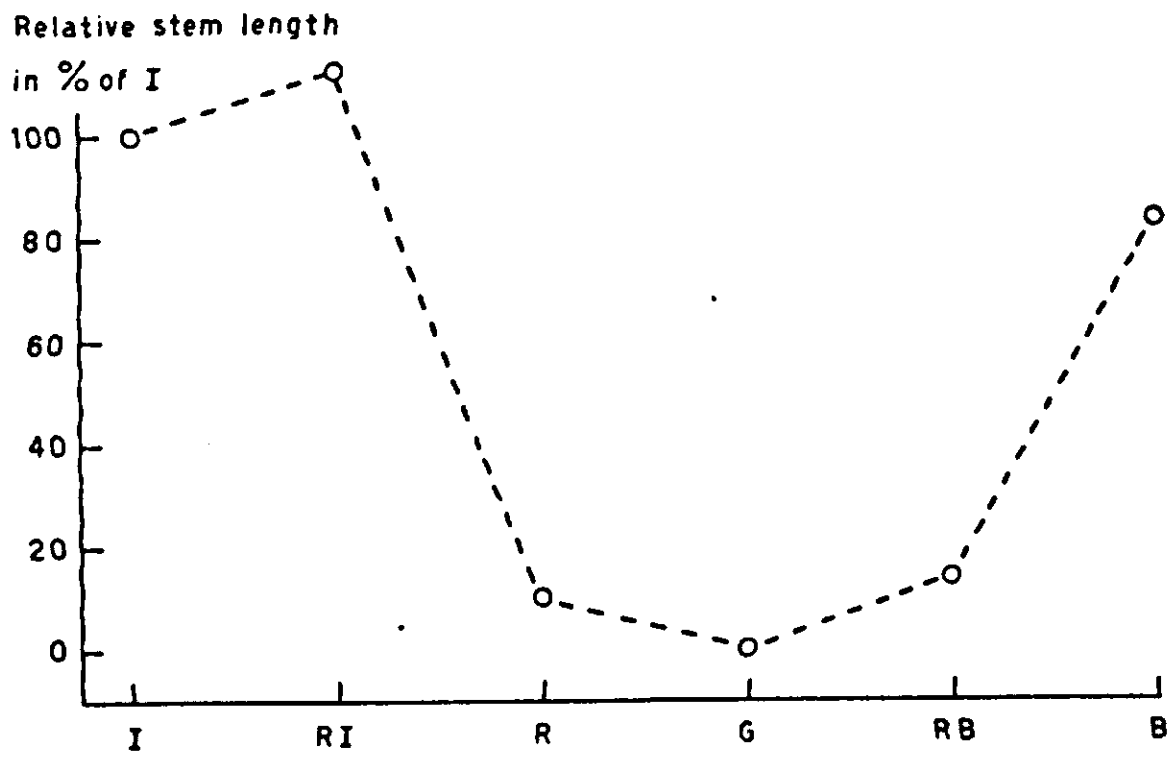

FIG. 4. Relative stem lengths in *Hyoscyamus*. Following a short-day treatment, the plants were irradiated 16 hours daily in the coloured light cabinets (*ca.* 9000 ergs/cm²sec) during 15 days. I = red and near-infrared mixture obtained with tungsten lamps, RI = red with near-infrared on top, R = red, G = green, RB = blue with red on top, B = blue. Hereafter, plants were kept in 10-hour days in white fluorescent light (*ca.* 20,000 ergs/cm²sec). Experiment started 28-9-'56.

The two red + near-infrared cabinets (one supplied with a red – near-infrared mixture of filament lamps [I], and a red cabinet with near-infrared from the top [RI]) yield equal stem elongation. This is in accordance with the observation of STOLWIJK and ZEEVAART (77), who reported that red in combination with 30 or 300 % near-infrared produces the same rapid stem elongation in *Hyoscyamus*. The elongation in blue (B), and the rosette growth in red (R) and green (G) light are in agreement with their results. The elongation in blue is strongly suppressed by a red top-irradiation (RB). This was observed already before, and once more afterwards (90). The blue used in this experiment was the "old" blue, containing 3 % near-infrared.

Since both blue irradiation types (with and without 3 % near-infrared) produce maximal stem elongation when used alone, it was of interest to study both in combination with the same red addition. It was then observed that the plants in impure blue elongated earlier than those in pure blue (89, Figure 10). The weak near-infrared contamination thus manifests a remarkably strong effect; evidently, the elongating tendency in blue is fairly weak with respect to that exerted by near-infrared.

From the above data, three important conclusions can be derived, *viz.*, 1) red light simultaneously given with blue light suppresses stem elongation (90); 2) in long-day blue irradiation stem elongation proceeds irrespective of an admixture of near-infrared; 3) admixture of red light inhibits stem elongation in blue light more strongly in the complete absence of near-infrared.

III.2.3. *Blue – red antagonism in supplementary irradiation*

In section III.2.2. it has been shown that red irradiation given *simultaneously* with blue, keeps the plants in the rosette state for longer times when near-infrared is completely removed from the blue. Results of *supplementary* red irradiation at high and low intensity are presented in this section. (Further details in chapter V, p. 31).

Plants were exposed to high intensity blue light (III.2.1., filter combination 3),

or red light (9500 ergs/cm² sec), in 14-hour days, nine plants in each colour. Following the high intensity exposure, the plants daily received low intensity irradiation with near-infrared or red during two hours, and darkness thereafter, or darkness immediately. The intensity of the supplementary irradiation (*ca.* 1000 ergs/cm² sec) may be accepted to be sufficient for maximum effects (49, 89). The results of this experiment are presented in table 2. Representative plants are shown on plate 1b.

TABLE 2. Days to stem elongation in *Hyoscyamus* under a daily irradiation schedule of 14 hours red, or blue, fluorescent light (9500 ergs/cm²sec) followed by 2 hours red, or near-infrared (1000 ergs/cm²sec), or darkness. Plants previously grown in 10-hour short days in white fluorescent light. Treatment started 15-1-'58, and ended after 60 days. Averages of 4 plants.

14 Hours basic illumination	2 Hours supplementary irradiation		
	red	near-infrared	dark
Blue	19	20	20
Red	>60	24	>60

The plants receiving red days reacted upon a supplementary treatment as expected from previous results (47). Plants in the high intensity red irradiation remain in the rosette state for very long periods. Daylength extension with red light of low intensity has no additional effect whereas a near-infrared supplement causes rapid stem elongation. Thus, for both red and near-infrared, the effect is the same as when following a basic period in white light. This result is in accordance with the observations of STOLWIJK and ZEEVAART (77) and of MEYER (56).

The results of the blue group, however, deviate from what might be expected. In all three treatments elongation started rapidly, being evident after about 20 days. This is important, because 2 hours (red) irradiation constitute a very short day, which of itself should have been strongly inhibitive (*cf.* 89, Figure 6B). This indicates that blue has not been photoperiodically inactive (as was a possible suggestion; *cf.* III.1.), but that it has antagonized the red function. It must be concluded, therefore, that, morphogenetically, even the purest blue does not act as complete darkness. This result resembles those obtained with near-infrared supplementary irradiation following white light (47). In that case controls on 8-hour days in white light remained in the rosette state, whereas those with 2 hours of near-infrared supplement showed stem elongation. Again, this is a demonstration for the similarity of blue and near-infrared action. (Further results along this line, see chapter IV.1.).

The foregoing experiment must be brought into connection with an experiment published by STOLWIJK and ZEEVAART (77), in which *Hyoscyamus* plants were irradiated with red light of high intensity. One group of plants, however, received 9 hours blue light each day instead of red, a group 9 hours blue every other day, and a third group 9 hours once every three days. All produced stems, and more rapidly so the more blue light they received. The control group in continuous red, and also plants receiving a 9-hour day in blue light only, remained in the rosette state. This experiment, again, demonstrates that blue, like near-infrared, antagonizes the red inhibition.

This experiment, however, was performed with the old blue glass filter. It was

interesting to see whether there would be any difference in reaction between old and new blue in such a type of experiment. Therefore, plants were given 16 hours red and 8 hours blue irradiation of high intensity, each day. Three blue types were used: old blue glass filter, the same combined with $1\frac{1}{2}$ cm copper sulphate solution, and the new blue plastic. The results are presented in table 3. Representative plants are shown on plate 1c.

TABLE 3. Stem elongation in *Hyoscyamus* in red light (16 hours per day) alternated with blue light (8 hours per day) of three different qualities. (See text, p. 13). Plants previously grown in the greenhouse in 8-hour days in daylight. Colour treatment started 28-10-'58. Averages of 3 plants.

Treatment	Days to stem elongation	Stem length after 35 days (mm)
16 h. Red + 8 h. blue (glass)	19	410
16 h. Red + 8 h. blue (glass and $CuSO_4$)	26	129
16 h. Red + 8 h. blue (plastic)	32	42

Certainly, there is a strong difference in reaction velocity between the plants in the various qualities of blue used. Plants in old blue elongated very quickly, in new blue much more slowly. Glass in combination with copper sulphate was in between.

Such results demonstrate that the quality of blue irradiation is more critical when red irradiation is applied either in between or simultaneously with blue irradiation.

It may be concluded that the elongating function of blue is similar to that of near-infrared. The effect of blue light is highly sensitive to the actions of red and near-infrared irradiation. It has been impossible to further decrease the elongating function of blue light by adding a copper sulphate solution layer to the new plastic filter, which demonstrates that this filter transmits a really pure blue.

III.3. DISCUSSION

In this chapter, results of a series of experiments on the quality of blue irradiation, with respect to near-infrared contamination has been presented. It was possible to produce a blue irradiation with no measurable near-infrared admixture (III.2.3., p. 14; 15), whereas the blue light originally used (89) contained an admixture of about 3% near-infrared.

Different from results obtained at low light intensities (49), the blue had an elongating effect when given at high intensity. Admixture of some red light to a blue irradiation or supplementary red light following a blue irradiation were strongly inhibitive to stem elongation. Any near-infrared contamination diminished the effect of the red action in these combinations of blue and red. These results prove that the elongation obtained at high intensity blue irradiation is of the near-infrared type.

This conclusion is in accordance with the results presented by MEYER (54) on elongation of the hypocotyls of gherkin seedlings. This author, using the same plastic filter, irradiated his plants during 8 hours per day with red and during another 8 hours with blue light. The red was given at high intensity; blue light directly following the red increased elongation in a range of low and

intermediate intensities upto 3000 ergs/cm² sec. Thus, blue antagonizes the inhibition caused by red.

Two hours of low intensity red illumination, following a basic photoperiod in blue light, do not inhibit stem elongation (see Table 2, p. 15). This seems to be against MEYER's supposition (56) that during the first hours after a blue irradiation the plants are highly sensitive to the inhibitive red action. It could be that the intensity of the red light used in our experiment was too low. This, however, is not likely in view of other cases in which a strong action of the same intensity of red light was observed (49, 89). Unfortunately, 2 hours low intensity red alone, which would be the necessary control treatment, could not be given, because the plants then starve, owing to shortage of photosynthates. In experiments of short duration, as described in chapter V (p. 31), this difficulty is irrelevant so that very low light doses could be tested.

The experimental technique presented in chapter V (p. 31) is of special interest for a better understanding of the results in short days in blue light obtained by STOLWIJK and ZEEVAART (77). They found that, in *Hyoscyamus*, in 8-hour days in blue light, leaf petioles elongate while stem elongation was absent. It is difficult to understand that 8 hours of blue light suppress stem elongation if blue is active only in the same way as near-infrared which, according to our views, removes inhibition. Also here, a special experimental procedure became necessary to overcome stringent photosynthetic requirements. Such experiments are described in chapter IV.2. (p. 18) and V (p. 31).

The experiments, described in the present chapter, show that the elongating, red antagonizing, activity of blue light is strongly reinforced by weak near-infrared admixtures, so that strong elongative action of blue irradiation in combination with red light was observed especially in case of a near-infrared admixture (94). Nevertheless, blue has a red antagonizing action itself. Whether this is a real blue effect or a fluorescence effect is not established as yet.

On the other hand, blue light seems to have a daylength action too, since short days in blue light are rosette-inducing (77).

The results on blue irradiation to be reported in the following chapters have all been obtained with the near-infrared opaque blue plastic filter.

CHAPTER IV

SHOOTING AS GOVERNED BY FORMATIVE AND PERIODIC EFFECTS OF LIGHT AND DARKNESS

IV.1. INTRODUCTION

DOWNS (17) reported that response to daylength in *Hyoscyamus niger* is mediated by the red – near-infrared system. Night interruption with red light brings about the long-day reaction which can be antagonized by near-infrared irradiation. This is the same behaviour as observed for a great variety of photoformative processes. These data suggest that supplementary red irradiation has the function of day extension, and that near-infrared antagonizes this.

STOLWIJK and ZEEVAART (77) have reported partly deviating results. Red light was most effective as night interruption, as usual, while admixture of near-infrared reduced this activity. Applied as low intensity supplementary irradiation, red and near-infrared (alone or in sequence) both more or less caused a long-day

reaction, and near-infrared much more strongly so than red. Exposure to long days in (high intensity) coloured light only, resulted in rapid flowering in blue, violet, and red + near-infrared, and also in continuous red with intermission of 9 hours blue. Stem elongation did not occur in short day (9 h.) in either of the colours (near-infrared and mixtures with this region have not been tested). Leaf petioles in short days in blue light, however, showed definite elongation. STOLWIJK and ZEEVAART concluded that some near-infrared (or blue) irradiation is essential for stem elongation, be it as supplement or admixture. An antagonism between red and near-infrared (or blue) irradiation could not be observed under their conditions.

VAN DER VEEN and MEYER (84) and MEYER (56) confirmed the results of STOLWIJK and ZEEVAART (77). They state that near-infrared (or blue) irradiation is essential for flowering, together with a short night, or a long night interrupted with red light. The short-day plant *Salvia occidentalis* remained vegetative under these conditions (55, 56). MEYER concluded that two different photoreactions with completely different spectral sensitivities, determine the photoperiodic effect.

From the experiments of STOLWIJK and ZEEVAART (77), it seemed that *Hyoscyamus* had a daylength reaction different from that of other plants. The colour dependence was equal to the one observed with cruciferous plants, *i.e. Brassica.* With supplementary irradiation, stem elongation proceeds most quickly in near-infrared. WASSINK, SLUIJSMANS and STOLWIJK (92) observed earlier that Cruciferae react strongly to the formative elongation reaction, but that they seem to be more or less indifferent to the photoperiodic flowering reaction. They concluded that these plants are quantitative long-day plants. From this point it was not too strange that flower stalks could develop under short days if these were supplemented with an elongation promoting irradiation. However, DE LINT (48) demonstrated that the qualitative long-day plant *Hyoscyamus niger* reacts in the same way, and that long-day requirement is not essential. If the last supplementary irradiation is in the near-infrared region (2 hours), stem elongation and flower bud formation occur irrespective of daylength.

WASSINK and SYTSEMA (96) reported on leaf petiole elongation upon interruption of long nights with red or near-infrared, or both. They observed elongation of leaf petioles according to the red – near-infrared antagonism, however, without stem elongation.

In the following sections, more evidence will be presented for the view that for *Hyoscyamus*, as for Cruciferae, besides the periodicity reaction also the formative reaction is of importance.

IV.2. WHITE LIGHT WITH SUPPLEMENTARY COLOURED IRRADIATION

In the following experiment,[1]) three different daylengths in white light (8, 11, or 14 hours) were combined with supplementary irradiation (4 hours, low intensity) in near-infrared, red, yellow, green, blue, violet, or dark. The experiment started 13-3-'58 and ended 20-5-'58, after 69 days. Results are presented in table 4 (a photograph of representative plants is published in [96, Plate 1]).

It is evident that, with a near-infrared supplement, stem elongation is ahead of that obtained with addition of any other wavelength region irrespective of

[1] This experiment was performed by Mr. W. SYTSEMA, in collaboration with the author.

TABLE 4. Days to stem elongation and flowering, and stem length in *Hyoscyamus* upon different daylengths (8, 11 and 14 hours) in white fluorescent light (*ca.* 13,000 ergs/cm²sec), supplemented with 4 hours of irradiation in narrow wavelength regions (1000 ergs/cm²sec). Experiment started 12-3-'57, and ended after 69 days. Averages of 4 plants.

Supplementary wavelength region	8 Hours white light			11 Hours white light			14 Hours white light		
	Stem length after 69 days (mm)	Number of days to		Stem length after 69 days (mm)	Number of days to		Stem length after 69 days (mm)	Number of days to	
		Stem elongation	Open flowers		Stem elongation	Open flowers		Stem elongation	Open flowers
Near-infrared	63*	58	b	61	56	b	327	27	41
Red	–	>69	v	–	>69	v	65	36	b
Yellow	–	>69	v	–	>69	v	120	30	58
Green	–	>69	v	–	>69	v	165	31	52+
Blue	–	>69	v	–	>69	v	106	30	59°
Violet	–	>69	v	–	>69	v	41	33	b
Dark	–	>69	v	–	>69	v	41	40	b

v = vegetative plants
b = after 69 days only flower buds

* = 2 plants dead
\+ = one plant still b
° = one plant dead, one b

daylength. Together with stem elongation, flower buds always appeared. With a basic irradiation of 14 hours, all supplementary treatments resulted in stem elongation. This observation is of importance with respect to the relation with the reaction in other plants, *e.g.*, in the short-day plant *Xanthium* (5).

In *Xanthium*, a basic period in white light just below critical daylength could be extended to above the critical value with red, and a too long one could be effectively shortened with a near-infrared supplement (5). Conversely, in *Hyoscyamus*, a brief near-infrared supplement produces long-day reaction when following 8 hours of white irradiation (48). However, supplementary red light does not give any retardation as compared with the dark control; on the contrary, it seems somewhat promotive (*cf.* Table 4). Similar results were obtained by STOLWIJK and ZEEVAART (77). Their supplementary red light contained some near-infrared admixture which, however, was removed in our experiments by a copper sulphate solution (1 cm, $^1/_8$th saturated).

The effectivity of a near-infrared supplementary irradiation seems to depend on the length of the basic light period (Table 4). Stem elongation starts later with shorter white days when combined with the same near-infrared supplement.

This observation justifies the following analysis. We will suppose that, in *Hyoscyamus*, red light is active in extending too short days, while near-infrared antagonizes this action. This photoperiodic system may run together with the photoformative inhibition of stem elongation by red and the antagonizing action of near-infrared upon this inhibition. Then, a complicated reaction will result. Plants receiving red extension, *e.g.*, may be in long-day conditions, but so strongly inhibited for elongation that stem elongation is impossible. On the other hand, near-infrared supplementary irradiation may be so strongly elongative that even in shorter days elongation occurs. It should be mentioned that this concept can be valid only if the daylength-dependence line has a rather flat slope, running down smoothly even to an 8-hour day. Evidence presented in

chapter V (p. 31) supports this supposition, which, moreover, is in accordance with BEST's data (2).

The following experiments demonstrate in a rather definite way that we are dealing with a combined action of the periodic and the formative reaction systems. In the first experiment plants were given 8 hours strong white fluorescent light, followed by 8 hours low intensity red irradiation. One set of plants received this treatment only (control group), the others hereafter received a time-series of near-infrared irradiation (1, 15, 30, 60, or 120 minutes; 1000 ergs/cm² sec). The results are presented in table 5. Representative plants are shown on plate 1f. Two hours of near-infrared resulted in stem elongation after 37 days, one hour only after 45 days and plants in shorter treatments remained vegetative for at least 78 days. Thus, stem elongation depends on the duration of irradiation with near-infrared.

TABLE 5. Days to shooting in *Hyoscyamus* upon daily 8-hour irradiation in white fluorescent light (20,000 ergs/cm²sec) supplemented with 8 hours red light (1000 ergs/cm²sec), and then followed by a time-series (0, 1, 15, 30, 60, or 120 minutes) of near-infrared radiation (1000 ergs/cm²sec). Started 17-10-'57, and ended after 78 days. Temperature *ca.* 20 °C. Averages of 4 plants.

Scheme of irradiation	Days to stem elongation
8 h. White + 8 h. red + 0 min. near-infrared	>78
8 h. White + 8 h. red + 1 min. near-infrared	>78
8 h. White + 8 h. red + 15 min. near-infrared	>78
8 h. White + 8 h. red + 30 min. near-infrared	>78
8 h. White + 8 h. red + 60 min. near-infrared	45
8 h. White + 8 h. red + 120 min. near-infrared	37

In the second experiment, a series of "daylengths", *viz.*, an 8-hour white day extended by 0, 2, 4, 6, or 8 hours of red light, all followed by 2 hours of near-infrared, were applied. The necessary controls were added (8 hours white only, 8 white + 8 red, 8 white + 8 near-infrared). The effect on the start of stem elongation is presented in table 6. Representative plants are reproduced on plate 1e.

TABLE 6. Days to shooting in *Hyoscyamus* upon daily 8-hour irradiation in white fluorescent light (20,000 ergs/cm²sec) supplemented with a time-series (0, 2, 4, 6, or 8 hours) of red light (1000 ergs/cm²sec), and then followed by 0, 2, or 8 hours near-infrared irradiation (1000 ergs/cm²sec). Experiment started 17-10-'57, and ended after 78 days. Temperature *ca.* 20 °C. Averages of 4 plants.

Scheme of irradiation	Days to stem elongation	Retardation as compared with the quickest group
8 h. White + 0 h. red + 8 h. near-infrared	43	6
8 h. White + 0 h. red + 0 h. near-infrared	>78	>41
8 h. White + 0 h. red + 2 h. near-infrared	50	13
8 h. White + 2 h. red + 2 h. near-infrared	46	9
8 h. White + 4 h. red + 2 h. near-infrared	42	5
8 h. White + 6 h. red + 2 h. near-infrared	41	4
8 h. White + 8 h. red + 2 h. near-infrared	37	0
8 h. White + 8 h. red + 0 h. near-infrared	>78	>41

Table 6 shows that the duration of the red daylength extension has a definite effect on the activity of supplementary near-infrared irradiation. The more red was intercalated between white and near-infrared, the sooner elongation started. This indicates that red was really active in day-extension, and that, moreover, near-infrared acts over a broad range of daylengths.

Part of the results presented in tables 5 and 6 are reproduced in figure 5.

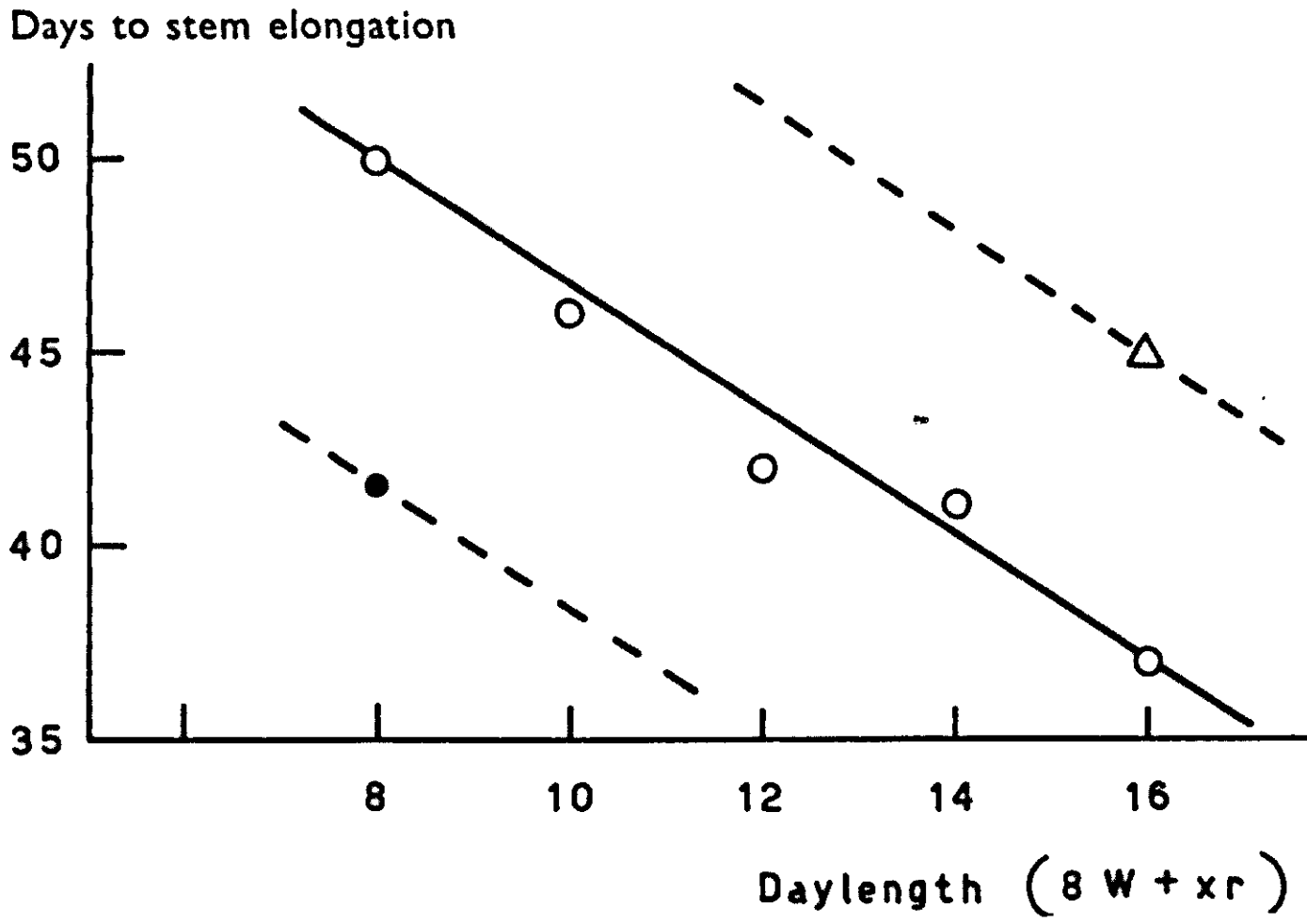

FIG. 5. Days to shooting of *Hyoscyamus* upon near-infrared supplementary irradiation, as influenced by intercalating x hours of low intensity red light between main and supplementary near-infrared irradiation. ● = 8, o = 2 and △ = 1 hour(s) near-infrared. Further description: tables 5 and 6.

This figure shows that the accelerating effect of near-infrared on stem elongation depends on daylength and on the near-infrared dosis.

The third experiment shows that the near-infrared effect can only be observed if the treatment is continued. There is no observable after-effect. Plants were given 8, 11, or 14-hour days in white light (fluorescent lamps) supplemented with 2 hours near-infrared, or red, or dark (control). These treatments were given during 20 days only; thereafter, the plants were placed in 8-hour days in white light from fluorescent tubes. The results are presented in table 7.

Only the 14-hour white + near-infrared group started elongation after 43 days, which is very slow. Moreover, this elongation stopped soon, so that stem length after 65 days still was only 10 mm. This strongly suggests that the action of supplementary near-infrared irradiation is a formative instead of a periodic one, because a long-lasting after-effect, known as irreversible induction, is absent. Later (IV.3.2., p. 25) it will be demonstrated, however, that this explanation is not fully valid because *Hyoscyamus* never shows any appreciable effect of inductive periods of limited duration, which is true also for white light treatment only (p. 27).

Subsequently, one could ask whether the accelerating effect of supplementary near-infrared radiation on stem elongation is active immediately from the beginning of the experiment, or only if the plants are in the reproductive phase, *i.e.*, after several days of photoperiodic induction. The last supposition not

TABLE 7. Stem elongation in *Hyoscyamus* upon different daylengths (8, 11, and 14 hours) in white fluorescent light (13,000 ergs/cm²sec) each supplemented with 2 hours red or near-infrared radiation (1000 ergs/cm²sec), or dark; thereafter all plants were exposed to 8-hour days in white fluorescent light. Treatment started 19-4-'57, and ended after 65 days.

Supplementary wavelength region	Basic treatment			
	8 Hours white	11 Hours white	14 Hours white	
	Appearance	Appearance	Days to stem elongation	Stem length after 65 days (mm)
Near-infrared	rosette	rosette	43	10
Red	rosette	rosette	rosette	
Dark	rosette	rosette	rosette	

being very reasonable (see Fig. 5), nevertheless the following experiment gave results strongly suggestive of this possibility.

Hyoscyamus plants were irradiated daily with 8 hours strong white fluorescent light (control), followed by 8 hours low intensity red (control), which combination was again followed by 1 hour red or 1 hour near-infrared (low intensity). The nine hour (8 + 1) red group consisted of a double number of plants, half of which were given this treatment during only 14 days, and thereafter 8 h. white + 8 h. red + 1 h. near-infrared. In this last group, treatment with the accelerating near-infrared irradiation, thus, started 14 days after the beginning of the long-day treatment. The results on stem elongation are presented in table 8.[1]) Representative plants are shown on plate 1d.

TABLE 8. Stem elongation in *Hyoscyamus* upon daily 8-hour irradiation in white fluorescent light (13,000 ergs/cm²sec), supplemented with 8 hours red radiation (1000 ergs/cm²sec), and then followed by 1 hour near-infrared, or red, or dark. A group of plants (5, see below) received treatment 3 during 14 days only and then were shifted to treatment 4. Stem lengths measured 30-7-'57, after 70 days of treatment. Averages of 4 plants.

Group	Scheme of irradiation		Days to stem elongation	Stem length after 70 days (mm)
	Basic	Supplementary		
1	8 h. White		–	–
2	8 h. White + 8 h. red		–	–
3	8 h. White + 8 h. red	1 h. red	–	–
4	8 h. White + 8 h. red	1 h. near-infrared	41	233
5	8 h. White + 8 h. red	14 × (3), then (4)	45	188

The number of days to stem elongation in the group receiving near-infrared immediately from the beginning is only 4 less than that in the group receiving near-infrared only from the fourteenth day onward. This seems possible only if the near-infrared should not have been active during some period like the first 10 days of long-day treatment. Later, however, it was envisaged that another

[1] Mr. W. SYTSEMA performed this experiment, in collaboration with the author

possibility remains open: being active in extension of the short day in white light, red light might have an appreciable action on stem elongation as compared with a short day in white light, be it less than if combined with near-infrared supplementary irradiation. More evidence for this suggestion will be presented in section V.2. (p. 33). It will then be shown that the periodic and formative stem elongation impulses run together, and are additive. With this statement, results described in the present section would be explained, *viz.*, that red light given supplementary to a short day in white light, diminishes the inhibition produced by the short-day treatment, be it less than near-infrared does.

IV.3. Permanent or temporary growth in coloured light only

Experimental data described in part 2 of this chapter have demonstrated that daylength dependence of shooting in *Hyoscyamus* can be strongly modified by photoformative action (Fig. 5, *cf.* p. 21), thus supporting the suggestion presented in chapter I according to which a periodic and a formative reaction should be distinguished. Secondly, results were suggestive of the idea that the floral induction had to last during the whole period until the beginning of stem elongation, and that the "after-effect" is only weak. More evidence for these two conclusions is presented in this part.

IV.3.1. *Continuous treatment with coloured light*

In the experiments to be described first, the plants during some time were exposed to a special wavelength region, *viz.*, blue or red radiation only. The red light contains a near-infrared admixture, which, however, under these conditions is almost ineffective. The blue contains no near-infrared, but has some red-antagonizing action (*cf.* Chapter III, p. 12).

The observation in chapter III that the elongating action of blue light is of the same nature as that of near-infrared, in combination with results from chapter IV.2. (p. 18), indicating that near-infrared antagonizes a short-day effect which may be interpreted as "red-inhibition", could lead to the conclusion that in blue light no (inhibitive) daylength action can exist. Stolwijk and Zeevaart (77) however, reported that, in short-day blue irradiation, no stem elongation takes place, be it that the inhibition seemed to be only weak because leaf petioles did elongate. It might be that in short-days in blue light, the tendency to stem induction, whatever this may be, accumulates too slowly, so that plants must be in it too long for normal further growth on to the moment elongation can start. But it is as well likely that blue light in this case has some red activity, owing to a weak admixture of green and yellow light. In the next experiment it is demonstrated that the above mentioned suggestions both are to be taken into account.

Plants were given a series of long (16 hours) or short (8 hours) days in blue light (0, 1, 2, 4, 8, or 15 days, or continuously), and hereafter were exposed to long days (16 hours), or to short days (8 hours) in red light. The treatment started 16-4-'58. Stem lengths were measured 21-7-'58, after 96 days. The number of macroscopically visible flower buds was also determined. The results are compiled in table 9. The exact moment at which stem elongation started was very difficult to observe because of the low rate of elongation. Representative plants are shown on plate 1g (long-day blue) and 1h (short-day blue), taken 14-8-'58.

TABLE 9. Stem elongation and flower bud initiation in *Hyoscyamus* in long (16 h.) or short (8 h.) days in red light after pretreatment with various numbers (0, 1, 2, 4, 8, or 15) of long or short days in blue light. Light intensities 9200 ergs/cm²sec. Temperature *ca.* 22°C. Experiment started 16-4-'58. Observations of 21-7-'58. Averages of 2 plants (one plant of each set remained in its red light treatment until 2-9-'58).

Pretreatment		After-treatment			
		16 Hours in red light		8 Hours in red light	
Hours daily in blue light	Number of days	Stem length (mm)	Number of flower buds	Stem length (mm)	Number of flower buds
–	0	–	¼	–	v
16	1	1	½	–	v
16	2	2	½	–	v
16	4	10	12	–	v
16	8	60	14	2	5
16	15	90	14	5	6
8	1	3	2	–	v
8	2	2	½	–	v
8	4	4	2	–	v
8	8	12	10	–	v
8	15	17	11	–	v

v = vegetative
¼ = not strictly v
½ = elongated apex

It is clear that both stem length and number of flower buds are favoured by longer duration of the pretreatment with long days in blue light. An effect along the same line is observed with short days in blue light, although it is weaker. Here, however, it is only manifest in the group receiving long red days afterwards. With long-day red after-treatment, the reaction is very slow, but it is still slower in the short-day red group. In this last group none of the plants of the short-day blue treatment had left the vegetative state at the moment the experiment ended, and of the long-day blue group only the plants having had 15 or 8 blue days had produced some flower buds.

In table 9, stem length is presented. This, however, is an arbitrary item, because stem length itself was, for all treatments, nearly zero. Elongation as measured was that of the main shoot of the inflorescence. (See especially plate 1g for this phenomenon; "normal" plants have stems of about 30 cm below the inflorescence [*cf.*, *e.g.*, Plate 2c]). This is a demonstration of the strength with which red irradiation inhibits the capacity for elongation produced in blue irradiation. The inhibition is stronger in short red days than in long ones. Plants in continuous long-day blue irradiation started stem elongation after about 25 days; plants having had 15 long days in blue light still could be inhibited almost completely by short-day red irradiation. A real reversal from flower induction back to vegetative growth could be observed in the group that received short days in red light after 8 long days in blue light. On plate 3a, an example of this reversal is shown. The little flower bud, mainly consisting of peduncle and calyx, between the two rosettes is the first one, and represents the tip of the original stem. The two rosettes, being lower axil shoots, grew strictly

vegetatively. Buds in red light never became open flowers. This reminds of an observation of LANG and MELCHERS (44) that defoliated plants produced buds consisting of a normal calyx only. In the above experiment the action of red light, thus, is definitely inhibitive, even after appreciable induction.

MEYER (*cf.* 16) has observed that, in much higher intensities of red light, elongation in long days occurs more quickly which may indicate a difference in saturation intensity between periodic and formative actions. It could be that the red function reaches a maximum at rather low intensities, whereas the near-infrared function (owing to a weak contamination) is gaining in strength up to still higher intensities.

The next experiment in part was a variation of the preceding one, with interconversion of red and blue irradiations. At the same time we wanted to find out the effect of darkness, given during a few days, for the following reasons. GENTCHEFF and GUSTAFSSON (27) obtained quick flowering in spinach seedlings, grown on sugar media in complete darkness. Since then, all types of plants (long-day, short-day) were shown to flower quickly in complete darkness throughout, when their young embryos were grown on a special sugar medium (65, 79). Also BEST's curves (2; Fig. 1, p. 3) compel to test darkness. The defoliation tests of LANG and MELCHERS (44) had presented evidence for the idea that darkness is active in the same direction as long days. The fact that flowering occurred only if the plants were defoliated indicates that an inhibitory action is exerted by the leaves after short-day treatment, and is stable in subsequent darkness. How many long days are necessary to destroy this, so that thereafter in darkness stem elongation can go on without defoliation?

Plants grown in short days in the greenhouse were given darkness or long days in red light, or combinations of both, for six days altogether. After these six days, the plants were given long days in blue light to obtain a quick completion of the reactions, leading to stem elongation. The combinations were: 6 days in dark (D), 6 long days in red light (LR), 1 LR + 5 D, 3 D + 3 LR, 3 LR + 3 D, and a control brought from the greenhouse immediately into long days in blue light. Two more groups were added, *viz.*, 15 LR and 15 short red days (SR).

The results are presented in table 10. They are not accurate to one day, but sufficiently so for a general conclusion. Further results of this type will be presented in section IV.3.2. and in chapter V (p. 31), confirming this first result.

There is a great difference in activity between long and short days in red light. Short days in red light have fully inhibited the induction, or almost so (7%) as compared with the control group. Long days in red light, at least up to 15, appear to be about non-inhibitive, provided they are followed by long days in blue light. Days in dark had the same effect as long days in red light, which does not agree with observations of LANG and MELCHERS (44) that plants with leaves in darkness do not produce stems.

IV.3.2. *The effect of a few days in coloured light between days in white light*

The foregoing section contains results from experiments in which plants, after having grown in short days in white light, were brought into a special light colour, and irradiated during a definite number of days with 8 or 16 hours per day of this light. Hereafter, these plants were transferred to another colour and given long or short days.

In this section, experiments of the same structure will be described, but now receiving the last mentioned long-day or short-day treatment in the greenhouse.

TABLE 10. Stem elongation in *Hyoscyamus* in 16-hour days in blue light (LB) after pretreatment during 6 or 15 days with either continuous darkness, or 16-hour days (LR) or 8-hour days (SR) in red light. Light intensities about 9200 ergs/cm²sec. Temperature *ca.* 22°C. Pretreatment started 2-5-'58. Averages of 3 plants.

Pretreatment	Days to stem elongation	Days in LB	Profits of pretreatment as compared with blue only	
			In days	In percent
0	26	26	–	–
15 LR	29	14	12	80
15 SR	40	25	1	7
6 LR	28	22	4	67
6 dark	28	22	4	67
1 LR + 5 dark	27	21	5	83
3 LR + 3 dark	26	20	6	100
3 dark + 3 LR	26	20	6	100

Plants were exposed to high intensity blue light for 16 or 8 hours per day during 0, 8, or 15 days, or until stem elongation started ("continuous"); also groups receiving 15 days of 8 or 16 hours of red light per day were added. Afterwards they were brought to the greenhouse, where they received natural daylight during 8 hours per day, or during full summer daylength. The experiment started 16-4-'58. The results are given in table 11. In short-day after-treatment none of the plants elongated, except those that received the pretreatment until elongation had already started. This indicates again that induction was not complete after 15 days, and moreover, that almost no additional induction took place during short-day after-treatment. (*cf.* p. 21).

In the 0-group, brought immediately into long-day greenhouse after short-day greenhouse exposure, stem elongation started after 21 days. All series with intermission of coloured light were slower, which may be a matter of temperature, or even photosynthesis. More interesting, however, are differences between the various colour treatments, especially the relation between long- and short-day exposures to blue light with after-treatment in long days. Short-days in blue light proved to be less active in elongation than long days in blue light. Long days in red light were hardly less elongative than long days in blue light, while short red days were fully inhibitive (*cf.* also Table 10). These differences are given in table 12, also in percentages.

The observation that a period of about two weeks of long days in red light – if followed by a suitable additional treatment – is favourable for shooting, whereas continued culture in long days in red light results in strongly retarded shooting, seems another case (*cf.* also p. 22), indicating that shooting in *Hyoscyamus* can only be described as the combined effect of two reactions which we have distinguished as "periodic" and "formative" respectively (p. 6).

The only plausible interpretation seems that in long days in red light (but not so in short days) some favourable reaction takes place, which, according to table 6 (p. 20), may be interpreted as a response upon the duration-ratio of light and darkness, and was denoted as periodic reaction. However, that this induction reaction cannot become effective during continued growth in long days in red light, must be due to another reaction denoted as formative reaction (p. 6) which, in red light, inhibits elongation. These observations strongly remind of

TABLE 11. Days to shooting in *Hyoscyamus* upon pretreatment with different numbers (0, 8, 15, or continuous) of long or short days in red or blue light (9500 ergs/cm²sec) followed by natural long days or 8-hour days in the greenhouse. Pretreatment started 16-4-'58. Averages of 3 plants.

Pretreatment		Second treatment	
		Days to stem elongation from beginning of pretreatment	
Hours daily in coloured light	Number of days	16 Hours white	8 Hours white
–	0	21	>80
16, blue	8	22	>80
16, blue	15	24	>80
16, blue	continuous	26	26
16, red	15	25	>80
–	0	21	>80
8, blue	8	24	>80
8, blue	15	28	>80
8, blue	continuous	>80	>80[1]
8, red	15	36	>80

[1] In blue light until the end of experiment.

TABLE 12. Stem elongation in *Hyoscyamus* as recalculated from the figures for long-day after-treatment of table 11. Effect of pretreatment expressed in days and as percent, compared with long day in white light.

Pretreatment		Effect of pretreatment as compared with long days in white light	
		In days	In percent
Hours daily in coloured light	Number of days		
–	0	0	**100**
16, blue	8	7	88
16, blue	15	12	80
16, blue	continuous	21	81
16, red	15	11	73
–	0	0	**100**
8, blue	8	5	63
8, blue	15	8	53
8, blue	continuous	–	–
8, red	15	0	0

the shooting reaction in wheat as described by STROUN, who distinguished a "photostade" and a "spectrostade" (78) (see also p. 6).

A series receiving a similar introductory treatment, now applying greenhouse daylight, showed the same result and corroborated the finding that in *Hyoscyamus* an "inductive" period not necessarily leads to elongation. Plants were grown under short days (8 hours) in the greenhouse. This treatment was interrupted with 0, 8, or 15 long (summer) days, or followed by continuous exposure to long (summer) days. The experiment started 21-5-'58. In plants of the "continuous" group, stem elongation started after 17 days and so did it in those receiving 15 long days. The 8 and 0 long-day groups remained vegetative

throughout. The rate of elongation was much lower in plants receiving 15 long days than in the continuous long-day group; the first never got open flowers. Thus, these results are equal to those obtained with after-treatment in short days in white light following treatment with long days in blue light, as can be seen from table 11 (as compared with table 10).

A last experiment of this series is concerned with the daylength dependence of stem elongation on irradiation with near-infrared (6000 ergs/cm² sec). Plants grown in 8-hour days in the greenhouse, received either darkness or 2, 10, or 14 hours of near-infrared per day during 7 days, while one group received 2 hours near-infrared every 12 hours. After this week the plants were put back into the greenhouse, now under long-day conditions. A control group was kept in short days in white light and then, together with the others, subjected to long days in white light. The experiment started 21-5-'58. Results are compiled in table 13. Days to stem elongation from the beginning of the treatment are shown in figure 6. Average plants are represented on plate 2a.

TABLE 13. Dependence of shooting of *Hyoscyamus* upon 7 days in dark, with 0, 2, 10, or 14 hours of near-infrared (*ca.* 6000 ergs/cm²sec) per day, or with 2 hours of near-infrared every 12 hours. Afterwards in long days in the greenhouse; a control series was moved directly from short days into long days in the greenhouse, at the same day as the other groups. Experiment started 21-5-'58. Averages of 4 plants.

Hours of near-infrared daily during 7 days in dark	Stem elongation		Number of days between short-day pretreatment and stem elongation	Stem length at	
	Date	Days after beginning of experiment		21-6 (cm)	24-6 (cm)
0	10-6	20	20	26	32
2	10-6	20	20	24	30
10	11-6	21	21	21	27
14	10-6	20	20	24	30
2 × 2	9-6	19	19	28	35
control (no dark)	17-6	27	20	9	13

Here again (*cf.* p. 25), the dark series (darkness during 7 days) has reacted as if it was in long days (as compared with the group that was kept in short days one week more). This confirms the last result of the foregoing section. All daylengths given in near-infrared produce about this same reaction. Only the 10-hour group is, may be, somewhat slower. We can say then, that, in this type of experiment, near-infrared has no evident action of itself. It is only possible to demonstrate the near-infrared activity by its antagonizing a preceding light treatment within the same daily cycle. Thus, a positive action of light (white, red) can be antagonized by near-infrared (and blue). Blue can produce both effects, *viz.*, it acts as light, and it can antagonize this action. A double action of near-infrared was demonstrated in *Avena* coleoptile growth (46); however, so far in *Hyoscyamus* no evidence for this was found.

IV.4. DISCUSSION

The data on shooting of *Hyoscyamus niger* in relation to wavelength and daily duration of irradiation, presented in this chapter, can only be under-

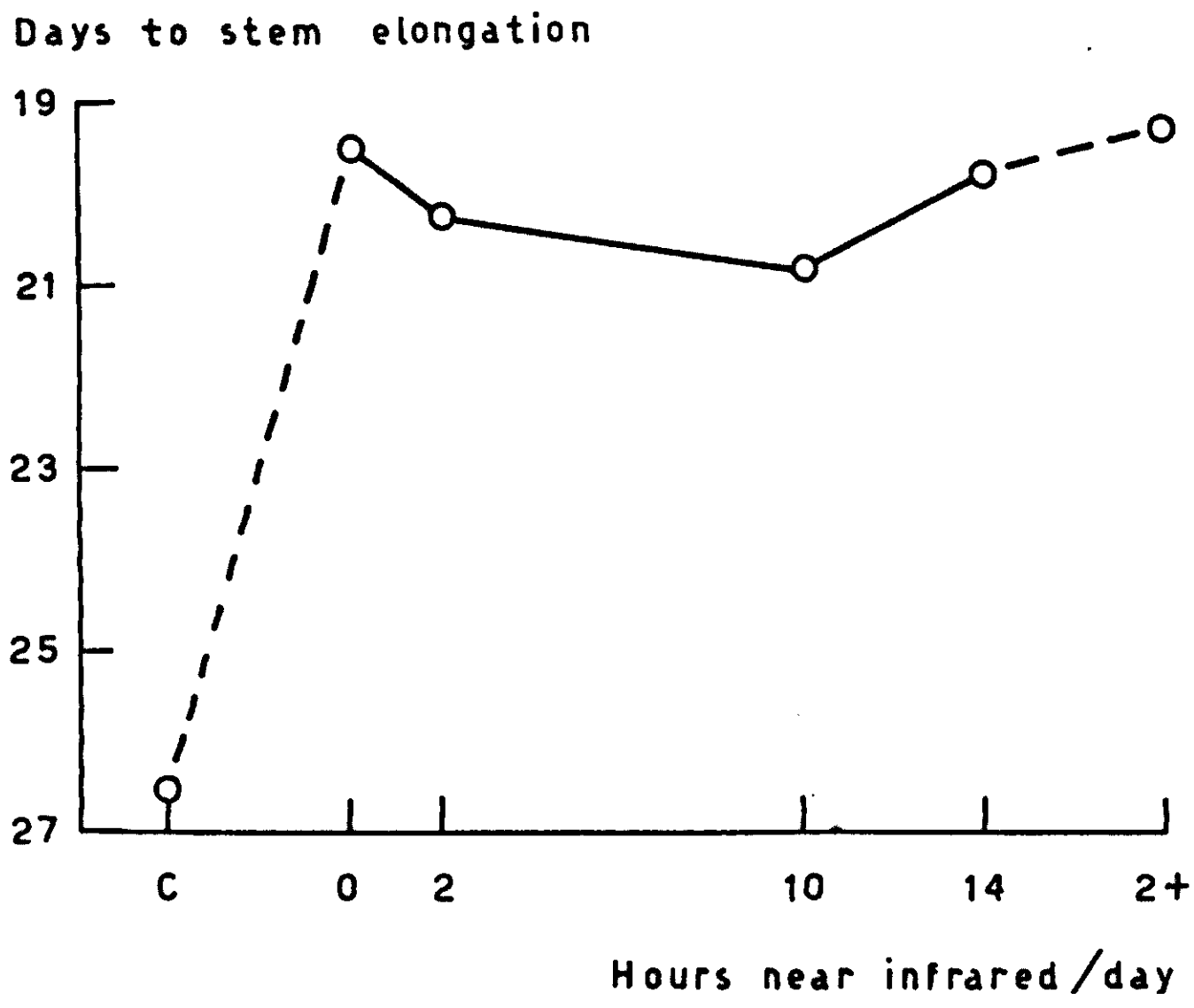

FIG. 6. Days to shooting of *Hyoscyamus* in long summer days, following 7 days in darkness with exposure to near-infrared radiation of different duration. See also table 13.

stood as the combined effect of two reactions, a periodic and a formative one.

The dependence of shooting on wavelength of supplementary irradiation following white light, *e.g.*, cannot be understood as a single reaction mediated by the red – near-infrared system. This dependence seems to be mainly due to the formative reaction, whereas daylength action is weak since red supplementary light has only a small promotive effect on stem elongation (*cf.* Table 4, and 77) which is strongly enhanced by a subsequent near-infrared supplement (*cf.* Fig. 5). Daylength, however, is of importance, which may be concluded from the observation that red light following 14 hours of white irradiation is not as effective in suppressing stem elongation as when following 8 or 11 hours of white light (p. 19). Moreover, larger doses of red light should be more strongly inhibitive than small ones, if they were merely formatively active. However, daylength extensions with red light increasingly favour flowering with increasing duration (p. 21). A reducing action of supplementary near-infrared on daylength could not be detected because of its strong photoformative activity. In all combinations, near-infrared irradiation resulted in promotion of stem elongation.

The above results are exactly equal to those obtained with *Brassica* and other Cruciferae, as STOLWIJK and ZEEVAART (77) already pointed out. Only one difference remains between the Cruciferae and *Hyoscyamus*, as far as can be seen now. *Hyoscyamus* under white irradiation, is a qualitative long-day plant, whereas Cruciferae in short days flower only somewhat slower than in long days. In chapter V, evidence will be presented for the statement that also *Hyoscyamus* by no means has an absolute "critical" daylength below which no flowering can occur, which statement also is in accordance with the curve presented by BEST (2).

Also shooting in coloured light only cannot be described as merely due to a

formative light action (p. 23). This was concluded from the observation that plants, kept continually in red light, remained vegetative for a long period, whereas a few long days in red light were favourable, as could be shown by adequate subsequent treatment (Table 10; *cf.* also 56 and 77).

That, on the other hand, the formative light action also plays an important rôle is demonstrated by the observation that the onset of shooting and the initiation of flower buds in equal daylengths is strongly dependent on the spectral quality of the light.

The effect of long-day treatment on stem elongation can be strongly reduced by the after-treatment. This could be observed in 8-hour days in white light on plants that before had been in 16-hour days in red or blue light or in 8-hour days in blue light for a limited period. Elongation in blue light (16-hour days) started after 26 days, but plants that had been in blue light (16-hour days) during only 15 days remained in the rosette state if subsequently kept in 8-hour days in daylight. In chapter V, it will be shown that the strength of this inhibition depends on the daylength of the last treatment. The stem promoting effect of a certain treatment is also influenced by the wavelength region of subsequent exposure. Red light has a highly inhibitive action, be it in long day somewhat less than in short day (*cf.* p. 24), and, according to MEYER, still less at higher intensity (*cf.* 16).

Plants that have been in blue light for 16 hours per day during 8 days, and then were placed in 8-hour days in red light showed a real regression from the flower producing state to the vegetative state. After a long time (all leaves originally present having died off) the plants consisted of two rosettes between which a flower bud (only the calyx being well shaped!) had developed. This bud, obviously, had originated from the 8 days in blue light while short-day red after-treatment had inhibited stem elongation and production of more flower buds. With long-day red after-treatment, the inhibition was somewhat less since flower bud production went on and inflorescences elongated, but again the main stems were very short. The numerous flower buds only had the calyx normally developed. The plants were growing vigorously (see Plates 1g and h, and 2a) so that sugar synthesis cannot have been too low for normal flowering. It must be concluded that the defective development is due to photomorphogenetic inhibition of normal flowering.

In all experiments in which this has been checked, stem elongation was strictly correlated with flower bud initiation, flower buds appearing shortly after the start of stem elongation. When stem elongation proceeded only slowly (or not), buds came in relatively earlier, but did not develop to open flowers under these circumstances.

Finally, some remarks should be made on the observation concerning the influence of days in darkness or of (photosynthetically inactive) near-infrared irradiation on stem elongation. It could be observed that 7 days in continuous darkness (following 8-hour days in white light) were active as 7 "inductive" days, and that the same was true for all near-infrared daylengths used, provided they were followed by favourable treatment.

The only possible remaining photoperiodic dependence now seems to be that *Hyoscyamus* plants, if not inhibited by light, are capable of quick flowering (*cf.* p. 2); light antagonizes this reaction effectively in short days, and less so in long days. This would be in accordance with the curve presented by BEST (2, *cf.* Fig. 1, p. 3). It was suggested (IV.3., p. 23) that near-infrared only has a

very weak inhibiting action (Table 13), that blue light is somewhat more inhibitive (Table 9), and that red light is strongly inhibitive, also in long days, and even in continuous treatment, be it decreasingly so. This idea could be substantiated by subsequent experiments, detailed discussion of which is to be found in chapters V and VI.

CHAPTER V

FURTHER ANALYSIS OF SHOOTING AS DEPENDENT ON THE TIME SCHEDULE OF IRRADIATION

V.1. Introduction

The mechanism of flowering in *Hyoscyamus niger* was described and discussed by Lang (43).

The action of a favourable treatment has an inductive nature, *i.e.*, flower bud initiation can take place under inhibitive conditions after a short period of such a favourable treatment. However, flowering is accelerated under continued favourable treatment of the whole plant. The induction can be built up by a series of groups of a sub-liminal number of favourable days, with inhibitive periods intercalated, until the critical dose is reached (fractional induction). Usually, the growing point needs not to be induced directly; the induction is transferred to it from the leaves. This transmissibility has also been demonstrated in grafting experiments. These results led to the assumption of a substantial floral stimulus.

According to Lang (43, p. 272), ... "an essential factor... is an inhibitory effect of long dark periods, an effect which under short-day conditions prevents the formation of the floral stimulus". This conclusion is based on the following data: continuous illumination is optimal for flowering, darkness is not necessary; short light periods in combination with sufficiently short dark periods result in flowering; the critical night length shifts to higher values at lower night temperatures.

However, short light periods in combination with very long dark periods are favourable for flowering, whereas, moreover, complete defoliation results in flowering under all light regimes. This renders it acceptable that inhibition only occurs in certain combinations of light and darkness. Owing to additional data, Lang (43, p. 274) tends to adopt the idea that an inhibitor which arises in the dark must be removed in the first hours of light. For this reason he could put only little stress on, *e.g.*, night-temperature results. Here, our view deviates from his in that we suppose that light gives rise to an inhibition by way of the production of an inhibitor-precursor. This precursor is assumed to be converted into an inhibitor mainly during darkness, and thus disappears, while also the inhibitor is used up in its action. The processes leading to induction of flowering thus are being inhibited or slowed down mainly during dark hours, immediately following a light period. This dark inhibition is a function of duration, intensity and quality of the preceding light treatment, and of internal and external factors obtaining during darkness.

The effect of supplementary near-infrared irradiation can easily be made to fit into this picture by the additional assumption that near-infrared destroys the inhibitor-precursor.

The daylength-dependence curve for long-day plants presented by BEST (2) was tested with a technique adapted to very short daily illuminations, *viz.*, treatment of a series of plants during a few days only with a range of daylengths, followed by an after-treatment in *long* day (and not in short day as is usual). Also the complementary treatment is possible: a sub-critical pre-treatment in long day, followed by a range of daylengths (in a series of plants) the induction values of which have to be investigated.

Theoretically, this method can be defended in the following way. Induction to flowering has to be built up in the plant from zero to 100%. The more inhibitive the conditions are (normal "short days"), the longer time it will take before 100% induction has been reached. Hence, the "critical" daylength moves to shorter days, the longer the treatment is applied. When the flower-inducing process is developing too slowly (at strongly inhibitive conditions), no flower initiation will occur, since, possibly, induced leaves are dying before full induction was reached, or owing to any other back reactions.

The total induction from zero to 100% can be built up at one daylength; but also in a combination of two different daylengths, a strongly inhibitive and a less inhibitive one. With the strongly inhibitive daylength, given during a limited period, *e.g.*, 10% induction can be accumulated, and the other 90% can be supplied thereafter quickly with weakly inhibitive (= "inductive") days. In this way, all daylengths result in flowering within reasonable experimental periods, with relatively small differences.

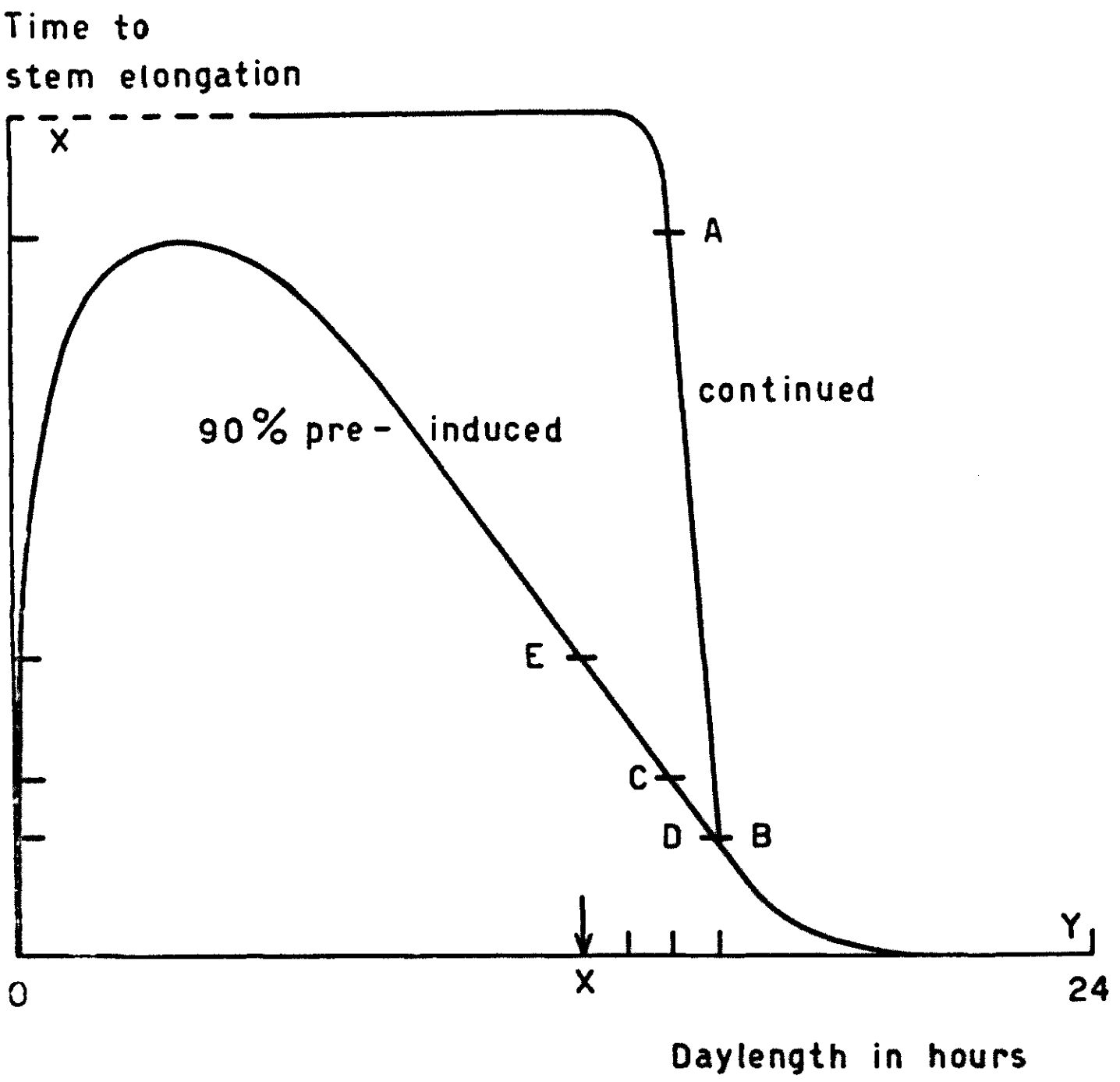

FIG. 7. Schematic representation of the difference in daylength dependence of shooting of a long-day plant between continued treatment and limited treatment, the latter preceded by induction at a definite daylength.

An example may illustrate the above proposition (Fig. 7). Curve XABY will be obtained with continuous treatment with the respective daylengths. The linear region AB represents only one-hour difference in daylength and results in a difference in time to shooting of, *e.g.*, 60 days. Curve OEY is obtained with plants pre-induced for 90%. Due to the pre-induction, slope CD has a steepness of $^1/_{10}$ of AB, assuming that the susceptibility to inhibition is the reverse of the degree of induction. CD presents a difference in time to shooting of 6 days only, through the same 1-hour difference in daylength. Through a range of 3 hours DE represents a difference of 18 days. This same range with continued treatment will only be finished after $3 \times 60 = 180$ days, or even longer, owing to possible back reactions.

V.2. Daylength dependence

The following experiments show the difference in daylength sensitivity of elongation in treatments given continuously or during limited periods only, as suggested in figure 7.

Plants grown in short days were subjected to continuous white light during 7 or 14 days. Hereafter, plants of both pretreatments were treated with a range of daylengths (0, $\frac{1}{4}$, 8, $12\frac{1}{4}$, $12\frac{1}{2}$, $12\frac{3}{4}$, 13, $13\frac{1}{4}$, $13\frac{1}{2}$, or 24 hours). Thus, the 24-hour treatment is a continuous one, whereas all other treatments are a combination of two differently inductive periods. The group which received a pre-treatment of 14 long days still has a steep switch-over from the vegetative to the flower initiating state around the "critical" daylength of 13 hours. In the group with 7 long days, the critical day is longer, and the slope is suggested to be even steeper than with 14 long days; this, however, is open to some doubt. Results of a stem length measurement are presented in figure 8. Average plants of this experiment are presented on plate 2c, d, and f.

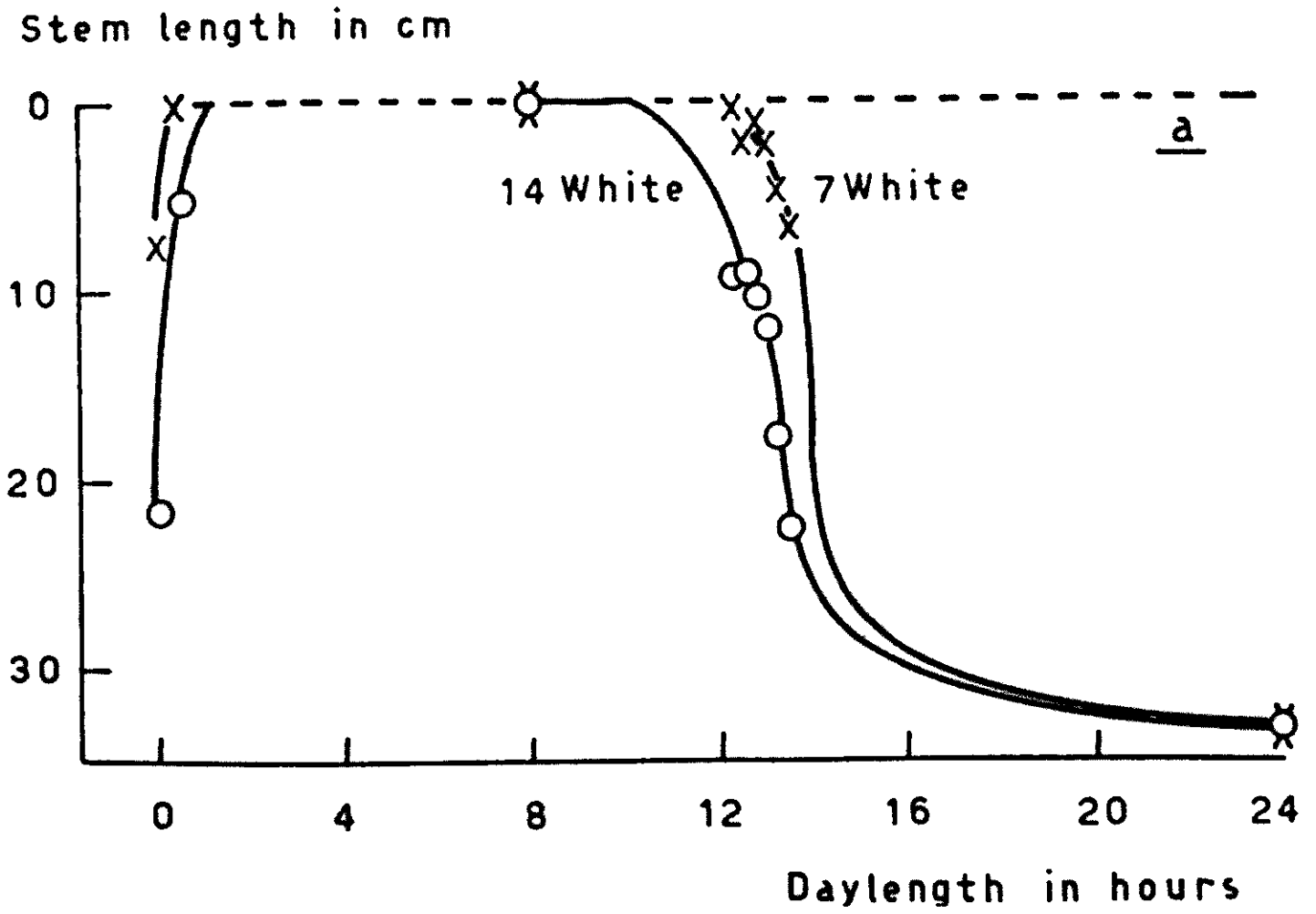

Fig. 8. Change in daylength dependence upon pre-induction in *Hyoscyamus*, in white light; × = 7 days continuous white light (*ca.* 20,000 ergs/cm² sec) before daylength treatment; o = same during 14 days. Experiment started 24-9-'58. Stem lengths measured after 54 days of treatment. Averages of 3 plants.

Pre-treatment for more than 14 days would result in still less steep curves. This is indicated by figure 9 which was obtained in a treatment during 14 days with a daylength range in red light, followed by continuous white light (*cf.* also Plate 2e). The results presented in figure 8 are obtained after pre-induction, those of figure 9 with after-induction. It will be clear that the results presented in figures 8 and 9 substantiate the curve proposed by BEST (Fig. 1, p. 3), and also the difference of the curves obtained with continuous and with limited treatment respectively, as suggested in figure 7 (p. 32).

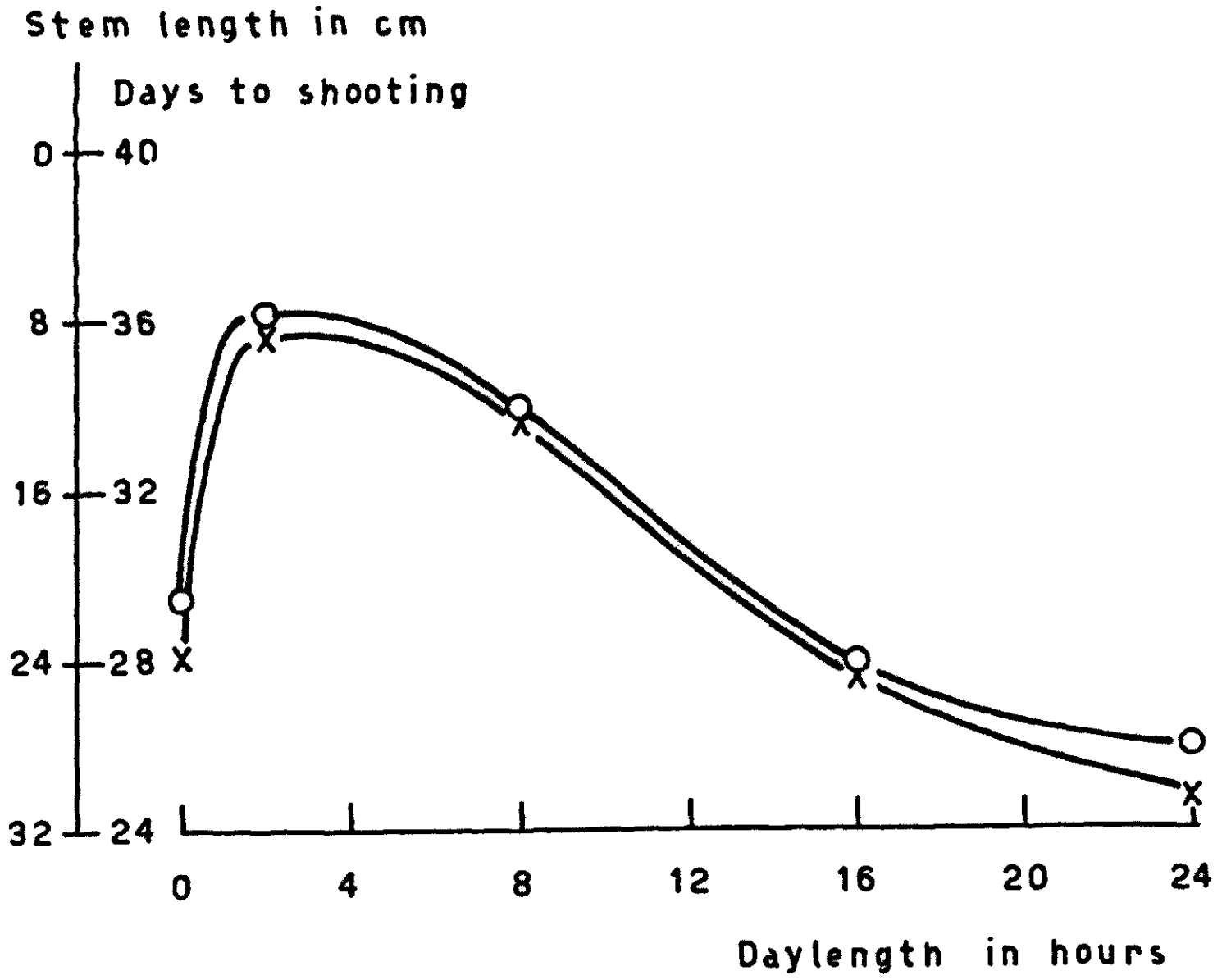

FIG. 9. Daylength sensitivity of time to shooting (×) and stemlength (o) in *Hyoscyamus*. Daylength treatment in red light (*ca.* 10,000 ergs/cm²sec) during 14 days; thereafter continuous white light (*ca.* 20,000 ergs/cm²sec). Experiment started 24-9-'58; measurement after 35 days. Averages of 3 plants.

Stem-length values recorded on a certain day are closely related to the numbers of days onto the moment of shooting (*cf.* Fig. 9). The dark-treatment is somewhat behind the long-day treatment. The exact dark position is hardly obtainable, owing to the very steep decay of the curve in short daylengths, so that a sufficient protection of the plants against minor illuminations is difficult to maintain; the slightest exposure to light will cause some inhibition already. Moreover, there is a considerable difference in photosynthesis between plants receiving either zero or 24 hours of light.

That, indeed the faintest irradiation seems to be of importance, could be concluded from the following experiment.

Leaf petioles of just full-grown leaves were measured at the end of a 10-day exposure to a series of daylengths in red light. The results are presented in figure 10. Graph *a* shows the result on a linear time-scale: at a daylength of about 4 hours a minimum is reached. Plotting the day-lengths on a logarithmic scale results in graph *b*. This result cannot be seen as a proof for log-linearity of the reaction, but it is indicated. The extrapolation to the zero-day stem-

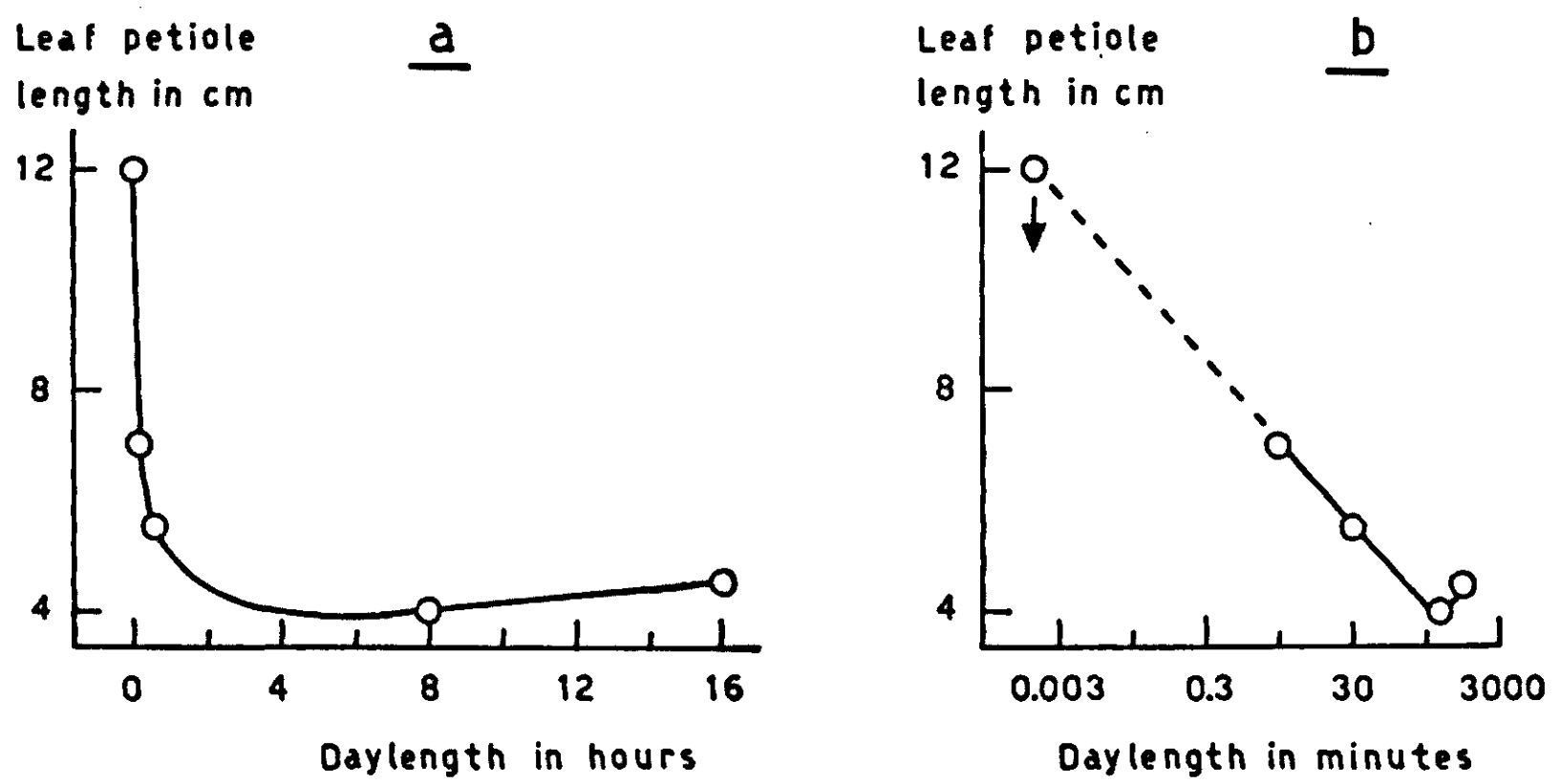

FIG. 10. Leaf petiole length in *Hyoscyamus* upon a 10-day treatment with red light (5500 ergs/cm²sec) during 0, 3, 30, 480 or 960 minutes. Measurement of 16-11-'58, averages of 4 plants; *a* and *b*, same data on a linear and a logarithmic time scale respectively.

length level (which extrapolation is somewhat hazardous) might indicate that irradiations as short as $^1/_5$ of a second already may have measurable effects, at least with the applied intensity of red light (*ca.* 5500 ergs/cm² sec).

Evidently, the method of partial induction of the plants, before or after applying a range of daylengths, allows the study of very short daylengths which was impossible with continuous treatment, owing to energy starvation of the plants. The results show that BEST's curve is valid for *Hyoscyamus*.

This method, however, can be used only if the plants remain sensitive to inhibitive treatment even after rather long inductive periods, *e.g.*, up to shooting, and will be valid only if inhibition has no after-effect, neither by remaining active during longer periods than its application, nor by destruction of previous induction. Both conditions are fulfilled, as is shown hereafter.

V.3. INDUCTION

In this section more data are presented showing that elongation can be influenced until it actually starts.

The first experiment is an extension of the last one described in section IV.2. (p. 22). Plants grown in short days were exposed to days in artificial light, composed of 8 hours strong white light, followed by 8 hours low intensity red irradiation. This photoperiod was extended with 2 hours of near-infrared irradiation, in various samples after a different number (0, 2, 4,...., 12, 21, 28, 35, or ∞) of days. (Infinite means that no near-infrared was applied).

The results of this experiment are presented in table 14, and those of a second one (basic daylength $13^1/_4$ hours white light only) in figure 11 (p. 36). Representative plants of the second experiment are shown on plate 2b.

It is clear from figure 11 that no initial period is apparent during which the elongating action of near-infrared supplementary irradiation is inactive. Obviously, the plants are susceptible to near-infrared immediately from te beginning of the long-day treatment. Thus, the elongating function obtained with near-infrared starts before long-day induction is accomplished, and irrespective

TABLE 14. Days to stem elongation in *Hyoscyamus* upon treatment with 8 hours in white fluorescent light (w; 20,000 ergs/cm²sec) supplemented with 8 hours in red light (r; 1000 ergs/cm²sec), and then followed by 2 hours near-infrared radiation (i; 1000 ergs/cm²sec) from x days after the beginning of long-day treatment onto the beginning of stem elongation (y days). Experiment started 15-1-'58. Temperature *ca.* 19 °C. Averages of 4 plants.

Treatment: x (8w + 8r) + y (8w + 8r + 2i)		
Days without near-infrared x	Days to elongation $(y + x)$	Days with near-infrared (y)
0	26	26
2	28	26
4	27	23
6	27	21
8	27	19
10	28	18
12	29	17
21	36	15
28	44	16
35	45	10
∞	46	0

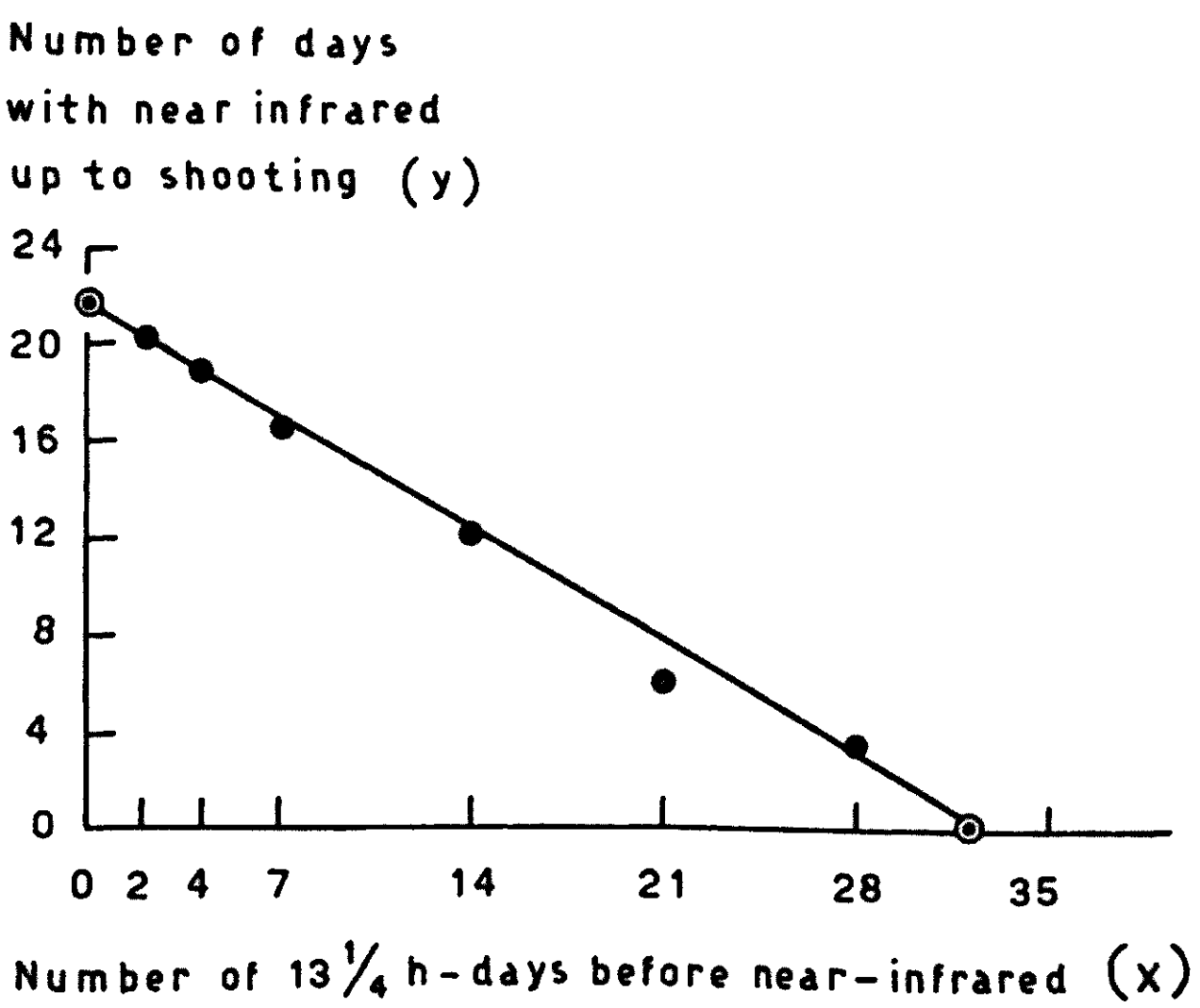

FIG. 11. Time to shooting in *Hyoscyamus* upon a daily photoperiod of $13^1/_4$ hours white light (*ca.* 20,000 ergs/cm²sec), after a different number of days supplemented with 2 hours near-infrared (*ca.* 6000 ergs/cm²sec). Experiment started 5-1-'59. Averages of 4 plants.

of daylength, as may be concluded regarding earlier results (Table 8). The same conclusion was drawn from figure 5 (p. 21).

The trick in this type of experiment is to find a basic treatment by which the plants proceed to shooting slowly, nevertheless within reasonable time. This is most easily accomplished by long days, obtained by low intensity red light supplement to a basic period in strong white light (Table 14). However, daylengths just above "critical" in white light only can be used as well (Fig. 11). The requirement of an intermediate reaction rate has a double reason. It is necessary to reach the onset of elongation of the group without near-infrared in due time. On the other hand, the elongation should not be too strongly induced by the basic treatment, because then near-infrared has too small an effect, owing to the fact that elongation of all plants already is nearly maximal.

These conclusions are supported by the results from the following experiment. Plants were brought indoors under 10-hour days in white light. After some days, one group received 6 summer days in the greenhouse, the others remained in short days in artificial light. Hereafter, all plants, except controls in short and long days, were treated with a range of daylengths in white light during 6 days. Finally, all plants were brought to the greenhouse in natural days (except controls in short days which did not start elongation for the duration of the experiment). Thus, at the moment at which the daylength treatment started, the plants had received either 6 long days, or none. In figure 12, the number of days between short-day treatment and the onset of shooting is plotted against the logarithm of the duration of the photoperiod applied during the last 6-day treatment. Average plants are presented on plate 2g (pre-induced by 6 long days) and 2h (not pre-induced).

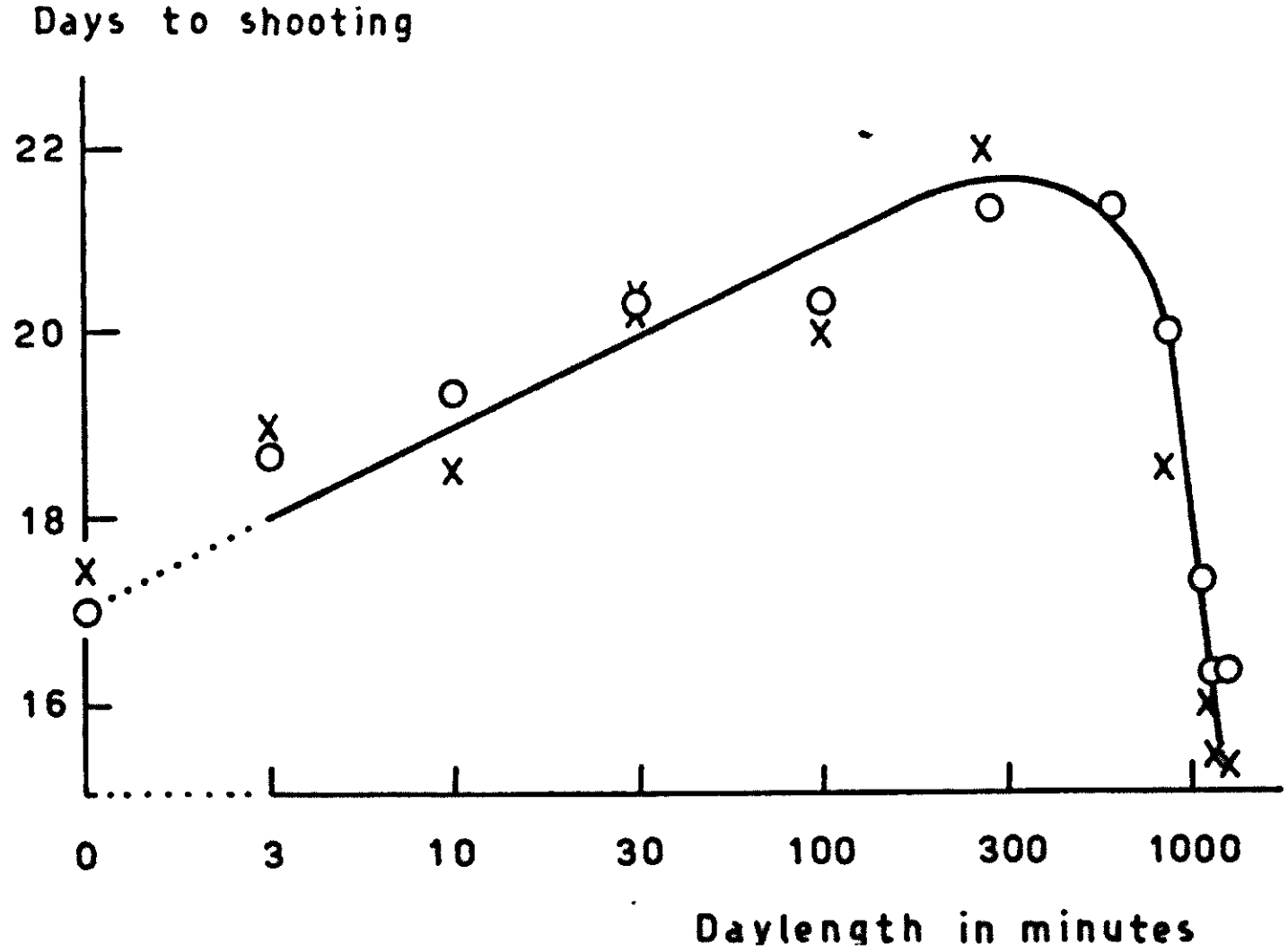

FIG. 12. Days to shooting in *Hyoscyamus* upon two different treatments in white light (*ca.* 20,000 ergs/cm²sec) following short days in greenhouse. x: 6 days, applying different daylengths, long summer days in greenhouse; o: as above, but 6 long summer days preceding the period of 6 days in different daylengths. Experiment started 11-8-'59 (×), or 17-8-'59 (o). Averages of 3 plants.

The result confirms those of the preceding section and also the curve suggested by BEST (2, *cf.* Fig. 1, p. 3).

It is clear that with this experiment, again, no limited induction period is demonstrated, since 6 long days caused insufficient induction to reduce the sensitivity of the plants to inhibition. Because both treatments resulted in equal curves (Fig. 12), it made no difference whether the plants had received 6 long days before or after the exposure to various daylengths. This allows the conclusion that no after-effect of inhibition was present, and that, obviously, long days applied immediately were, or remained fully effective. Thus, it can be concluded that the effect of any treatment is fixed irreversibly within the same day, or approximately so, in *Hyoscyamus*.

An additional experiment, dealing with the problem of after-effects of inhi-

bitive days, was carried out to see whether there really is no after-effect into subsequent days in darkness. Plants that had received 6 summer days in the greenhouse, were placed in darkness for 6 days, and received a series of daylengths (including zero daylength) on the second day only, after 40 hours of darkness. After the 6 days, all plants were returned to the greenhouse in long days. Results are reproduced in table 15, presenting days to the beginning of stem elongation, and stem lengths after 28 days from the beginning of the experiment. Representative plants of an earlier experiment of the same type are shown on plate 3c.

TABLE 15. Days to stem elongation, and stem length in *Hyoscyamus* upon specific combinations of light and dark periods. After 6 long summer days, darkness was applied during 6 days, except for an interruption with red light (9500 ergs/cm²sec) starting on the 40th dark hour and lasting 0, 3, 10, 30, 100, 300 minutes, 12 or 24 hours, respectively, for different groups. Experiment started 7-9-'59. Stem length measured 28 days after beginning of long-day treatment. Averages of 3 plants. Temperature *ca.* 25 °C.

Daylength (minutes or hours)	Days to stem elongation	Stem length after 28 days (cm)
0	17	21
3	20	18
10	19	15
30	20	14
100	19	15
300*	19	13
12 h.	20	14
24 h.+	17	16

* one plant only
\+ two plants only

All groups elongated at about the same day which indicates that none of the treatments had a much stronger inhibitive effect than any other. The subsequent dark days were equally inductive after all daylengths, or previous induction was equally persistant with all daylengths. Thus, the inhibition has no appreciable after-effect, neither directly, nor by annihilating previous induction.

From the series of experiments presented in this section (*cf.* Fig. 12 and Table 15), it can be concluded that the fraction of the necessary induction which is formed per day, is fixed in a very short time, most probably within the same day (or may be even during the "really inductive hours").

Fully induced plants (or plants with induced leaves grafted upon them) become "day neutral". In these plants flowering can no more be inhibited by light. This can be concluded from grafting-experiments (102) and from some "after-effect" results (9), and is once more demonstrated by the following experiment.

Fully induced plants, with stems of about 30 cm were decapitated so that stems of about 2 cm length remained (Plate 3b). From the moment of decapitation onwards, the plants were subjected to a range of daylengths (0, 3, 10, 30, 100 or 300 minutes, and 14 or 24 hours) during 10 days. Hereafter, all plants were placed in natural summer days in the greenhouse. Stem lengths after 27 days from the moment of decapitation are presented in figure 13. Representative plants are shown on plate 3d.

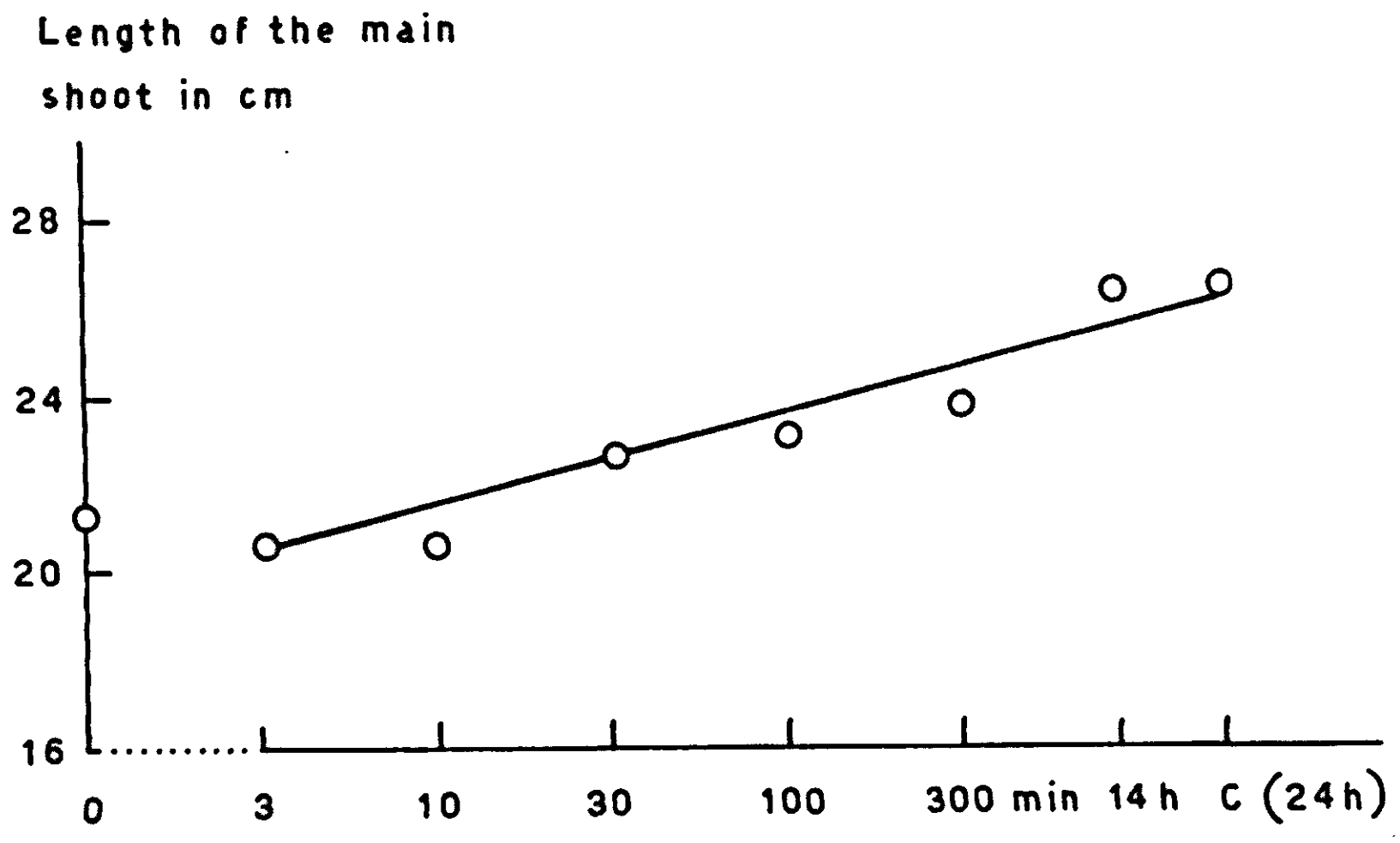

FIG. 13. Shooting of second order stems of *Hyoscyamus* after decapitation, upon a daylength treatment in white light (*ca.* 20,000 ergs/cm²sec) during 6 days after which the plants were kept in continuous white light. Before the experiment, plants had received 30 summer days and had developed stems of about 30 cm which were cut down to 2 cm. Experiment started 6-9-'59, measurement of 3-10-'59.

The dependence on daylength of elongation of secondary stems clearly differs from the elongation of stems of not fully induced plants. This time, a slight and gradual promotion of stem elongation up to continuous illumination is observed, which could be a result of differences in vigour, *e.g.*, due to photosynthesis. However, the photoperiodic inhibition curve with a minimum at about 5-hour daylengths is fully absent. This can be so only if the induced state somehow remains, most probably in the leaves. A further conclusion from this experiment can be that curves of the type of those in, *e.g.*, figure 12 should be corrected with the one presented here (Fig. 13), so that continuous darkness and continuous light have their onset of shooting at the same day really, so representing the induction reaction more accurately.

In this section, it could be demonstrated that the induced state is gradually produced, and that it is stable. Secondly, it was concluded that the inhibition of the induction reaction does not function *via* a long-lasting inhibitor. Finally, it can be stated that the plants become insusceptible to inhibition as soon as they are induced.

V.4. ANALYSIS OF THE DAYLENGTH-DEPENDENCE CURVE

In sections V.2. and V.3., the daylength-dependence curve proposed by BEST (2) for long-day plants was shown to be valid for *Hyoscyamus*. Treatment with continuous darkness for a limited period produced a strong stem elongation impulse; stem elongation is increasingly inhibited with increasing duration of daily illumination up to about 5 hours. With still longer daily illuminations, the inhibition decreases about linearly to approximately the darkness level at continuous irradiation. An analysis of this curve was suggested in

chapter I (p. 3). The rise of the inhibition to high values should be the same effect as light inhibition of etiolation. The decrease at still longer photoperiods should be due to shortage of darkness, assuming that the inhibition by light is favoured by subsequent darkness. Both parts of the curve are somewhat further analized in the following experiments.

During 6 days, plants received a series of short days of various durations, in two intensities of red light (Fig. 14), or in either red of blue light of equal intensities (Fig. 15). After these 6 days, the plants were under natural summer days.

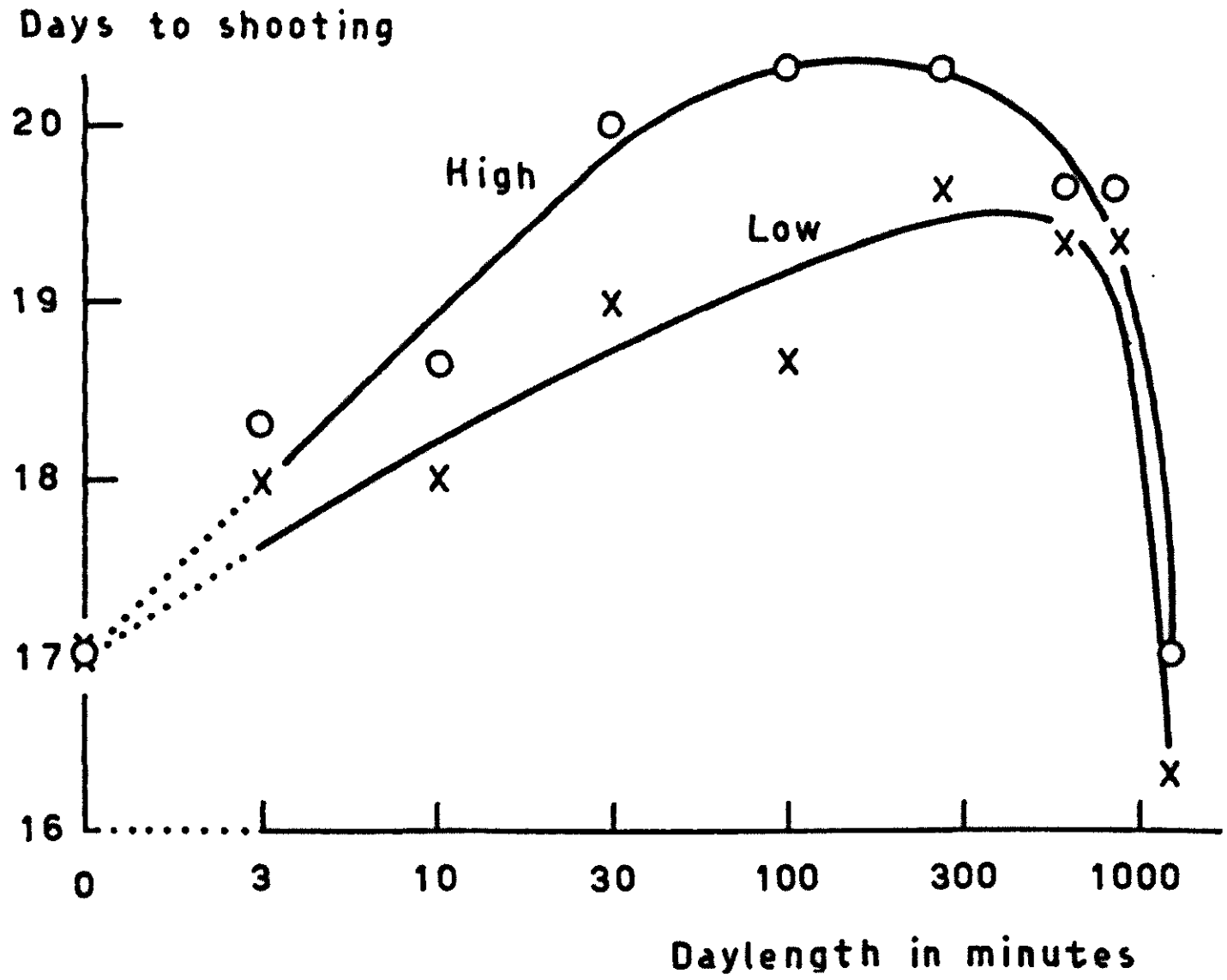

FIG. 14. Days to shooting in *Hyoscyamus* upon a daylength in red light treatment, during 6 days, at two light intensities (o = *ca.* 9500 ergs/cm²sec, × = *ca.* 4250 ergs/cm²sec). Experiment started 17-8-'59. Averages of 3 plants.

In figure 14, the curve representing "days to shooting" rises less sharply at the lower light intensity than at the higher one. The maximum inhibition is found at somewhat longer days in the low intensity curve, and remains below the high intensity effect. The relation between red and blue is the same as that observed between high and low intensity within the same colour (Fig. 14 and 15).

These results are in agreement with those from experiments in which the reduction by light of elongation of plant parts was studied (12; *cf.* Chapter I, p. 4).

It can be concluded from figure 15 that blue light requires longer "short days" to produce maximal inhibition than red light of the same intensity, and maximum inhibition is less than in red light at the same daylength.

The next important feature to be studied is stem elongation in relation to cycle length, and the effect of the latter on the "critical" daylength.

This problem can be easily approached with our technique. If the light in fact leads to the production of an inhibiting after-effect during the subsequent dark period (Chapter I, p. 2; Chapter V.1., p. 31), the length of the dark period should be a regulating factor if combined with short illumination periods. The next experiment gives information on this question. Various daylengths in white

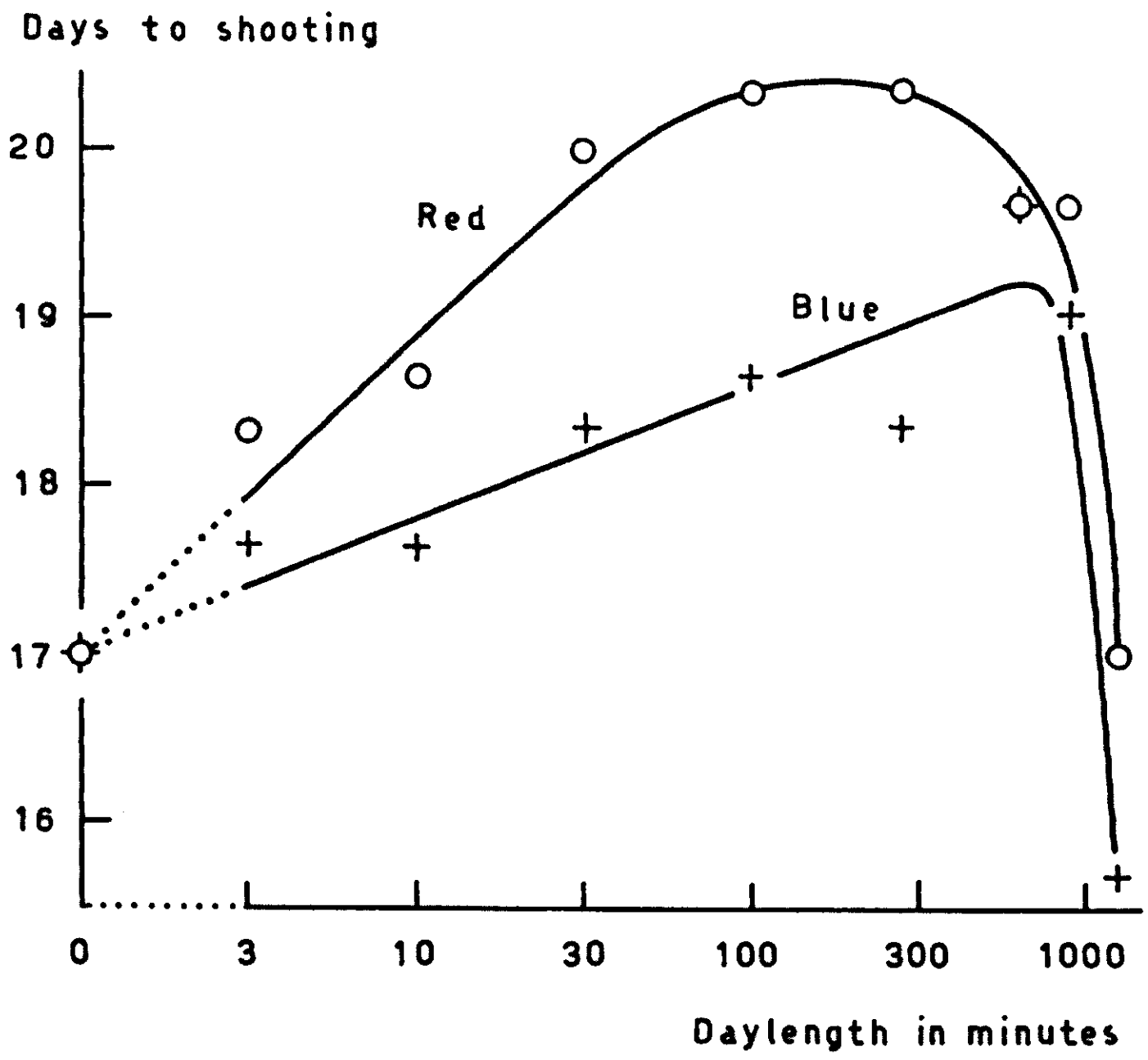

FIG. 15. Days to shooting in *Hyoscyamus* upon a daylength treatment during 6 days, with red (o) or blue (+) light at the same intensity (*ca.* 9500 ergs/cm²sec). Experiment started 17-8-'59. Averages of 3 plants.

fluorescent light (0, 1, 3, 10, 30, 100 minutes, 4, 8, 12, 16, 20, 24, 32, 40 hours, or continually; intensity 20,000 ergs/cm² sec) were applied to *Hyoscyamus* plants in combination with different cycle lengths, *viz.*, 12, 24, 36, or 48 hours, during 6 days. Hereafter, summer days were given in the greenhouse. The results are presented in figure 16.

Inhibition appears to develop more easily in shorter cycle lengths. The maxima in the curves shift to longer days with increasing cycle length. However, the maximum in the 48-hour cycle is at a shorter day than that in the 36-hour one. The maxima of the two shorter cycles are about equally high; those of the longer ones are lower.

We may presume that the relation between the curves presented in figure 16 results from two influences. The first is the fact that strongly inhibitive periods are less frequently returning in longer cycles, so that the curves rise more slowly in longer cycles. The second is due to the fact that with longer dark periods a larger amount of inhibitor-precursor and thus a longer light period can be effectively utilized, which causes a shift of the maxima to longer days. This influence, however, reaches a maximum because light periods also are favourable for stem elongation since only darkness converts the as such inactive inhibitor-precursor into an active inhibitor. That the maximum decreases with longer cycles seems to be the combined effect of the reduced slopes of the inhibition lines and the fact that both during long light periods and during long dark periods no sufficient inhibition gets a chance to develop.

Finally, some additional experiments regarding the red – near-infrared (blue) antagonism were made.

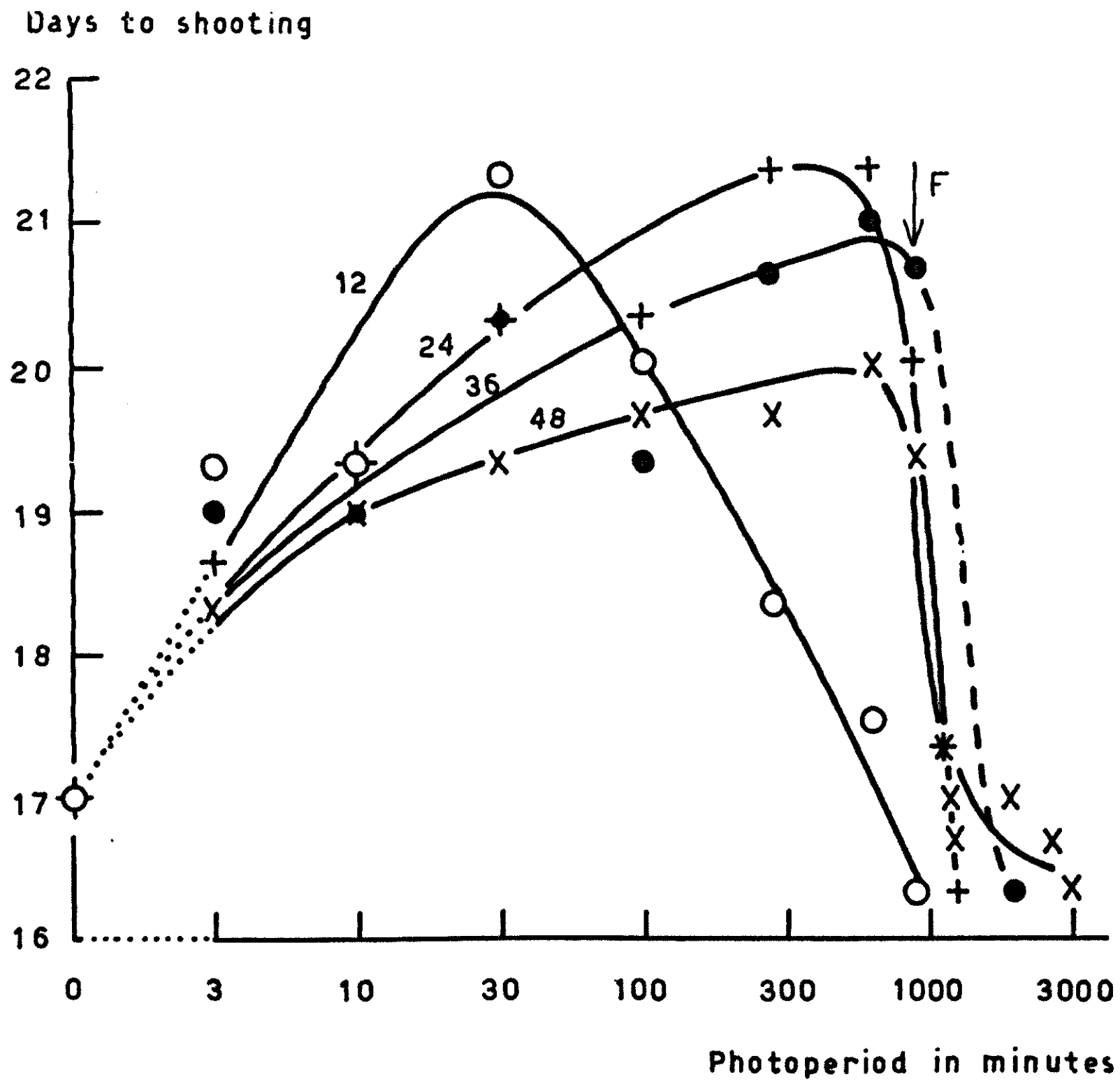

FIG. 16. Days to shooting in *Hyoscyamus* upon a daylength treatment in white light (*ca.* 20,000 ergs/cm²sec) during 6 days with cycle lengths of 12 (o), 24 (+), 36 (●), or 48 (×) hours. Experiment started 17-8-'59. Averages of 3 plants.

From the experiment of STOLWIJK and ZEEVAART (77), discussed in chapter III, (p. 12), and from a similar experiment with three different qualities of blue light (p. 15), the conclusion was reached that the stem elongating effect of near-infrared and blue is a formative one, which antagonizes the inhibitive action of a red irradiation (*cf.* III.3., p. 16).

An interesting question then is how much time daily is required for an induction to result in a definite acceleration of elongation as compared with the control in experiments of usual duration. According to STOLWIJK and ZEEVAART (77), 9 hours of blue light per day are fully sufficient. An experiment was performed, therefore, in which red light was interrupted with blue light each day. The blue light was given during 0, $\frac{1}{3}$, 1, 3, 10, 30, 100 or 300 minutes, or 24 hours (blue-only control). Results are presented in figure 17a; representative plants are shown on plate 3e.

It follows from these data that the inductive irradiation (the blue light interruption) must be given during at least 100 minutes in order to obtain a noticeable acceleration of elongation above that of the red-only control.

However, it is not strictly certain that at least 100 minutes of blue light are necessary for a measurable acceleration of elongation. It might well be that below this duration, the light dose is not yet limiting, but that the 100-minutes

limit is determined by some essential reaction time in the plant. This question is answered by the following experiments.

Plants, having been in 6 summer days before, were irradiated daily with 19 hours of red light supplemented by a series of blue or near-infrared irradiations which combination was followed with darkness so as to complete the 24-hour cycle. The durations of the blue and near-infrared irradiation were 0, 3, 10, 30, 100, or 300 minutes. Results are presented in figure 17b. Average plants of the near-infrared group are presented on plate 3f. Already 3 minutes of near-infrared appear to be maximally active in this combination. Blue is less active, but with 30 minutes saturation is reached which is a somewhat shorter time than the 100 minutes necessary in the experiment of figure 17a. This difference, however, is too small to be sure that in figure 17a elongation was limited by the duration of the interruption and not by the amount of blue light. For this reason a fourth series was run.

Plants were pretreated with 6 summer days and thereafter subjected to red light, which was interrupted for 0, 3, 10, 30, 100, or 300 minutes daily (*cf.* Fig. 17c). During the interruptions the plants received 3 minutes of near-infrared, followed by darkness. Representative plants are shown on plate 3h.

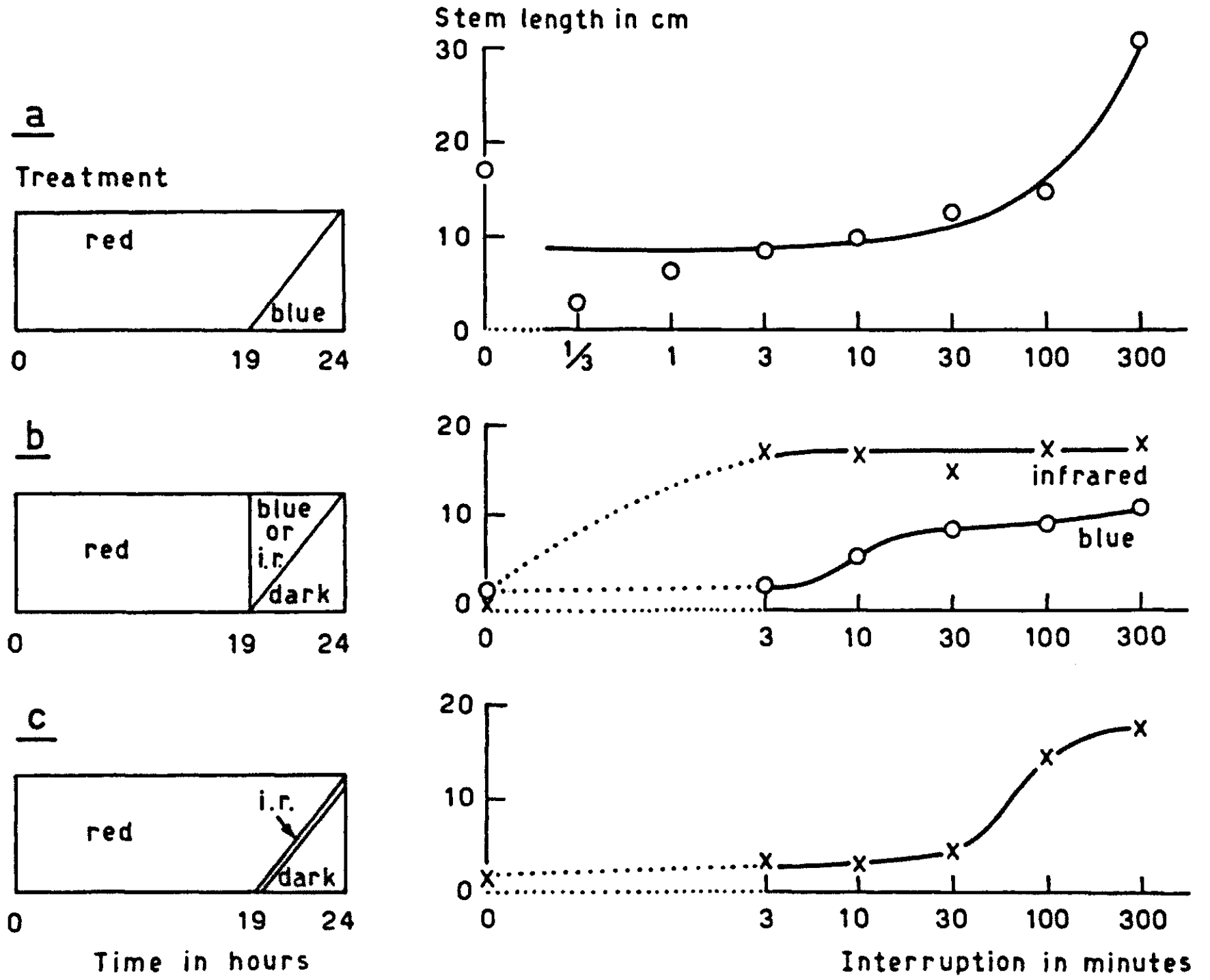

FIG. 17. A series of experiments on stem elongation in *Hyoscyanus* (right side) upon red (*ca.* 9500 ergs/cm²sec) irradiation which was interrupted with blue (*ca.* 9000 ergs/cm²sec), near-infrared (*ca.* 6000 ergs/cm²sec) or dark periods, as indicated in left diagrams. Plants were pretreated (except for [a]) with 6 summer days in the greenhouse. Measurements after 58 (a), 28 (b) and 24 (c) days of treatment. Experiments of 6-1-'59, 11-8-'59, 23-8-'59, respectively. Averages of 4 (a) or 3 plants.

The effect is clear: acceleration of elongation above that of the control group (red only) occurs with interruptions of the order of 100 minutes or more. We might state, however, that this duration will depend on the strength of the inhibitive impuls created by the photoperiod used.

Thus, stem elongation relies upon the annihilation in darkness of the formative inhibitory action of red light. The sensitivity of the plants to this inhibition gradually decreases irreversibly under non-inhibitive conditions, since more extensive pre-induction results in smaller differences between the reactions upon different subsequent daylengths (Fig. 8, p. 33). This is due to continued accumulation of induction.

V.5. Discussion

In this chapter, daylength dependence of shooting in *Hyoscyamus* was analyzed with the aid of a method allowing observations on the effect of very short light periods and of continuous darkness. This method consists in the application of a certain treatment during a limited number of days, after which the plants were placed in inductive conditions instead of inhibitive conditions, so that sufficiently quick stem elongation was obtained in all cases.

The curve proposed by Best (2) for long-day plants was found to hold for *Hyoscyamus* (V.2.). With logarithmically increasing (short) daylengths a linearly increasing inhibition of stem elongation up to a maximum was observed. With still longer photoperiods the inhibition-curve falls to about the continuous dark level at continuous irradiation. The maximum inhibition obtained with weakly active illumination remains below the maximum attained with more strongly active radiations, while in the first case the maximum is situated at longer daylengths. These results are in agreement with the assumption that light creates an impulse for producing inhibition in subsequent darkness (*e.g.*, in generating a precursor which develops into an inhibitor in subsequent darkness, *cf.* p. 3).

The activity of this inhibitor, however, only lasts for a few hours, as was concluded from table 15 (p. 38). This seems to be in contradiction with the observation (44) that plants flower in darkness only upon defoliation which should prove that the leaves develop an inhibitive action of *long* duration. We may suppose that, in long-lasting experiments in continuous darkness, the inhibitive leaf function is non-specific with respect to the photomorphogenetic reaction, and *e.g.*, due to additional respiration of energy reserves as compared with defoliated plants.

The activity of an illumination *(e.g.*, the position of the maximum of inhibition) depends on light intensity (Fig. 14), wavelength region (Fig. 15), and cycle length (Fig. 16).

Daylengths of maximal inhibition in relation to cycle length is shown in figure 18; this curve may be extrapolated to shorter cycle lengths.

It seems possible to interpret the results obtained with night-interruption by red light in combination with a stem inducing treatment (8 hours white + 2 hours near-infrared) (48) with the aid of figure 18. The region of night interruptions between 1 and 30 minutes was found to be inhibitive for stem elongation, whereas 1 and 2 hours were not. This seems to be in agreement with figure 18, because the maximum inhibition in a 7-hour cycle (the number of hours remaining from the start of the interruption!) is found at daylengths (= irradiation times of night interruption) of about 5 minutes. Night inter-

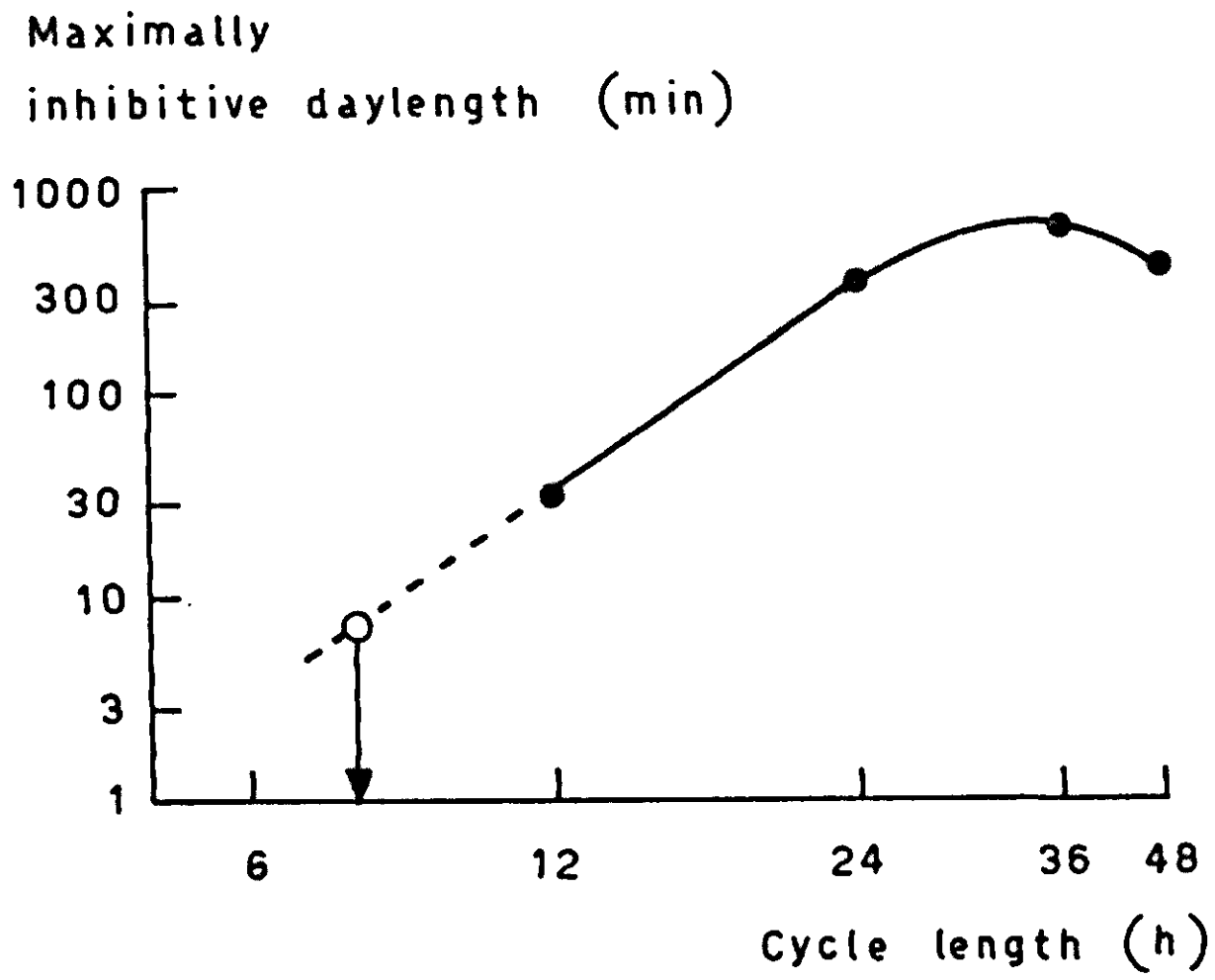

FIG. 18. Relation between maximally inhibitive daylength and cycle length in *Hyoscyamus*. Taken from figure 16 (●), o see text.

ruption, thus, is seen to have two functions, *viz.*, being the photoperiod of a new cycle beginning at the start of the interruption until the next light period, and secondly, shortening the original cycle by taking off some of its night-hours. A treatment with night interruption then is an alternation of two cycles each with specific photoperiodic effects. An experiment with a series of night interruptions of different lengths may then be considered to consist of a cycle of constant composition alternated by another cycle in which the light period is subjected to variation in duration and thus comparable to the type of treatment as shown, *e.g.*, in figure 14 or 15.

Figure 16 is in agreement with the finding of CLAES and LANG (*cf.* 43) that the "critical" daylength is shorter in 48-hour cycles than in 24-hour ones. Probably, the authors would even have obtained flowering in all daylengths of the 48-hour cycle, if their experiment had lasted a few weeks more. A result obtained by FINN (23) that 30-hour cycles are maximally inhibitive for stem elongation in *Hyoscyamus* as compared with a broad range of other cycles to both sides, is also in accordance with figure 16, because FINN used a 12-hour photoperiod. In figure 16, arrow F shows that at a 12-hour daylength the 36-hour cycle point has the highest position. Both the 24-hour cycle and the 48-hour one are less inhibitive at that point.

A third problem dealing with cycle length is the "endogenous rhythm" concept of BÜNNING. We have not tried to work on this problem, but certainly, the method presented here can be useful for experiments dealing with BÜNNING's hypothesis. Growth in darkness for a period of one or two weeks, in which at any moment light flashes may be supplied to interfere with the endogenous sensitivity or reactivity, followed by an after-treatment which is favourable for shooting, seems to be a promising tool in research on this problem.

Our picture becomes still more complicated, owing to the action of near-infrared in antagonizing the inhibition. The amount of the agent, generated by light (the inhibitor-precursor) which brings about inhibition by way of a dark reaction, is decreased by near-infrared. This will result in a reduced inhibition, *i.e.*, in promotion of stem elongation.

For stem elongation, *Hyoscyamus* requires about 100 minutes daily of non-inhibitive conditions (Fig. 17c). This may explain the rise of the curve for long-day plants above 22 hours, and demonstrates once more the validity of the curve suggested by BEST (Fig. 1, see also plate 3g). The length of the non-inhi-

bitive period will be determined by the reaction velocity of a plant to the non-inhibitive situation. May be, this might contribute to an explanation of the difference between long-day and short-day plants, assuming that short-day plants react upon favourable conditions much more slowly and, thus, need a longer dark period.

The sensitivity of the plants to light inhibition can be altered. After a period of inductive treatment, the plants were shown to be less inhibitable (Fig. 8, p. 33), while after a sufficiently long induction period they become completely insensitive (Fig. 13, p. 39). Other desensitizing agents seem to be gibberellic acid, and sometimes vernalization (85).

CHAPTER VI

GENERAL DISCUSSION

The graph, describing the response of floral induction to daylength, as proposed by BEST (2; *cf.* Fig. 1, p. 3) for long-day plants, was found to apply to *Hyoscyamus* also (Figs 9, 12, 14, 15, 16). The resemblance of this curve to those obtained for certain photoperiodic phenomena in animals is striking (*cf.* 45).

The reaction upon different cycle lengths can be understood on the assumption that the inhibitive action of an illumination, *viz.*, the formation of an inhibitor-precursor, is available during a certain time only, since it disappears by reaction. During longer nights, more hours of light (more inhibitor-precursor) can be effectuated, shifting the maximum retardation of elongation to longer photoperiods. In a special experiment, with the light intensity and light quality used, this was valid up to a cycle length of 36 hours. In the 48-hour cycle (Fig. 16) the daylength of maximum inhibition is smaller than in the 36-hour cycle and, moreover, the retardation is reduced. This reminds of the observation of CLAES and LANG (*cf.* 43) that the critical daylength is shorter in 48-hour cycles than in 24-hour ones. It is also in accordance with the observation of FINN (23) that, with 12-hour light periods, the 30-hour cycle is most inhibitive among cycles varying from 12 to 72 hours. From the foregoing considerations it is to be expected that maximum inhibition would be obtained in cycles of more than 36 hours if higher light intensities were used. This, however, will be so only if not too much near-infrared is admixed to the incident radiation, because near-infrared counteracts the inhibition of stem elongation by light. This may explain MEYER's finding that high intensity red radiation, if given continually or as long day, is less inhibitive than red light of lower intensities (*cf.* 16), assuming that the effect of a possible admixture of near-infrared becomes increasingly manifest with increase in light intensity.

As pointed out before (46), all visible wavelength regions are inhibitive for stem elongation as compared with darkness, with an optimum in red.

A second light action is that this inhibition can be annihilated by near-infrared and blue. Thus, with near-infrared admixture, inhibitive radiation can produce only a reduced inhibition, while supplementary irradiation with near-infrared may annihilate inhibition almost completely. In the latter case, the near-infrared is supposed to function by affecting the concentration of inhibitor-precursor, present at the moment the light is switched off.

Blue light also has an inhibition-annihilating action, comparable to that of near-infrared, but weaker. However, the inhibitive action of blue light is stronger

than that of near-infrared (p. 31) so that its antagonizing activity is not as strong.

In our experiments, *Hyoscyamus* required about 2 non-inhibitive hours per day for stem elongation. In combination with strongly inhibitive red light the activity of supplementary blue light reached saturation in about 30 minutes irradiation and that of near-infrared in about 3 minutes, but, nevertheless the interruption of the red irradiation must last about 2 hours (*cf.* Fig. 17, p. 43) as was demonstrated in experiments in which the remainder of this period was filled with darkness.

From the viewpoint of distinction between effects of duration of photoperiod and strictly formative effects, it was expected that blue or near-infrared radiation, given during interruptions noticeably shorter than 2 hours, might annihilate the "photoperiodic" effect of the last hours of red light, and thus shift the internal status of the plant somewhat towards that of short-day treatment. It was observed, however, that plants to which such short blue or near-infrared interruptions were applied, elongated after the same number of days as all-red controls (Fig. 17a). This indicates that these interruptions do not antagonize the daylength effect of the previous red light period.

If a periodic reaction, inhibiting flower bud initiation, and a formative reaction, inhibiting elongation exist, they obviously are cross-linked in *Hyoscyamus* and could not yet be separated experimentally (p. 6).

We might suggest that the two reaction types mentioned by MEYER (56), *viz.*, a red and a near-infrared one (both antagonizable with the other colour), result from the fact that the daylength activity is divided into two parts which are manifest from 0-hour to the optimum inhibition and from the optimum inhibition to continuous light, respectively. Moreover, these reactions may be identified with our periodic and formative reactions. If so, however, MEYER's conclusion that both reactions must be equally fulfilled to obtain flowering does not always hold. From the results presented in this paper, it follows that the near-infrared function is only necessary to de-inhibit stem elongation, whereas the red activity contributes to stem elongation only in extending inhibitive short days. Moreover, some of the results described do not agree with the suggestion presented by MEYER, *viz.*, stem elongation in complete darkness, and under daylengths obtained with near-infrared irradiation only. His suggestion that a red function must be fulfilled together with a near-infrared one, within the same day, seems to be valid only as far as daily treatments with inhibitive irradiations are concerned.

Some critical remarks also can be made with respect to the work published by KÖNITZ (42) which has relations to BÜNNING's concept of endogenous rhythms. It is clear that a rhythmic light treatment is no prerequisite for flowering because some consecutive days of darkness are inductive when following a treatment with any daylength (Table 15), and because near-infrared irradiations are inductive irrespective of the duration of its daily application (Fig. 6, p. 29). Moreover, DE LINT's results (48) on red night-interruptions in *Hyoscyamus* do not agree with the suggestions put forward by KÖNITZ. Near-infrared, directly following a short day was found promotive for flowering, while red night-interruptions can be either inhibitive or promotive, dependent on their duration (48). Moreover, the results presented in the present paper lead to the conclusion that an inhibitive irradiation and the subsequent dark period constitute an entity, the effect of which can be modified (within the limits of reaction of the

plant) by additional irradiations. However, observations are available on rhythmic differences in sensitivity to light, depending on temperature (14, 62, 63). Such differences may be due to changes in pigment concentration (57), while intervention of an as yet unknown external factor is not excluded (7).

It has been reported in this paper that stem elongation can be influenced by light treatment all the time until elongation factually starts. This means that no initial induction period can be distinguished after which flowering later on necessarily follows, but that, with the inductive treatments applied so far, a period of induction is required of the same order of duration as the time required for elongation to become manifest, considered from the beginning of the inductive treatment. This is in accordance with results published by other investigators with other plants (8, 30, 73).

It is clear that there is no reason to postulate an after-effect of any extent on the degree of induction itself. In cases in which a reaction finally becomes manifest, this must be due to additional induction during subsequent treatment which may take place when such treatment is not strictly inhibitive (p. 27). Thus, in *Hyoscyamus*, the induction can be increased almost continuously. Contrary, it decreases only slowly in strongly inhibitive days as may be inferred, *e.g.*, from CARR's experiments (9). In short-day plants, however, a strong decrease could be observed (73, 98).

Of course, the induction reaction leads to metabolic changes which can be duely indicated by the term after-effect, and it may be assumed that this phase of the reaction chain can hardly be modified any more by an irradiation. As soon as a plant has reached this stage, it becomes "day-neutral", *i.e.*, its reaction can no longer be inhibited by any specific daylength treatment.

A summary of the inhibitive action of a periodic light treatment on the production of the induced state may be given with the aid of figure 19.

Within a cycle, an inhibitor-precursor is produced during the photoperiod which, in the subsequent dark period, is converted into an inhibitor, and then utilized in an inhibition reaction. The left side of the figure is a recapitulation of the results obtained with time series of irradiation at different light intensities and in different wavelength regions. Line rh, *e.g.*, represents the formation of inhibitor-precursor by a highly inhibitive illumination which started at X. With shorter irradiation times, the amount of inhibitor-precursor formed is smaller (illumination during the period XO', instead of XO). With the longer irradiation time, induction can only start beyond position B, with the shorter one already beyond position C. It should be pointed out in addition that the experimental results in this paper lead to the assumption that induction to flowering may be produced both in darkness and in light, so that, in our figure, in any period between B (or C, respectively) and X as well as between X and O (or O', respectively), a positive contribution to the induction may be expected aside of the already discussed production of an inhibitor-precursor during the light period.

Within a cycle either of the two photoperiods considered above, may be most inhibitive, depending on the cycle length used: the greater the number of hours through which the decay line runs (actually inhibitive hours), the stronger the inhibition will be. In general, maximal inhibition will be attained when the amount of inhibitor-precursor just lasts onto the moment at which the following light period starts. If darkness lasts longer than this decay-time, the remaining dark hours are inductive. If the decay-line still is at some level at the end of the

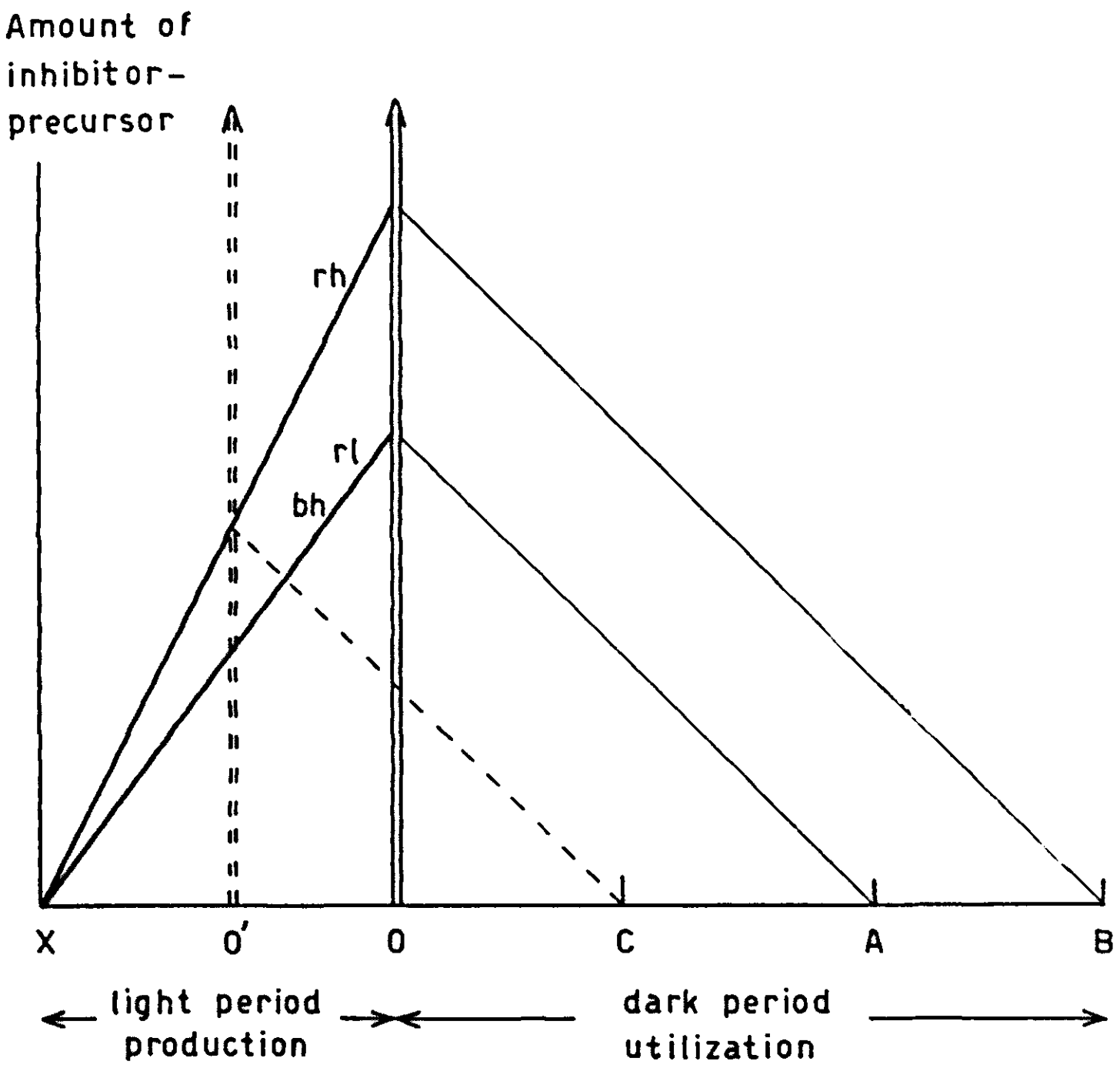

FIG. 19. Schematic presentation of the photoperiodic reaction system demonstrated in stem elongation. Explanation: see text.

cycle, too much light was given, which is again favourable for flowering, because light hours as such are not inhibitive. We may say: the greater the ratio of inhibitive to non-inhibitive hours, the stronger the inhibition. This interpretation is especially supported by the results of time-series of illumination at a given intensity (Fig. 13), and of an experiment on cycle length (Fig. 16).

The relation between the effects of low and high light intensity can be seen from the lines rl and rh. Only in cycles longer than XA hours, the lower intensity is less inhibitive as can be seen from the lines representing the decay of inhibitor-precursor. This clearly may serve as an explanation why short cycles are maximally inhibitive with short (or low intensity) photoperiods. The same is true for the relation between the effects of (high intensity) red and blue light (rh and bh). Most probably, a similar explanation holds for the night-interruption effects reported by DE LINT (48): in this case a night interruption may be seen as the photoperiod of a new cycle which lasts until the end of the original night, and which cycle alternates with another cycle consisting of the original photoperiod with a reduced dark period, ending where the night interruption starts. The reaction of the plant, then, will be the combined effect of both cycles.

In our concept, the normal effect of (red) night interruptions in long-day plants can be understood in case they are of fairly long duration; if not it seems necessary to assume that a night interruption, causes an additional, enhanced effect into the adjacent dark period, thus effectively decreasing the number of inhibitive hours. This however, was not further analyzed.

The action of near-infrared following an inhibitive illumination may be

assumed to consist in a rapid diminution of the amount of inhibitor-precursor, which results in more inductive (dark) hours, favouring stem elongation. Admixture of near-infrared to inhibitive colours results in lower values of the rate of production of the inhibitor-precursor, and, thus, in a weaker total inhibition.

Inhibition of flowering by near-infrared has not been found in our experiments; under certain conditions, however, near-infrared may strengthen the inhibition due to an illumination (56). This points to the existence of another mechanism which, obviously, results in partial destruction of the inductive effect of the light period. This fits well with the assumption of the foregoing paragraph which implies that the non-inhibitive situation lasts some time during the first dark hours following an illumination, since then not much of the inhibitor has as yet been produced.

Not much can be said as yet concerning the mechanism of the inhibition. According to THOMSON (81, 82) we are dealing with the production of a stimulator rather than an inhibitor. Whether the active principle is a single substance or a specific condition resulting from the interaction of several substances, can be left out of consideration for the moment since, in each case, one compound will constitute the limiting factor for photomorphogenesis.

A further question is that concerning the relation between the active principle and the photoreceptive pigment system. This relation, probably, is a rather close one, since the absorption of near-infrared may annihilate, or at least reduce, an inhibition of flowering or of stem elongation. Since, most probably, the primary action of near-infrared is the destruction of the infrared-absorbing pigment, it seems reasonable to assume that an inhibitor-precursor is closely related to the infrared-absorbing pigment. Several detailed concepts may be envisaged. The most likely assumption would appear to be that the infrared-absorbing pigment is an active principle (*e.g.*, a coferment or electrontransporter) in a biochemical reaction chain resulting in the production of an inhibitor which ultimately leads to the observed final effect. One may envisage the question whether the infrared-absorbing pigment can be identified with the primary inhibitor-precursor. Strictly speaking, of course, the pigment initiating the inhibition-producing reaction chain may be qualified as inhibitor-precursor. However, since further pertinent biochemical data are lacking until now, it does not seem useful at the moment to enter into further consideration of the consequences of this speculation. The adjacent reaction chain, of admitted amplifier nature, clearly is driven by compounds, ultimately derived from previous photosynthesis.

It is generally assumed that the near-infrared-absorbing pigment is formed from a red-absorbing pigment, mainly by the absorption of red radiation, while it can be brought back to the previous stage by the absorption of near-infrared radiation. The effect of blue light is comparatively small. Since, however, as has been discussed earlier in this paper (Chapter 3, p. 12), blue light may show either "red" effects or "near-infrared" effects, it may be visualized that the generally observed low activity of the blue light is due to the counterbalancing activities of absorption of blue light both in the "red-absorbing" and the "near-infrared-absorbing" pigment. Thus, the absorption peak of any of the two pigments in the blue may well be much higher than might be derived from its observed activities. This might suggest that the red and infrared pigments are more closely related to the chlorophylls than is generally assumed.

The difference between an induced leaf and its non-stimulus-producing state is unknown. The same holds for the floral stimulus produced by induced organs; it could be a stimulator or the absence of an inhibitor (75). Similarly, the biochemical difference between apices all or not "stimulated" to flower can as yet not be defined.

Whether the description of the photoperiodic and formative reactions of *Hyoscyamus* is valid for other plants needs additional research. So much seems certain, however, that long-day plants forming rosettes react as *Hyoscyamus*.

Flowering in *Lemna gibba* depends in the same way upon light (40).

If stem elongation is a prerequisite for flowering, near-infrared and gibberellic acid are favourable (11, 13, 15, 33, 80). *Rudbeckia* (60) and *Spinacia* (87) seem to differ somewhat, because in these species stem elongation does not seem to be such a strong prerequisite for flower bud initiation. Differences in overall sensitivity may exist, as *e.g.*, between *Hyoscyamus* and *Brassica*.

The system operative in short-day plants does not seem to differ greatly since SCHWABE (73) presented a hypothesis on flowering in *Kalanchoë* which is about equal to the one described here, and also because within the Lemnaceae both long-day and short-day species exist (38), as within *Nicotiana* (102). However, in quantitative aspects the daylength-dependence curve is different for long-day and short-day plants (*cf.* Fig. 1, p. 3). Another unexplained phenomenon is the reaction system of plants flowering only upon a sequence of both short and long days (2, 71, 100).

In accordance with the suggestions developed in the introductory chapter, photo-control of floral induction can be described as a process of inhibition because the reaction resulting in the induced state, is inhibited. Induced plants produce a floral stimulus irrespective of daylength, and this stimulus is effective under all light treatments. Thus, flowering is a stimulus process, originating from an autonomous induction which is sensitive to light. It is inhibited by light of certain wavelength regions and by special combinations of light and dark periods.

SUMMARY

The present paper is concerned with shooting of *Hyoscyamus niger* as a reaction upon radiation with coloured or white light in various combinations of intensity and duration.

In long days in blue light, rapid shooting occurs, also in blue light which is not contaminated with near-infrared (Table 1). Admixture of some red light to the blue strongly delays stem elongation (Fig. 4), however, less so the more near-infrared is present (*cf.* 89). Plants in short days in blue light remain vegetative, whether near-infrared is admixed or not (77). If alternately irradiated with red light (16 h.) and blue light (8 h.), plants elongate the stronger the more near-infrared is admixed to the blue (Table 3).

The inhibition of shooting in short days in white light can be only slightly reduced by red extension (77), and strongly reduced with near-infrared (Fig. 5, Table 4). Shooting-promoting treatment must be continued onto the onset of shooting in order to become effective (Table 7).

Retardation of shooting in long days in red light (15, 77) is strongly reduced by supplementary near-infrared; this reduction was concluded to be active from the first long day on (Fig. 11, Table 14), although the near-infrared treatment,

if starting *ca.* 2 weeks later, was able to "recover" several of the "lost" earlier days (Table 8). Long days in red light up to *ca.* 2 weeks favour shooting; in longer periods an inhibitive effect predominates which can be annihilated by near-infrared supplements. The following results support this interpretation. If followed by long days in white light or in blue light, a period of about 2 weeks of long days in red light favours shooting (Tables 11 and 12), whereas short days in red light are inhibitive; in blue light, long days fully favour shooting and short days do so to some extent (Table 9). Also a few days in darkness (Table 10) with or without some supply of near-infrared (Table 13, Fig. 6) favour shooting if followed by favourable treatment.

Plants which receive, during one or two weeks, treatments inhibiting shooting to different degrees, and which thereafter or partly before, are subjected to non-inhibitive treatment, show rapid stem elongation. The differences in onset of shooting closely correspond to the degree of inhibition applied, and they not exceed in number of days the total duration of the variable treatment (Fig. 12). Daylength sensitivity is reduced inasmuch as more favourable days precede the variable treatment (Fig. 8). Shooting of secondary stems of decapitated flowering plants of *Hyoscyamus* is not inhibited in short days (Fig. 13). An interpretation of this phenomenon is suggested in figure 7. These plants were so thoroughly induced to flower that subsequent inhibitive days did no more prevent shooting. Elongation of leaf petioles is extremely sensitive to light (Fig. 10).

Daylength dependence of the number of days to shooting and of stem length on a certain day are similar (Fig. 9). It is in accordance with BEST's suggestion (Fig. 1), for white (Fig. 8), red (Fig. 9) and blue light (Fig. 15), and also resembles the dependence of some reproduction phenomena in insects (*cf.* 45). The effect of one inhibitive day is not appreciably more than one day retardation of shooting (Table 15). The inhibition of shooting produced by short daily irradiations increases about linearly with the logarithm of the duration (Fig. 12) and increases with increasing intensity (Fig. 14); red light is more active than blue (Fig. 15), while near-infrared seems inactive (Fig. 6); moreover, a certain intensity is more strongly inhibitive and the maximum of the curve is reached with shorter photoperiods the shorter the experimental cycle (Fig. 16). Extrapolation of the relation between cycle-length and the maxima of the curves is applied for an interpretation of night-interruption effects (Fig. 18). The inhibitive activity of long days in red light can be reduced by supplementary irradiation with blue (30 minutes) or near-infrared (3 minutes), provided the red light is interrupted for about 100 minutes (Fig. 17), the remainder of which is filled with darkness.

The above data give rise to the following interpretation.

Hyoscyamus will grow vegetatively if floral induction (an accumulative and autonomous process comprising flower bud initiation and a shooting impulse) is inhibited, and also if stem elongation underlies formative inhibition (suppression of etiolation). Thus, vegetative growth may be due either to unsuitable photoperiod, primarily inhibiting floral induction, or to unsuitable light quality, primarily causing formative inhibition of stem elongation. In *Hyoscyamus*, stem elongation and flower bud initiation always appear together. Nevertheless it seem evident that the entrance of periodic influences into the process is *via* flower bud initiation (entailing some shooting impulse) while the entrance of formative influences is *via* stem elongation (suppression of elongation). In

Hyoscyamus, thus, cross-links between the periodic and the formative inhibition processes must exist.

Inhibition is due to the production of an inhibitor-precursor, which becomes active mainly in darkness, following a light period. In dark, the precursor will be gradually transformed into an inhibitor which itself has no measurable persistence, so that in continued darkness the plant is no longer inhibited, no more than during light (see Fig. 19). The pigment system mediating precursor production is that known to be active in photomorphogenesis with a maximum in the red and another weak one in the blue (24). The inhibition increases with irradiation time as in formative light action. The inhibition observed, most probably, results from increased physiological activity (82). Near-infrared may be assumed to antagonize inhibition by inactivating the precursor. In the ascending part of the curve from 0 to about 4 hours (Figs 9, 12, 14, 15, 16), light is limiting the light-dark interaction; in the descending part darkness curtails the effect. In extreme cases, *e.g.*, long-lasting exposure to red light of high intensity, the very high precursor concentration will give rise to an appreciable amount of inhibitor already during the illumination, resulting in inhibition of shooting in long days.

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SAMENVATTING

De waarnemingen over het optreden van vegetatieve groei of bloei van *Hyoscyamus niger*, als reactie op bestraling met gekleurd (Hfdstn 3 en 4) of wit licht (Hfdst. 5), kunnen als volgt worden samengevat:

In lange dagen in blauw licht schieten de planten snel, ook wanneer het blauw niet verontreinigd is met nabij-infrarood (Tabel 1). Toevoegen van enig rood aan blauw licht vertraagt de strekking sterk (Fig. 4), maar minder naarmate een sterke bijmenging van nabij-infrarood aanwezig is (89). In korte dagen in blauw licht, ook bij aanwezigheid van enig nabij-infrarood, blijven de planten vegetatief (77). Afwisselende bestraling met rood licht (16 uur) en blauw licht (8 uur) resulteert in strekking, en sterker naarmate meer infrarood in het blauw aanwezig is (Tabel 3).

Verlenging van korte dagen in wit licht met rood licht van lage intensiteit geeft slechts een geringe (77), verlenging met nabij-infrarood een sterke strekking (Tabel 4), waarbij de grootte van het effect afhangt van de daglengte (Fig. 5). Behandelingen die strekking toelaten moeten voortduren om effectief te worden (Tabel 7). Het trage schieten in lange dagen in rood licht (15, 77) wordt zeer versneld door supplementair nabij-infrarood; gevoeligheid voor deze invloed is vanaf de eerste lange dag aanwezig (Fig. 11, Tabel 14), men kan echter ook zonder veel vertraging de nabij-infrarood behandeling eerst *ca.* 2 weken later laten beginnen (Tabel 8). Lange dagen in rood licht zijn dus gunstig

voor schieten tot een periode van *ca.* 2 weken, daarna worden ze remmend, welke remming met supplementaire nabij-infrarode bestraling opgeheven kan worden. De volgende resultaten versterken deze interpretatie.

Ook een periode van *ca.* 2 weken in lange dag in rood licht, gevolgd door lange dagen in wit of blauw licht bleken bevorderlijk voor schieten (Tabel 11 en 12), korte dagen in rood licht waren evenwel remmend; in blauw licht waren lange dagen in sterke mate en korte dagen tot op zekere hoogte gunstig voor strekking (Tabel 9). Behandeling gedurende enkele dagen met donker (Tabel 10) of nabij-infrarood (Tabel 13, Fig. 6) bevorderde het schieten, indien daarna een gunstige behandeling volgde.

Planten welke behandelingen van verschillende werkzaamheid ontvangen gedurende een periode van een of twee weken en daarna, of deels vooraf, onderworpen worden aan een behandeling welke het schieten niet remt, gaan allen snel over tot stengelstrekking, met tijdsverschillen welke de werkzaamheid der gevarieerde behandeling weerspiegelen en in het algemeen niet groter zijn dan de duur van deze (Fig. 12). Naarmate er meer gunstige dagen voorafgaan aan de gevarieerde behandeling is de gevoeligheid der planten voor de werking der remmende dagen geringer (Fig. 8). Het schieten van planten, welke langdurig in lange dag stonden en reeds enige stengelverlenging vertoonden, kon niet meer geremd worden, want na afsnijden strekten de secundaire stengels ook in korte dagen (Fig. 13). Een interpretatie van dit verschijnsel kan ontleend worden aan figuur 7. De strekking van bladstelen is zeer gevoelig voor licht (Fig. 10). Het aantal dagen tot schieten en de lengten der stengels op zekere dag vertonen een gelijke afhankelijkheid van de daglengte (Fig. 9). Deze bleek in overeenstemming met de door BEST voorgestelde relatie (Fig. 1), zowel voor wit (Fig. 8), rood (Fig. 9) als blauw licht (Fig. 15), en kwam ook overeen met die van enkele reproduktie-verschijnselen bij insecten (45). Een dag onder bloei-remmende condities vertraagt het schieten voor slechts ongeveer een dag (Tabel 15). De remming in zeer korte belichtingen neemt ongeveer lineair toe met de logaritme der daglengte (Fig. 12) en is sterker naarmate de lichtintensiteit hoger is (Fig. 14), waarbij rood licht sterker werkt dan blauw (Fig. 15) en nabij-infrarood geheel onwerkzaam is (Fig. 6); bovendien werkt een bepaalde intensiteit sterker en wordt het curve-maximum met kortere fotoperioden bereikt naarmate de cyclus korter is (Fig. 16). Het verband tussen cyclus lengte en curve-maximum kan, geextrapoleerd, een verklaring geven van de werking van nachtflitsen (Fig. 18). De remwerking van lange dagen in rood licht kan opgeheven worden door supplementaire belichting met blauw (30 minuten) of nabij-infrarood (3 minuten), wanneer er tenminste gedurende *ca.* 100 minuten geen rood licht, noch enig ander remmend licht gegeven wordt (Fig. 17).

Het bovensamengevatte feitenmateriaal heeft tot de volgende interpretatie aanleiding gegeven.

Hyoscyamus groeit vegetatief wanneer de bloeiinductie (een accumulatief en autonoom proces, wat resulteert in bloemknopaanleg en een daarmee gepaardgaande schietimpuls) geremd wordt, en ook wanneer de stengel formatief geremd wordt (etiolementsremming). Vegetatieve groei kan dus een gevolg zijn van ongunstige fotoperiodische behandeling, welke primair de bloeiinductie remt, en van ongunstige lichtkwaliteit, waardoor de stengel primair formatief geremd wordt. Daar bij *Hyoscyamus* stengelstrekking en bloei steeds samengaan moeten deze twee remsystemen gekoppeld zijn. Het lijkt evenwel duidelijk, dat de fotoperiodische invloed in het strekkingsproces grijpt via bloemknop-

aanleg, terwijl de formatieve invloed meer direct op de stengelstrekking werkt.

Remming treedt op, doordat in licht een remstofprecursor ontstaat welke in hoofdzaak actief wordt *na* de belichting. In het donker zal na enige tijd de precursor omgezet zijn (de remstof heeft geen meetbare levensduur maar wordt meteen als "remming" geëffectueerd), zodat de plant in verder voortdurend donker niet meer geremd wordt, evenmin als tijdens de belichting (zie Fig. 19). De remwerking van de korte fotoperioden neemt toe met de belichtingsduur. De waargenomen remming berust waarschijnlijk op verhoging der physiologische activiteit van de plant (82). Het pigmentsysteem dat een rol speelt bij de precursorproductie is gekenmerkt door het fotomorfogenese spectrum met een top in rood en een zwakkere in blauw (24). Nabij-infrarood en ook blauw antagoneren dit remsysteem, waarschijnlijk doordat de precursor wordt geinactiveert, zodat er geen na-effect (remming!) van een belichting kan optreden. In het opgaande been van de curven (Fign 9, 12, 14, 15, 16), van 0-4 uur, is het licht beperkend voor de licht-donker interactie; in het neerdalende is de lengte der donkere periode limiterend. In extreme gevallen (o.a. langdurig sterk rood), waarbij reeds tijdens het licht remming optreedt, zal de hoge precursor-concentratie ook in het licht in merkbare mate aanleiding tot remstofvorming kunnen geven.

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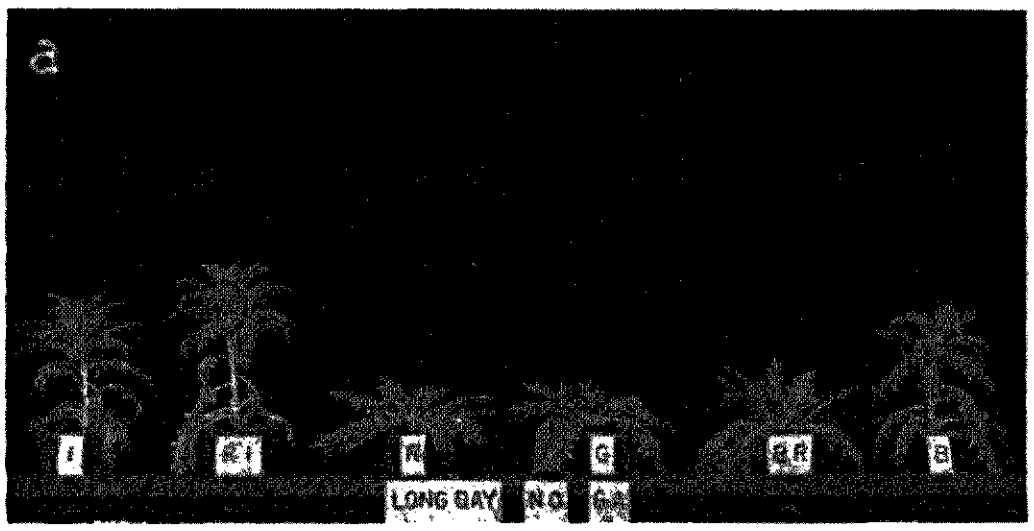

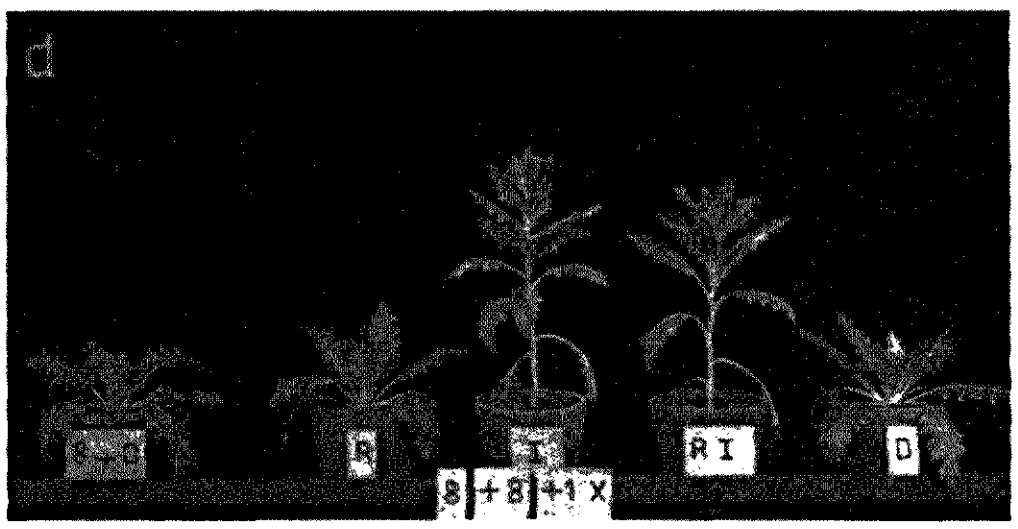

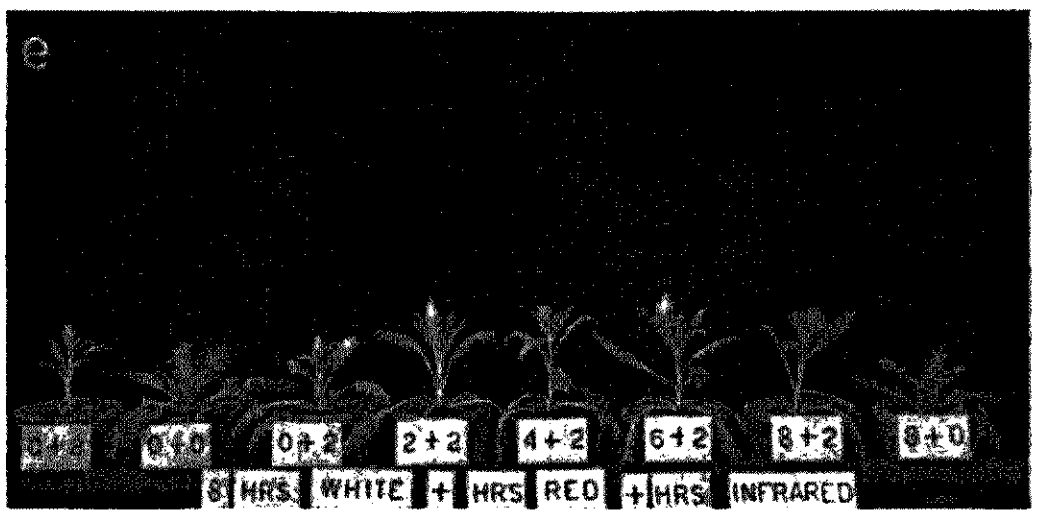

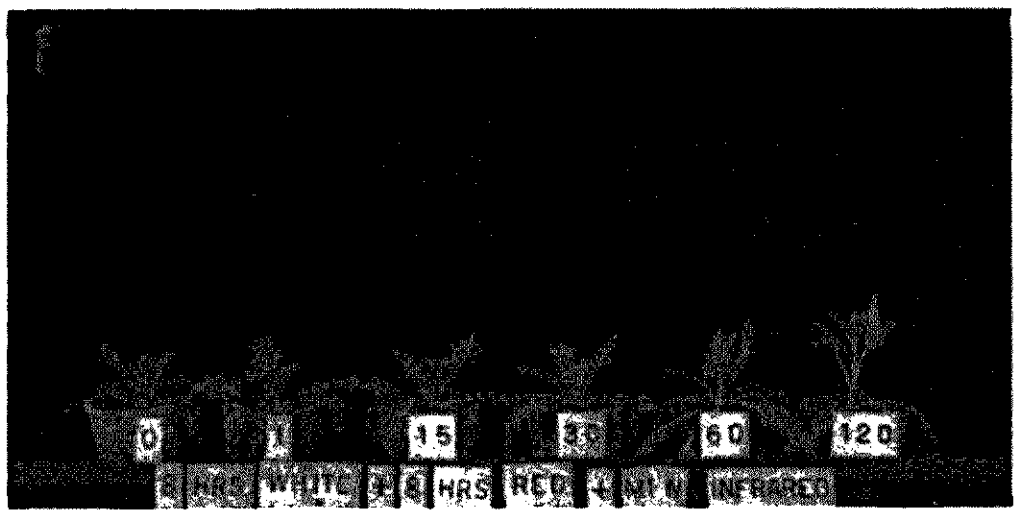

PLATE 1. *a.* 15-Day treatment with 16 hours of coloured light daily, followed by short days in white light. See figure 4, p. 14. Photographed 24-10-'56, after 26 days.

b. Continued treatment with 14 hours red (R) or blue (B) light daily, supplemented with 2 hours red, or 2 hours near-infrared, or darkness only. See table 2, p. 15. Photographed 6-3-'58, after 51 days.

c. Continued treatment with 16 hours red and 8 hours in 3 qualities of blue light (G+ glass filter, G– glass filter with copper sulphate, and P– plastic filter). Photographed 1-12-'58, after 35 days.

d. Treatment described in table 8 (p. 22). Left plant daily received 8 hours white light only (8+D). The other four received 8 hours white light supplemented with 8 hours red, followed by another hour of red (R) or near-infrared (I) or darkness (D). Plant RI received treatment R during 14 days, thereafter treatment I. Photographed 2-8-'57, after 55 days.

e. Continued treatment with 8-hour days in white light, followed by a series of hours in red light (0–8), and 2 hours of near-infrared. See table 6, p. 20. Photographed 11-12-'57, after 73 days.

f. Continued treatment with 8-hour days in white light, supplemented with 8 hours red and a series of minutes (0–120) of near-infrared. See table 5, p. 20. Photographed 11-12-'57, after 73 days.

g. A series of 16-hour days in blue light (0–15) were given, preceding a treatment with 16-hour days in red light. See table 9, p. 24. Photographed 14-8-'58, after 121 days.

h. A series of 8-hour days in blue light (0–15) were given, preceding a treatment with 16-hour days in red light. See table 9, p. 24. Photographed 14-8-'58.

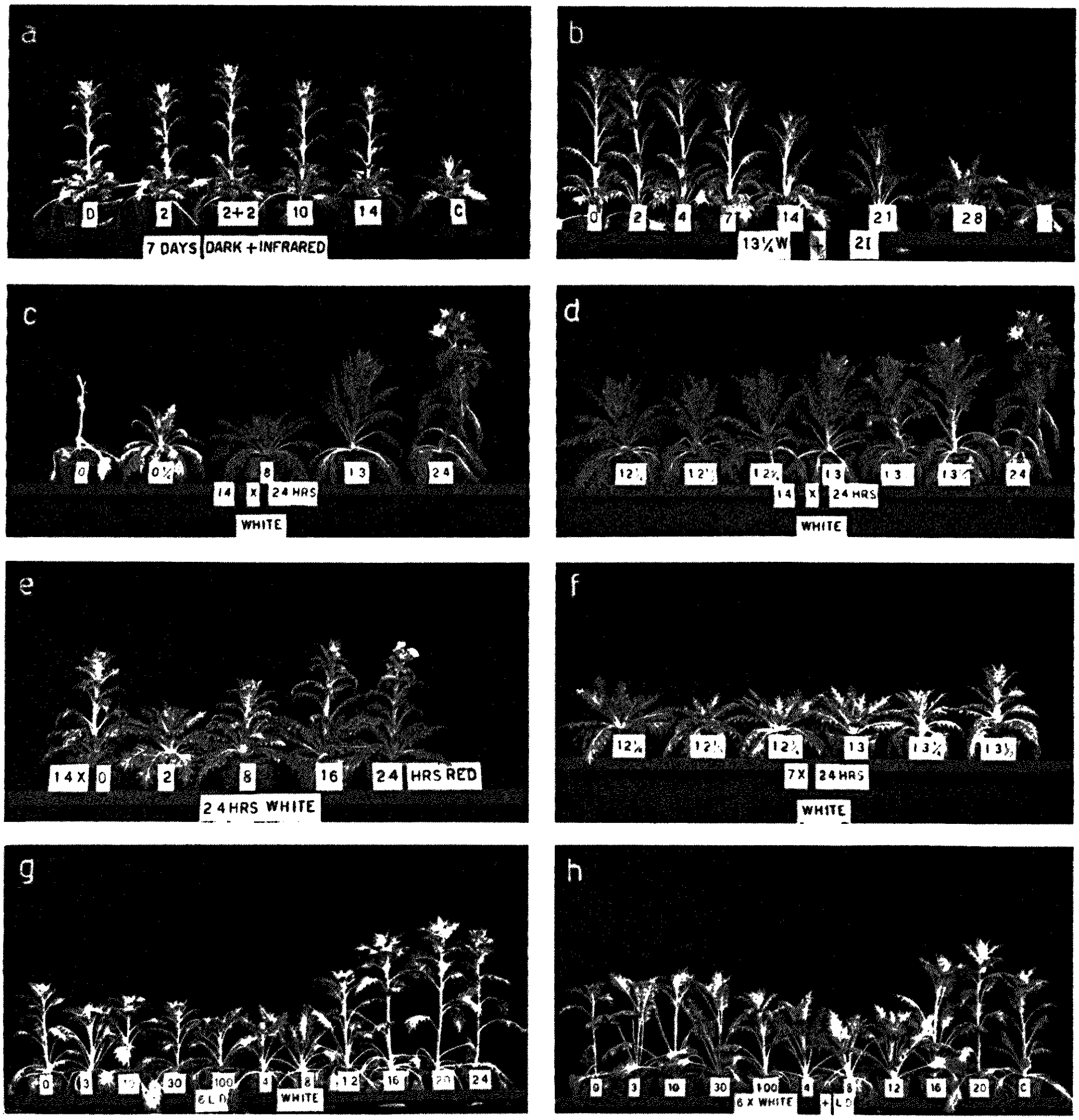

PLATE 2. *a.* Near-infrared only was given, during 7 days (0–14 h. per day; 0 h. = darkness only [D]; C did not receive this treatment but remained in short day), after which all plants were placed in long summer days. See table 13, p. 28. Photographed 21-6-'58, after 32 days.

b. Days of 13¼ hours in white light were supplemented with 2 hours near-infrared starting after indicated numbers of days. Photographed 9-2-'59, after 36 days. See figure 11, p. 36.

c. d. Following a treatment with continuous white light during 7 days, a daylength series (daylength indicated in hours) was given. See figure 8, p. 33. Photographed 30-10-'58, after 37 days.

e. A daylength series (daylength indicated in hours, 0 = continuous darkness) in red light was given during 14 days, and followed by continuous exposure to white light. See figure 9, p. 34. Photographed 5-11-'58, after 43 days.

f. Following a treatment with continuous white light during 7 days, a daylength series in white light (daylength indicated in hours) was given. See figure 8, p. 33. Photographed 15-11-'58, after 53 days.

g. After 6 long summer days, a daylength treatment in white light (daylength indicated in minutes or hours from left to right, respectively) was given during 6 days, and again followed by long summer days. See figure 12, p. 37. The very first long day was 11-8-'59. Photographed 7-9-'59.

h. A daylength treatment in white light (daylength indicated in minutes or hours) was given during 6 days, whereafter long summer days followed. See figure 12, p. 37. Treatment started 17-8-'59. Photographed 14-9-'59.

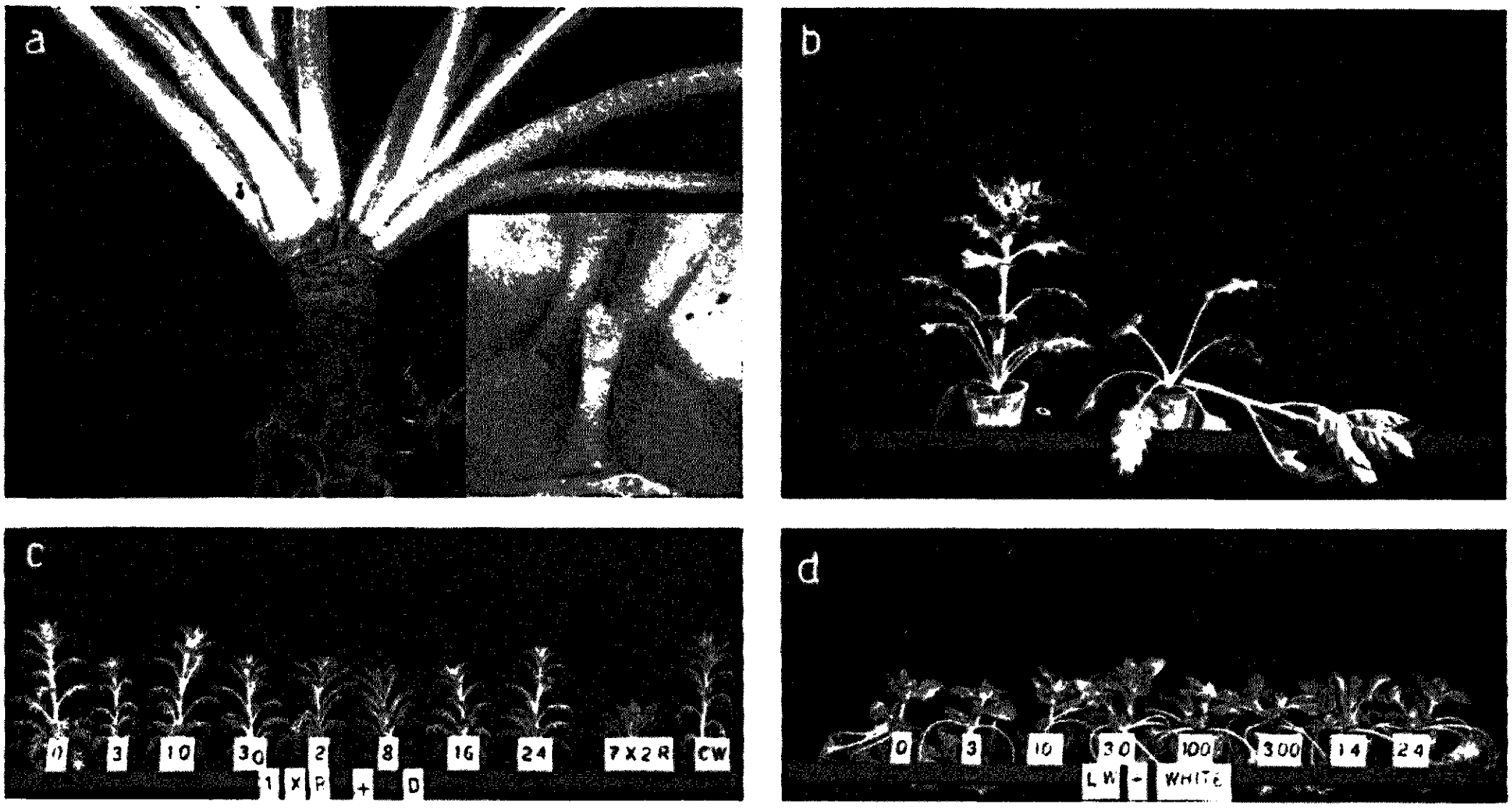

PLATE 3. *a.* Reversal to vegetative growth in 8-hour days in red light after treatment with eight 16-hour days in blue light. Photographed 2-9-'58, after 140 days.

b. Plants of plate 3d just before (left) and after decapitation (right). Photographed 5-9-'59.

c. Plants once received the indicated daylengths (minutes or hours from left to right, respectively) in red light (9500 ergs/cm²sec), thereafter they were in darkness for 6 days, and finally were placed in long summer days. Controls received 7 successive 2-hour days in red light (7 × 2R), or continuous white light (*ca.* 20,000 ergs/cm²sec) CW). See p. 38. Photographed 9-2-'59, after 35 days.

d. Decapitated plants (Plate 3b) received 6 days of indicated daylength (minutes or hours, respectively) and then were placed in continuous white light (*ca.* 20,000 ergs/cm²sec). See figure 13, p. 39. Photographed 22-9-'59, after 18 days.

e. Red irradiation interrupted daily with blue periods (minutes indicated) in continued treatment. Continuous blue (CB) and continuous red (0) are controls. See figure 17a, p. 43. Photographed 5-2-'59, after 31 days.

f. After 6 long summer days (started 11-8-'59), a daily treatment of 19 hours red light, supplemented with a range of near-infrared irradiations (minutes indicated) followed. See figure 17b, p. 43. Photographed 5-9-'59.

g. Daily, 24 hours of light were given in a combination of red (hours indicated) and blue. Light intensities *ca.* 9500 ergs/cm²sec. Photographed 1-12-'59, after 35 days.

h. After 6 long summer days (started 29-8-'59), a treatment in red light was given, interrupted daily during indicated periods (minutes) and supplemented with 3 minutes near-infrared, *c.q.*, followed by darkness (0 only received 24 hours of red light daily). See figure 17c, p. 43. Photographed 22-9-'59.