FLOWER INITIATION IN *HYOSCYAMUS NIGER* L. AS INFLUENCED BY WIDELY DIVERGENT DAYLENGTHS IN DIFFERENT LIGHT QUALITIES

M. K. JOUSTRA

Dit proefschrift met stellingen van

MARTEN KORNELIS JOUSTRA,

landbouwkundig ingenieur, geboren te Hoorn op 29 maart 1938, is goedgekeurd door de promotor Dr. E. C. WASSINK, hoogleraar in het Plantenphysiologisch Onderzoek en de Physiologie der Planten.

De Rector Magnificus van de Landbouwhogeschool,

J. M. POLAK

Wageningen, 2 november 1970.

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FLOWER INITIATION IN *HYOSCYAMUS NIGER* L. AS INFLUENCED BY WIDELY DIVERGENT DAYLENGTHS IN DIFFERENT LIGHT QUALITIES

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWWETENSCHAPPEN
OP GEZAG VAN DE RECTOR MAGNIFICUS, MR. J. M. POLAK,
HOOGLERAAR IN DE RECHTS- EN STAATSWETENSCHAPPEN
VAN DE WESTERSE GEBIEDEN,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG 18 DECEMBER 1970 TE 16.00 UUR

DOOR

M. K. JOUSTRA

H. VEENMAN & ZONEN N.V. - WAGENINGEN - 1970

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STELLINGEN

I

De remming van de bloemaanleg bij lange-dag planten in korte dag (in wit licht), komt tot stand onder invloed van een 'factor' gevormd via fotosynthese-systeem II en van fytochroom in de donker-rood absorberende vorm.

Dit proefschrift.

II

De bevordering van de bloemaanleg bij lange-dag planten in lange dag (in wit licht), wordt beheerst door pigmenten, behorend tot fotosynthese-systeem I, tezamen met fytochroom in de donker-rood absorberende vorm.

Dit proefschrift.

III

Violaxanthine speelt een rol in het bloeimechanisme van daglengte-gevoelige planten.

Dit proefschrift.

IV

De door SPRUIT en RAVEN voorgestelde verklaring voor de 'lag phase' bij de chlorophylsynthese onder invloed van licht, in kiemplanten, die in het donker zijn opgekweekt, verdient de voorkeur boven die van GASSMAN en BOGORAD.

C. J. P. SPRUIT and C. W. RAVEN, Acta Bot. Neerl. **19**, 165–174 (1970).
M. GASSMAN and L. BOGORAD, Plant Physiol. **42**, 781–784 (1967).

V

De opvatting van VAN DE VOOREN, dat deblokkering van het bloei-inducerend mechanisme bij *Silene armeria* plaats vindt in de fotofase en blokkering in de nyctofase, is onjuist.

J. VAN DE VOOREN, Z. Pflanzenphysiol. **61**, 135–139 (1969).

VI

De toepassing van chemische snoeimiddelen, vooral bij de pereteelt, biedt mogelijkheden ter beteugeling van de junirui.

A. VARGA, 22ste Symp. Fytofarmacie en Fytiatrie, mei 1970, Gent.
Meded. Rijksfaculteit Landbouwwet. Gent, 1970 (in druk).

VII

Een vroege selectie op resistentie tegen ziekten en plagen is riskant op grond van gevonden verschillen in de gevoeligheid van planten in de jeugdfase en volwassen planten.

M. K. JOUSTRA, Landbouwdocumentatie **20**, 483–488 (1964).

VIII

Het belang van methylbromide bij de bestrijding van virusziekten in champignons wordt door HAYES sterk overschat.

W. A. HAYES, Mushroom Growers' Association Bulletin 234, 240–243 (1969).

IX

De overdracht door thripsen is geen onvoorwaardelijk criterium in de karakterisering van het bronsvlekken virus bij tomaat.

R. J. BEST, Advances in Virus Research **13**, 65–146 (1968).

X

Het verdient aanbeveling de term mergstraal te bewaren voor groepen van parenchymatische (en bij Gymnospermae tracheïdale) elementen, welke gevormd worden door in longitudinale richting korte initialen in het cambium.

XI

Het is een kwestie van fatsoen, dat gekozenen in volksvertegenwoordigende lichamen bij uittreding uit de groepering die hen candidaat had gesteld, hun zetel ter beschikking stellen. Een wettelijke regeling ter handhaving van deze norm is gewenst.

XII

De 'Varsity' is van groot belang geweest voor de aansluiting van het Nederlandse bij het internationale roeipeil.

Proefschrift van M. K. JOUSTRA
Wageningen, 18 december 1970.

VOORWOORD

Bij het afsluiten van mijn proefschrift, bedank ik in de eerste plaats mijn ouders voor alles wat zij voor mij hebben gedaan.

Voor de aansporing van Prof. WELLENSIEK, om aan promotie-onderzoek te beginnen, ben ik hem zeer erkentelijk.

Het spontane aanbod van mijn promotor, Prof. WASSINK, om bij hem een promotie-assistentschap te aanvaarden, heb ik zeer gewaardeerd. Voor de vele waardevolle suggesties tijdens het onderzoek en de uitvoerige en critische aandacht bij de vormgeving van mijn manuscript ben ik hem zeer dankbaar.

De voortdurende belangstelling voor mijn werk van Dr. Ir. P. J. A. L. DE LINT, heeft mij steeds weer gestimuleerd. Zijn vele suggesties en zijn critische opmerkingen naar aanleiding van een voorlopig manuscript, zijn voor mij van grote betekenis geweest.

Al mijn collega's van het Laboratorium voor Plantenphysiologisch Onderzoek ben ik dank verschuldigd voor hun hulp en voor de vele vruchtbare discussies. Ook alle overige personeelsleden stonden mij steeds op een bijzonder plezierige wijze terzijde. Met name wil ik hier noemen de goede verzorging van enkele tienduizenden proefplanten; het verzetten van de planten van licht naar donker en weer terug in de bijna honderd proeven geschiedde tot op de minuut nauwkeurig. Veel dank aan allen die bijdroegen aan dit *Hyoscyamus*-5-jarenplan.

De steun tijdens het werk en het geduld waarmee mijn vrouw de onvermijdelijke onregelmatige werktijden opving, zijn voor mij onmisbaar geweest.

CONTENTS

1. INTRODUCTION

1.1. Brief remarks on literature

The influence of light on plant growth and flower initiation has been studied by numerous investigators for many years. Already in the 17th century, Ray (117) recognized light inhibition on elongation. In 1912, Tournois (140) described clearly the photoperiodic control of flower formation; six years later, Klebs (75) also detected the importance of light for plant growth and development. But it was to Garner and Allard (48), in their paper of 1920, to introduce the term photoperiodism for the response of an organism to the relative length of day and night. In 1923 they also recognized two photoperiodic response types, the long-day and the short-day plants (49), which can be considered as the basic photoperiodic response types, not only in plant flowering, but in organisms in general. Later, other photoperiodic response types have been found, most of which are derived from the two types first described.

In the second half of the last century, the mechanism of flower initiation was already under investigation. In 1863–1865, Sachs (121, 122) published his hypothesis about flower forming substances, produced by the leaves in the light. But it was only in 1934 that Knott (77) demonstrated that optimal flowering response can be obtained in both long-day and short-day plants, if only the leaves are exposed to the inductive daylength conditions. From the experiments with short-day plants in 1936 by Moshkov (107), Chailakhyan (15), and Psarev (114) it became still more evident that the leaves, and not the apical buds, are the receptive organs of the photoperiodic stimulus. This work became the basis for a flower hormone concept in photoperiodism, thus supporting the hypothesis of Sachs. In 1937, Chailakhyan (16) proposed to call the concerned hormone 'florigen', meaning 'flower-former'. From grafting experiments (discussed in great detail by Lang (81)), much evidence in favor of the existence of a florigen was gained. It was assumed to be formed in the leaves and transported to the growing points; the nature of florigen was supposed to be the same in various plant species (80). Efforts to isolate florigen, however, were rather unsuccesful. Only, in 1961, Lincoln, Mayfield, and Cunningham (83) reported on the preparation of an extract from flowering *Xanthium pennsylvanicum* plants which, when applied to non-induced individuals, lead to flower initiation in some cases. In 1962, Lincoln et al. (84) and, in 1966, Biswas et al. (6) succeeded in inducing flowering in non-induced plants even with extracts from other species. However, the stimulus obtained was weak. Further concentration and purification of the extract and identification of the hormone is needed in order to explain the function of florigen in the flowering process.

Some investigators (115, 79, 82, 51, 54) expressed a different viewpoint, viz., the flower-inhibition hypothesis, clearly defined by von Denffer (24) in 1950. He suggested that a plant is always capable of flowering, but that flowering may be inhibited by a particular factor, the formation of which in photoperiodically

sensitive plants should be favoured by photoperiods unfavourable for flowering. WELLENSIEK and coworkers (157, 152, 151, 153) also assumed the removal of an inhibition during the photoperiodic induction. These ideas resulted in the hypothesis that, at least in the long-day plant *Silene armeria*, inductive treatments cause the destruction of an inhibition after which the synthesis of a transportable floral stimulus can start (154, 155, 156). This stimulus, which might be a substance with auto-catalytic properties, should cause the realisation of flower initiation in the growing point. So, in the hypothesis of WELLENSIEK, both the theory of flower-inhibition and that of flower forming substances are combined.

Since it is not the aim of this study to copy already existing reviews on this subject, reference is made to the papers of: BOPP (1966: 8), BORTHWICK and PARKER (1950: 12), BORTHWICK and HENDRICKS (1960: 10), LANG (1952: 80 and 1965: 81), LIVERMANN (1955: 89), MELCHERS and LANG (1948: 98), MOHR (1962: 104), SALISBURY (1961: 123; 1965: 124 and 125), WASSINK and STOLWIJK (1956: 148), and ZEEVAART (1962: 163).

1.2. SCOPE OF THE INVESTIGATIONS

Much of the earlier work on photoperiodism of flower intiation has been concerned with the effects of night interruptions in the frame of 24-hour cycles. The effect of light quality of the main light period has also been studied in more recent years (e.g. 147, 132, 85, 100, 127, 28), but its duration has not often been

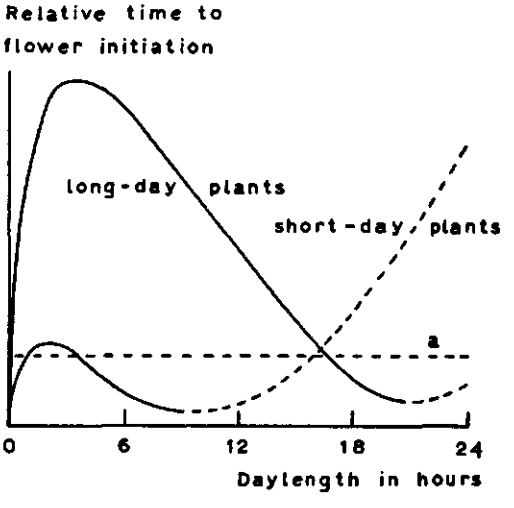


FIG. 1. Schematic daylength dependence curves of flowering in long-day and short-day plants, as proposed by BEST (5). Line *a* added by DE LINT (87); the critical daylengths between vegetative and generative growth were characterized by the level *a*, the position of which should depend upon experimental conditions and duration of treatment. In daylengths in which the response curves rise above *a*, plants remain vegetative.

the subject of investigation. In 1960, BEST (5) and DE LINT (87) investigated the main light period somewhat further. BEST, on the basis of extensive observations in rice, has presented photoperiodic response curves for flowering of both short-day and long-day plants. Photoperiods in his experiments were between 4 and 24 hours. For the part of the curve between 0 and 4 hours, which at that time he still called hypothetical, he made the assumption 'that under conditions of continuous darkness flowering occurs early, provided that the plant is continuously supplied with sufficient carbohydrate'. This assumption was based on publications about flower initiation in total darkness in short-day as well as in long-day plants (50, 82, 137, 138, 59). Interruption of the otherwise continuous dark period by only a few seconds or minutes of light in a 24-hour cycle should, according to BEST (5), markedly delay flower initiation. On this basis, and supplemented by the observations of DE LINT (87), BEST presented photoperiodic response curves of short-day and long-day plants over a range of photoperiods between 0 hours (continuous darkness) and 24 hours (continuous light). These curves are presented in fig. 1.

Somewhat in contradiction with these curves is the observation of HARDER and GÜMMER (55) that the short-day plant *Kalanchoe blossfeldiana* will not flower in continuous darkness, but that it needs at least some light, be it even as little as 1 sec. of sunlight. This observation has been confirmed by FREDERICQ (43).

Working with the long-day plant *Hyoscyamus niger*, DE LINT (87) confirmed the BEST-curve for long-day plants; one of his curves is shown in fig. 2. DE LINT explained his observations by suggesting that inhibition of flowering was due to the production during the light period of an inhibitor-precursor, which

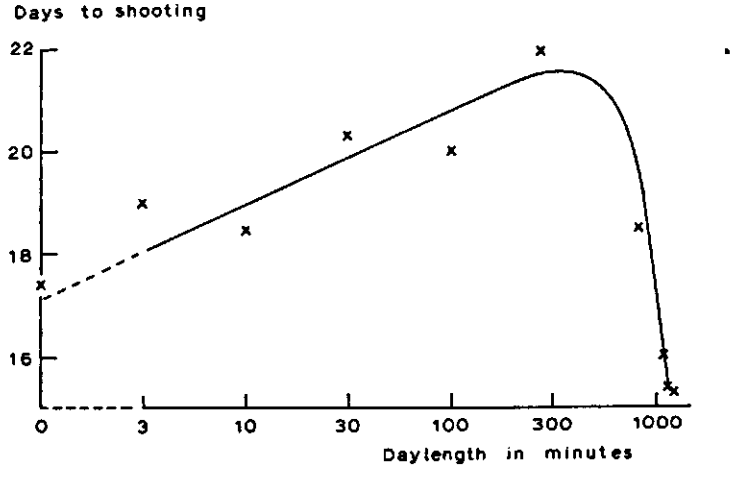


FIG. 2. Days to shooting in *Hyoscyamus* upon a daylength treatment in white light (20,000 ergs. $cm^{-2}.sec^{-1}$) during 6 days, following short days in the greenhouse. After-treatment in long summer days in the greenhouse (as given by DE LINT (87)).

should become active as an inhibitor in the dark, following that light period. The inhibitor, formed from the precursor in darkness, should have no measurable persistence, so that in continued darkness the plant, being exhausted of precursor, was no longer inhibited. For the main pigment system, mediating precursor production, DE LINT pointed to the reversible red/far-red (R-FR) pigment system which was known to exist in plants since the discovery of FLINT and MCALISTER in 1935 (38) and became well known under the name phytochrome* (10, 62, 130, 67).

In our investigation, we have again studied the daylength dependence of flower initiation under white light (cf. Chapter 3), as well as under light of restricted spectral regions (cf. Chapter 4) in the annual strain of the 'qualitative' long-day plant *Hyoscyamus niger*, in order to try to contribute to a better understanding of the flowering mechanism.

* It is worth noting that this name had already been used in 1883 by ENGELMANN (29) for all pigments in plant cells.

2. TECHNIQUES, MATERIALS, AND PARAMETERS OF FLOWER INITIATION

2.1. General planning of the experiments

In an investigation of the daylength dependence of flowering in *Hyoscyamus niger*, continuous treatment cannot be applied since plants, grown in very short daylengths or otherwise unfavourable photosynthetic conditions, will soon die. To avoid this, DE LINT (87) subjected his experimental plants to specific daylength treatments during 6 days only, after which limited period all plants were exposed to flower inducing long days. In the present series of experiments, this method was used also. But even this treatment often caused the plants to become very weak, especially in far-red irradiation. Therefore, we wanted to explore in how far this method could be improved by intercalating days with a non-inductive light period in order to improve the photosynthetic conditions of the plants.

Summation of effects of inductive photoperiodic cycles in *Hyoscyamus* has been investigated by LANG and MELCHERS (82); these authors concluded that the effects of series of limited numbers of inductive cycles are additive, evidently due to the formation of a stable substance, and also that reversal or annihilation of induction by intercalated SD will not take place. The restriction has to be made that they did not investigate this possibility for single LD, nor for pairs of LD with more than 10 inserted SD. The same phenomenon was examined in greater detail by CARR (14), who concluded that the effects of photoperiodic stimuli are additive in the LDP *Hyoscyamus niger* as well as in the SDP *Chenopodium amaranticolor*. CARR assumed that the precursors of the flower hormone are stable and retained in the apical meristem. Only the diluting effect of continued cell division was supposed to be a limitation. Also DE LINT (87) in his experiments found no reduction by 6 inhibitive SD on the inductive effect of preceeding LD. He concluded that the inductive effect of any treatment is fixed irreversibly within the same day, or approximately so, in *Hyoscyamus*.

It seemed therefore justified to repeatedly interrupt photoperiodic treatments by some short days (i.e. with 9 hours of light) in order to keep the plants in a better condition during the experimental period. This enables us to apply photosynthetically unfavourable cycles in larger numbers than is possible by continuous application. As in the work of DE LINT, after-treatment was given in LD, in order to obtain flower initiation in all treatments. This method already was shown to be satisfactory (87, 72).

During the treatment days (TD), circumstances like light quality were varied, but inserted days always were SD in fluorescent light (except in the experiments presented in subsection 3.3.4.) at 20 °C. Illuminations in SD, LD, and TD started between 8 and 10 o'clock in the morning as much as possible, so that a new cycle for all plants started at about the same time.

Abbreviations used in this paper are listed below:

ATP adenosine-5'-triphosphate;

dATP 2'-desoxyadenosine-5'-triphosphate;

HER	high energy reaction(s);
ID	intercalated day(s);
LD	long day(s): ca. 16 hours of light per day;
LDP	long-day plant(s);
NADP	nicotinamide adenine dinucleotide phosphate;
P_{fr}	far-red absorbing form of phytochrome;
P_r	red absorbing form of phytochrome;
P_{total}	$P_r + P_{fr}$;
SD	short day(s): ca. 9 hours of light per day;
SDP	short-day plant(s);
TD	treatment day(s).

2.2. Techniques

2.2.1. *Introductory remarks*

Plants of the annual strain of *Hyoscyamus niger* L. were, prior to the experiment, grown in the phytotron at about 20 °C, mostly for 3 to 4 months under a 9-hour day. During this period they were transplanted twice into larger plastic pots. The light intensity was kept somewhat lower directly after transplanting (30,000 ergs. cm^{-2}. sec^{-1}) and thereafter raised to 50,000 to 60,000 ergs. cm^{-2}. sec^{-1}. The light was obtained from fluorescent lamps, Philips TL 33/120W or TL 33/40W.

Daily irradiation during the precultivation period, and during experiments ended either by automatically switching off the lights, or by moving the plants into a dark room. During experiments with very short irradiations or with coloured light, transport of the plants was always made in complete darkness. In all experiments, temperature was maintained at 20 °C. During the precultivation period, as well as during the experimental period, air humidity was not always under control; it mostly was around 65 %.

Occasional appearance of aphids on the plants could not completely be avoided. In that case the plants were treated with vapourized 'Liro-Nogos', containing DDVP (dimethyl-dichlorovinyl-phosphate) as the active compound. The treatment was carried out in a greenhouse under natural light and was only done during a part of the day at which the plants otherwise would have been under white light conditions in the phytotron. When aphids appeared on the plants during an experiment, they were removed by hand or the plants were treated as mentioned above some days later when they were in white light again.

When, at the end of an experiment, leaf- and flower primordia had to be counted, all plants in the experiment were harvested on the same day and put into 70 % ethanol.

Counting of leaf- and flower primordia, and examination of the growing points was carried out under a binocular microscope.

2.2.2. *Equipment used for irradiation during the experiments*

During the experiments, white light always has been given from above. It was

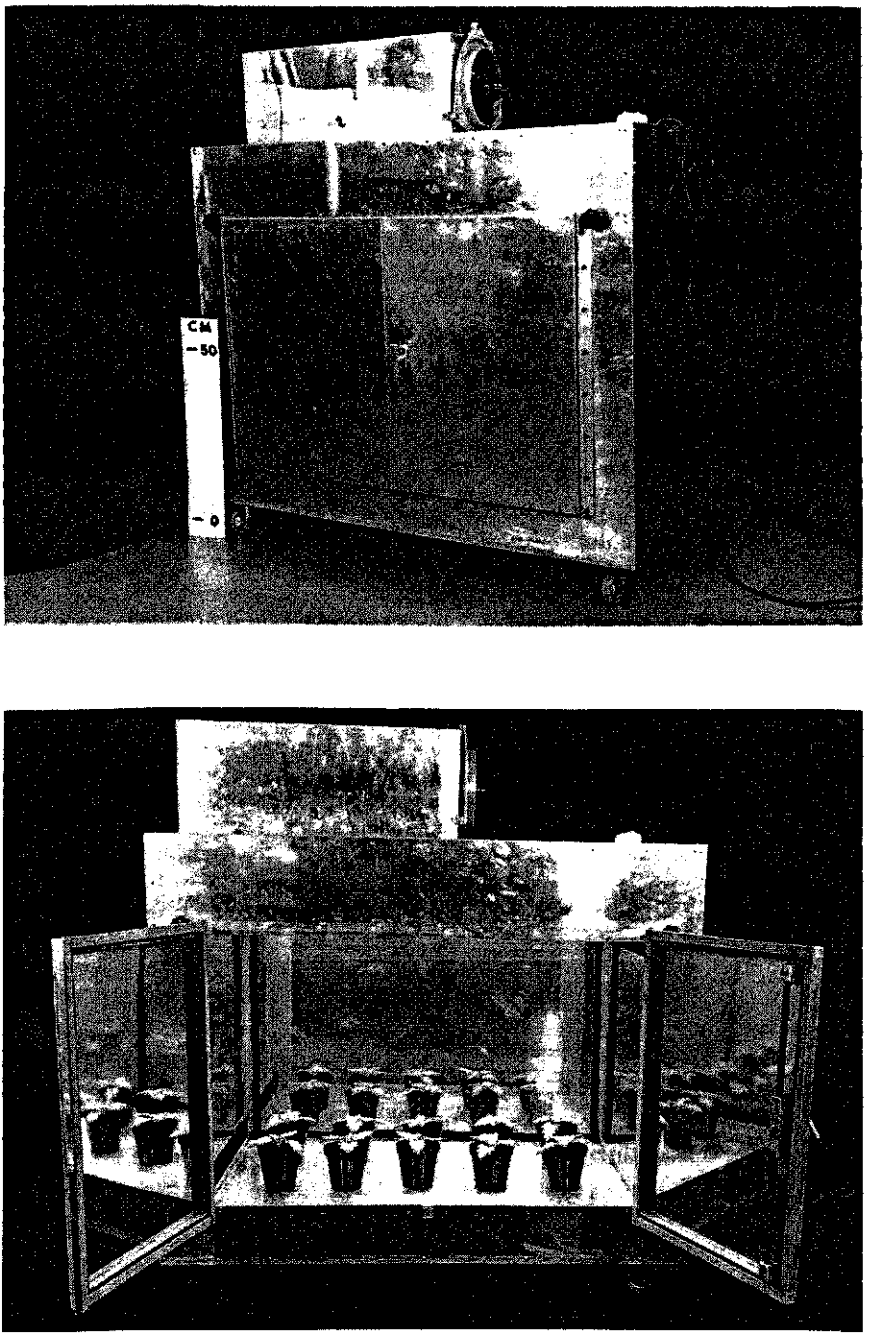


PLATE 1. Movable cabinet for irradiation in narrow spectral regions.

supplied in the phytotron rooms of this laboratory by fluorescent lamps of the type mentioned in subsection 2.2.1. In order to add some more red and far-red, we sometimes combined the fluorescent lamps with incandescent lamps, mostly PHILIPS 'Argenta super lux', 75 W. The spectral composition of the fluorescent radiation is shown in fig. 3*a*, in which also the effect of the addition of incandescent light is demonstrated.

Irradiation in light of narrow wavelength bands was supplied in separate cabinets for each colour. In one experiment, the cabinets for low light intensity, already described by DE LINT (87), were used.

In each of these cabinets specific colour 'monophosphor' fluorescent lamps are installed on top, the far-red cabinet having incandescent lamps. In each cabinet, the lamp compartment was separated from the plant compartment by one or more glass or 'plexiglas' filters, and in the far-red cabinet also by an 8 cm water filter.

In figs. 3*b*, *d*, *e*, and *f* the spectral characteristics of the incident irradiations in the different compartments are presented. In most experiments, however, a new type of movable coloured light cabinets was used (Plate 1).

The inner dimensions of these cabinets are: 109 cm long, 69.5 cm wide, 68 cm high. The inside of a cabinet is completely aluminum-lined, so that the light distribution on plant level (plants placed at the bottom) is very uniform. Lamps are mounted at the top of the cabinet. By means of a fan in the upper part of the cabinet, air is sucked through the cabinet from one side near the double bottom of the plant compartment, to the other side near the top, from where it is expelled via the lamp compartment. The temperature inside the plant compartment does not differ more than 1 °C from that of the air space in which the cabinet is placed. Thus, by placing a cabinet in a temperature controlled phytotron room, we obtain a temperature controlled coloured light cabinet. The lamp compartment is separated from that of the plants by 'plexiglas' filters. In the lamp compartment, a maximum of 12 fluorescent lamps of 40 W each can be installed. The same types of 'monophosphor' fluorescent lamps as in the earlier cabinets have been used. In the far-red cabinet fourteen 60 W incandescent lamps have been used in combination with 'plexiglas' filters. A water filter was not included.

Most filters were 'plexiglas' RÖHM and HAAS filters (Darmstadt, Germany). To obtain far-red radiation, filters nr. 501 and nr. 627 were used; red: filter nr. 501, combined with PHILIPS TL 103339/40 W as a light source; blue: filter nr. 248, combined with PHILIPS TL 18/40 W as a light source. In figs. 3*c* and *d*, the spectral characteristics of the incident irradiations in the movable cabinets are presented. For the green light, PHILIPS TL 17/40 W lamps were combined with a glass filter, reducing the radiation to the region between ca. 500 and 600 nm (see fig. 3*e*).

2.2.3. *Light intensity measurements*

The incident intensities of irradiation have been measured just above soil level in ergs.cm^{-2}.sec^{-1} (1 erg = 10^{-7} W. sec) with a cosine corrected photocell (57). The photocell is calibrated with a standardized thermopile for each wavelength combination. Intensities of mixed radiations were determined by separate measurement of the components.

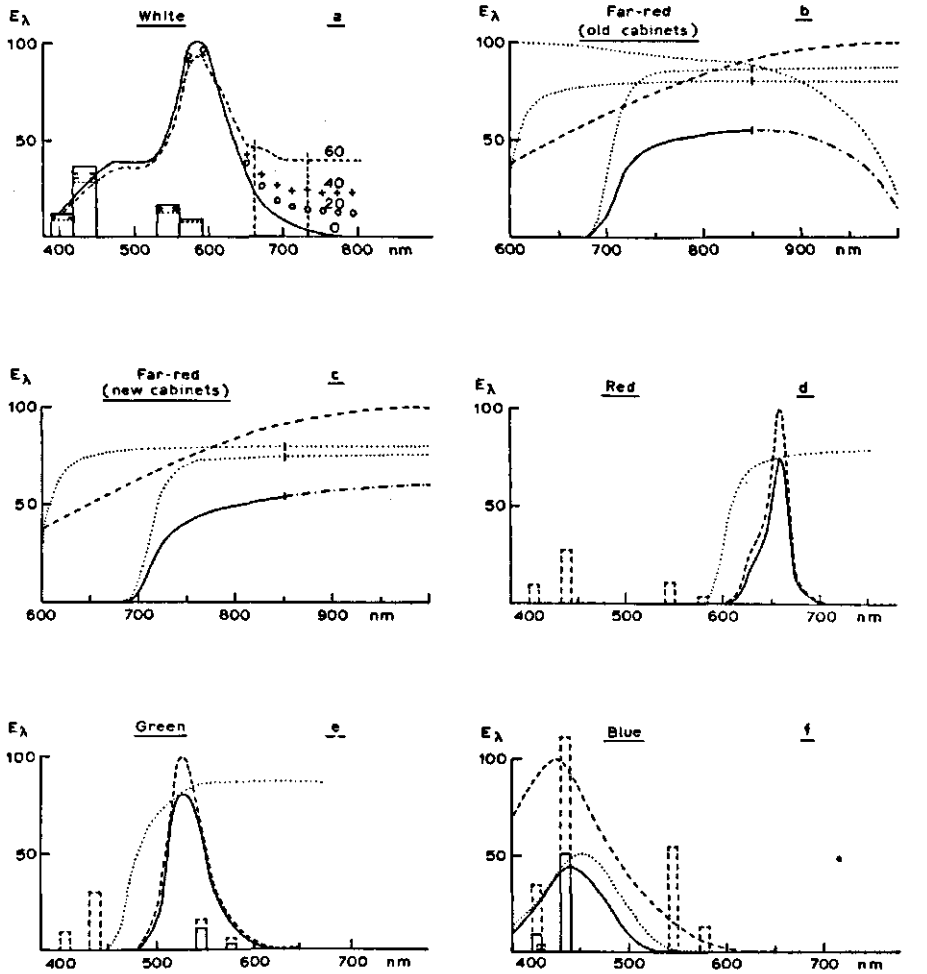


FIG. 3. Spectral composition of the irradiations; *a*: for TL 33/40 W, with 0, 20, 40 or 60 % of the, in the main illumination installed power, installed in incandescent lamps (from GAASTRA (46)); *b*, *c*, *d*, *e*, and *f*: for colour light; ——— energy distribution spectra of incident irradiations; – – – – emission spectra of the lamps; . . . . . transmissions of the filters. The transmission of the 'plexiglas' filters has been measured up to 850 nm; in figs. *b* and *c* the curves are extrapolated up to 1000 nm (–.–.–). The emission spectrum of the incandescent lamps was estimated from data of DE VOS (141); that of the red fluorescent lamps as well as the entire fig. 3*e* has been taken from DE LINT (87). The emission spectrum of the blue fluorescent lamps was obtained from the PHILIPS Laboratories.

In general, the light intensity values of the experiments are given in the legends of the figures.

2.3. PLANT MATERIAL

2.3.1. *General*

Hyoscyamus niger exists in an annual and a biannual form; the last one needs a period of cold before being responsive to long-day induction. According to a study of CORRENS, in 1904 (20), the two forms differ only in one pair of genes. MELCHERS, in 1936 (96), showed that the biannual form is not absolutely dominant over the annual one.

The plant type used throughout this investigation is an annual, yellow flowering strain of *Hyoscyamus niger* var. *pallidus*. Seeds were originally obtained from Dr. A. LANG (133). It is known as a qualitative long-day plant. The same strain was used in all earlier work on *Hyoscyamus* from this laboratory (142, 133, 23, 85, 150, 86, 145a, 87, 88, 28), and in many cases elsewhere (e.g. 82, 111, 100, 127, 128).

2.3.2. *Remarks on plant age*

In experiments on *Hyoscyamus*, mostly plants of some months old are used; sometimes plants of only one month old were used (68). LANG and MELCHERS (82) mentioned that the annual strain of *Hyoscyamus niger* already in early stages of growth reacts upon daylengths, and in 1965 LANG (81) stated that, after completion of the juvenile phase, with progressing age the minimum number of inductive cycles decreases further.

In order to obtain more information about the duration of a so-called juvenile phase, plants of six different ages, 41, 31, 21, 17, 14, and 12 days old respectively (from sowing, and grown in SD), were exposed to several LD and then placed in SD again. The experiment started 7-3-'66 and lasted 50 days. The two groups of oldest plants neither showed any difference in days to shooting, nor in flower initiation. LD treatment of younger plants, however, was less effective. Shooting was slower and the critical number of inductive cycles needed to obtain generative development and shooting, was higher. This effect of plant age was observed with the mixture of fluorescent and incandescent light as well as with fluorescent light only; in the latter, reactions were somewhat slower. When applied in the SD after-treatment, the two different light qualities did not show any difference in effect.

Averages of some data are given in fig. 4.

From this experiment it is concluded that, under our experimental conditions, no absolute juvenile phase was evident, but that, with increasing age, up to some 30 to 40 days, *Hyoscyamus* responds somewhat better to inductive treatment.

In other experiments, plants of 3 to 4 months old were mostly used, because these survived better than plants of 1 to 2 months under low light energy conditions, such as very short daylengths.

2.4. PARAMETERS OF FLOWER INITIATION

In an earlier paper (72), parameters of flower initiation have been briefly discussed. In general, the leaf or node index (cf. e.g. 81) is the most reliable para-

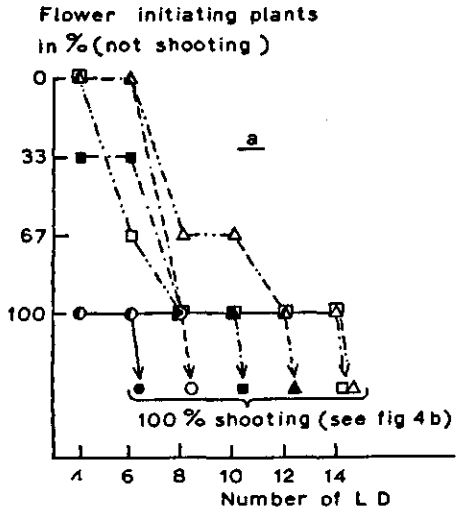
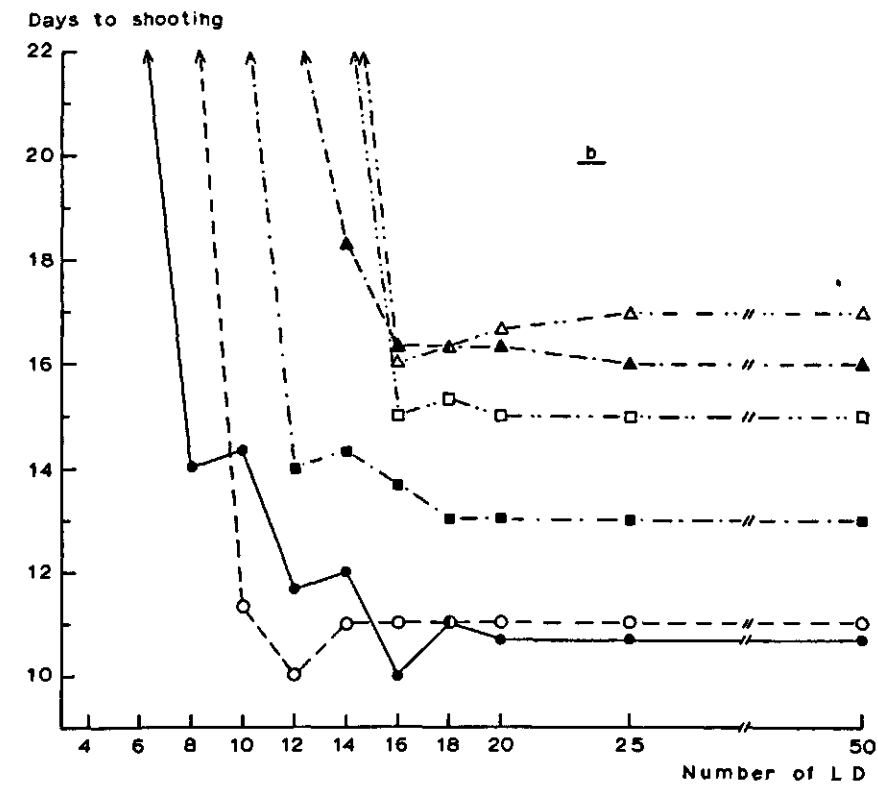


FIG. 4. Flower initiation (a) and shooting (b) of *Hyoscyamus* plants of 6 different ages: 41, 31, 21, 17, 14, and 12 days old (after sowing), subjected to different periods of LD (4, 6, 8, 10, 12, 14, 16, 18, 20, 25 or 50 LD), with an after-treatment in SD. LD as well as SD were applied in a mixed radiation of fluorescent and incandescent lamps (total irradiation <700 nm: 80,000 ergs.cm^{-2}.sec^{-1} and 17,500 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm). Averages of 4 plants. See also text.

●——● : 41 days old
○- - - - ○ : 31 days old
■-.-.-.-. ■ : 21 days old
□-..-..-□ : 17 days old
▲- - . - -▲ : 14 days old
△- - . . - - △ : 12 days old



meter of flower initiation, reflecting differences in the relation between vegetative growth and the beginning of flower initiation. Thus, possible environmental effects on developmental rates in later stages of flower development are eliminated (81). However, in section 3.2. of this paper, it will be shown that the leaf index neither is a fully reliable parameter since, in *Hyoscyamus*, leaf initiation and flower initiation both depend on daylength, but in different ways.

For *Hyoscyamus* 'leaf increment' is determined rather than total number of leaves. When plants with numerous leaves, like *Hyoscyamus*, are used, variation in the number of leaves may be considerable already at the start of an experiment. Therefore, total number of leaves is an inaccurate parameter for the determination of the relative moments of floral induction. It is better to use 'leaf increment' which, however, neither is always sufficiently accurate as a parameter of flower initiation.

For this reason, in the present paper, the number of flower primordia on the main flower stalk is used as the principal characteristic. To determine its value, the production rate of flower primordia has been investigated in LD and also in SD after different numbers of LD (fig. 5). In the second case, counts were made after equal total numbers of days, including both LD and SD.

For this experiment, the plants were sown directly in pots and grown at 20 °C under fluorescent light in SD. The experiment started 28-9-1966, when the plants were 1½ month old. SD was then given as a mixture of fluorescent and

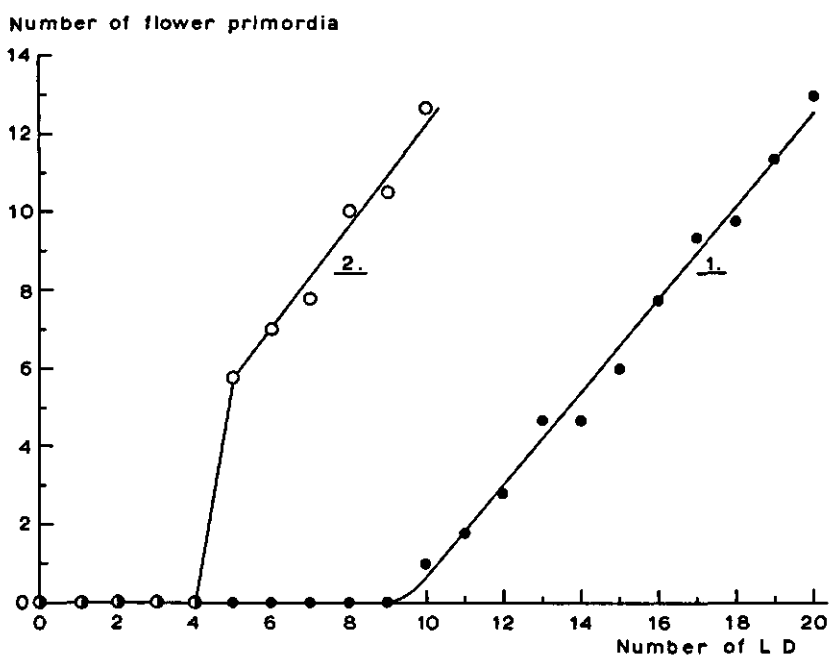


FIG. 5. 1. Rate of flower initiation of *Hyoscyamus* in LD, and 2. number of flower primordia after x LD and (25-x) SD. Averages of 5 plants. See also text.

incandescent light (total irradiation < 700 nm: 81,000 ergs.cm^{-2}.sec^{-1} and 17,500 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm) for 9 hours, while LD was given by extending the same short day with 7 hours of low intensity incandescent light (< 700 nm: 1000 ergs.cm^{-2}.sec^{-1} and between 700 and 1000 nm: 2700 ergs.cm^{-2}.sec^{-1}).

At the start of the experiment, the plants were placed in LD, and daily (up to 20 LD) 5 plants were harvested and the number of flower primordia was counted (see fig. 5, curve 1). From this curve it is clear that after a certain number of inductive cycles (10 LD), the first flower primordium is seen and production then increases linearly with increasing number of LD.

Besides, every day (up to 10 LD) groups of plants were transferred back to SD, and 25 days after the first LD, 5 plants of each of these groups were harvested, and flower primordia counted (see fig. 5, curve 2). Curve 2 shows that also with a limited number of LD, with SD after-treatment, the number of flower primordia recorded after a fixed total number of days, reflects the duration of the inductive treatment. The demonstrated results indicate that at least 10 days are necessary for a flower primordium to become apparent and, moreover, that 5 inductive days are sufficient for the production of 6 flower primordia, and 10 days for 13 primordia, after a (total) reaction time of 25 days. The latter difference can be interpreted as the effect of increased induction (the difference be-

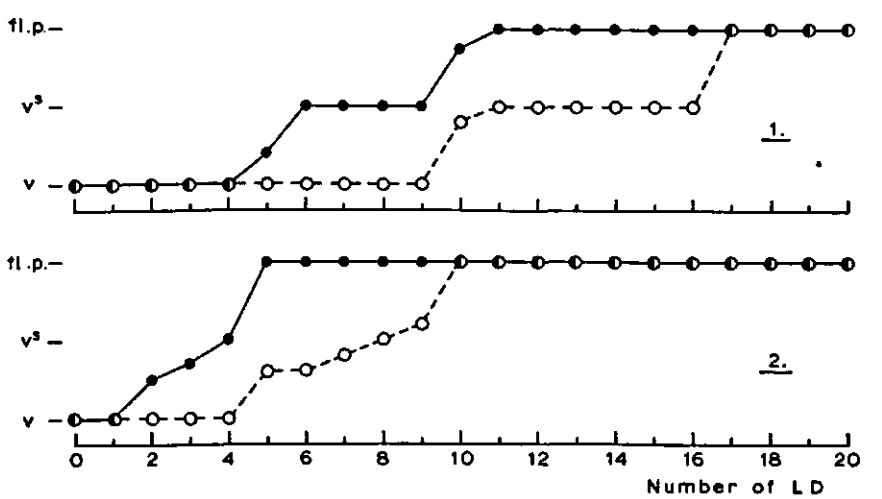


FIG. 6. Macroscopic and microscopic examination of the state of the growing point of *Hyoscyamus*: 1. during induction in LD; 2. after x LD and (25-x) SD;
v: vegetative in macroscopic and microscopic examination; no visible stem elongation;
v^s : stem elongation, but no flower primordia visible in the examination used;
fl. p.: flower primordia visible in the examination used.
●———● : microscopic examination
○- - - -○ : macroscopic examination
See also text.

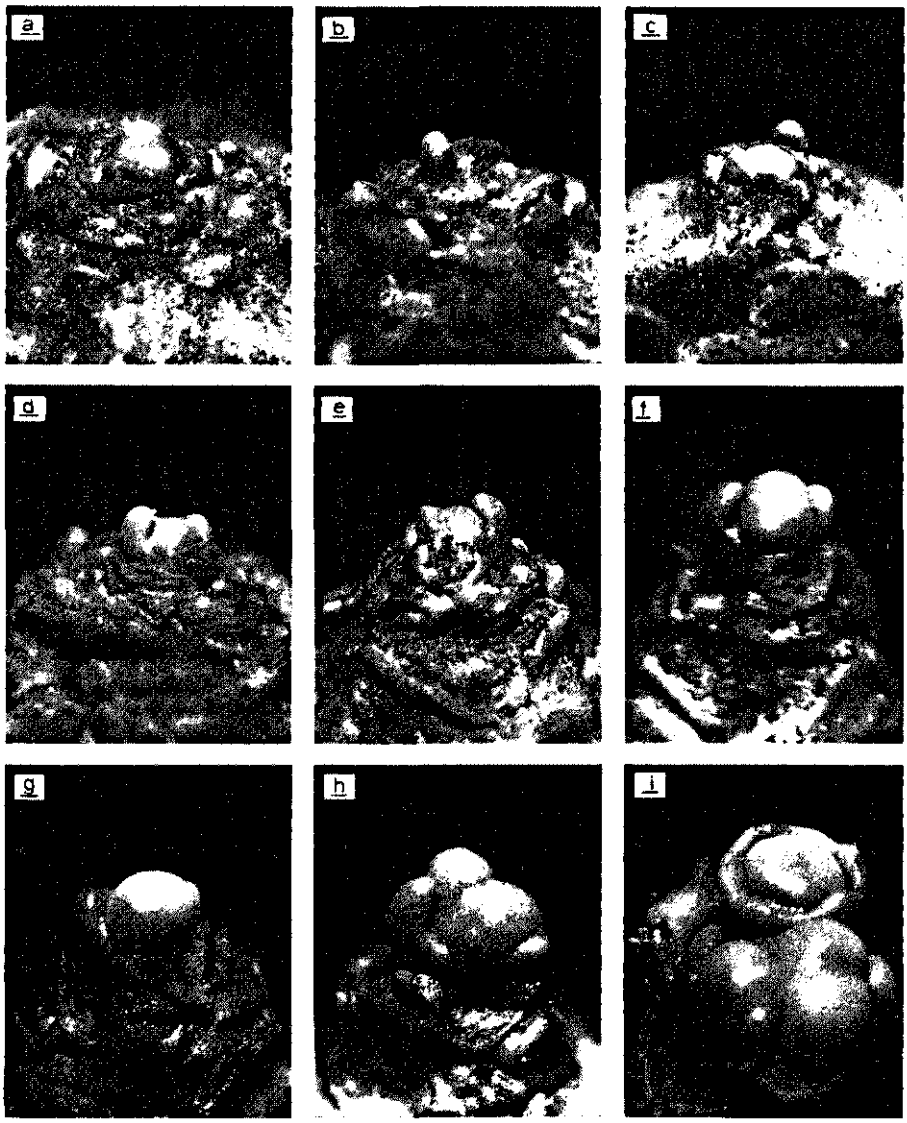


PLATE 2. Growing points of *Hyoscyamus* plants after different numbers of LD: *a.* 0; *b.* 2; *c.* 4; *d.* 5; *e.* 7; *f.* 9; *g*, *h*, and *i.* 10 LD. Enlargement: 1:47.

tween 5 and 10 LD), whereas, in curve 1, it seems that the effect contains a mixture of increase in induction and in reaction time. It is remarkable, from this viewpoint, that both curves are very nearly parallel.

In the same experiment, a comparison was made between macroscopically visible shooting and the microscopical examination of flower bud production. For the daily harvests of plants in continuous LD, visible shooting was nicely

correlated with flower bud production as found under the binocular microscope (see fig. 6,*1*). However, with limited LD treatment and SD after-treatment (fig. 6,*2*), only 60–80% of the plants which had received 5, 6 or 7 LD, had started shooting, but all showed flower primordia. So, in these cases, the correlation in time between the two criteria is somewhat less apparent.

Growing points of plants in LD were harvested daily and photographed (Plate 2). Until after 4 LD, in this experiment, the growing point remained rather flat (Plate 2*a-c*). Only after a greater number of LD it became more and more globular, and stem elongation seemed to start (Plate 2*d-g*); flower initiation started after about 10 LD (Plate 2*h-i*).

From the foregoing, it can be concluded that as long as the plants are in inductive conditions, 'number of flower primordia' and 'days to shooting' are equivalent parameters to determine flower initiation in a qualitative way. Also, 'number of unexpanded leaves' has shown to be a useful parameter (72). 'Leaf increment' is not always as good as the other parameters, while the influence of daylength on the growth rate (leaf increment) is somewhat different from that on the rate of flower initiation (82). Therefore, in cases that leaf increment is used in this paper, at least one other parameter will also be used.

3. DAYLENGTH DEPENDENCE OF FLOWER INITIATION IN WHITE LIGHT

3.1. INTRODUCTION

Amongst the extensive literature about light influences on flowering, surprisingly little information on the response curves of daylength dependence of flowering is found. Mostly, the daylength dependence was examined to establish the critical daylength (49). For *Hyoscyamus* this has been done in great detail by LANG and MELCHERS (82). They established the critical daylength with the aid of two parameters, 'days to shooting' and 'leaf increment'. Under their experimental conditions, at various temperatures, they found a slightly shorter critical daylength for leaf increment. For both parameters, the critical daylength was longer at higher temperatures. At 20 °C, they determined the critical daylength to be about 9 hours 40 minutes.

Under his experimental conditions, at 20 °C, EL HATTAB (28) found the critical daylength to be between 10 and 12 hours; the daylength reaction was the same under fluorescent light and under a mixture of fluorescent and incandescent light, with a slightly faster response under the mixture. The difference between LANG and MELCHERS, and EL HATTAB may be due to slight differences in growth of the plants (97) or to small differences in experimental conditions.

However, the critical daylength is not completely fixed in *Hyoscyamus*, and the term 'critical daylength' is rather senseless in view of recent photoperiodicity research, especially that in narrow spectral regions (148, 85, 28), but when a description of the photoperiodic response includes a specification of the environmental conditions, 'critical daylength' still is a useful term.

In section 3.2., an experiment is described in which the critical daylength has been established in the two white light qualities used throughout most of our experiments.

In section 3.3., some experimental factors possibly influencing the daylength dependence of flower initiation in white light are examined in greater detail, and in section 3.4., the influence of light intensity on the daylength dependence of flower initiation is demonstrated.

Some investigators examined the influence of daylength on flower initiation below the critical daylength. As already mentioned in section 1.2., BEST (5) and DE LINT (87) presented complete photoperiodic response curves for long-day plants (fig. 1, p. 2 and fig. 2, p. 3). Also LONA (92), with the LDP *Urtica pilulifera* L. and PARLEVLIET (113), with spinach, studied very short daylengths and, like the previous authors, found decreased inhibition of flower initiation in very short photoperiods.

3.2. THE EFFECT OF TWO WHITE LIGHT SOURCES ON THE CRITICAL DAYLENGTH

In this experiment, two light qualities were used. First, fluorescent light was applied at an intensity of 53,000 ergs.cm^{-2}.sec^{-1}, being about the same as that

under which the plants were grown before the experiment. Secondly, a mixture of fluorescent and incandescent light was applied at an intensity of 25,000 ergs.cm^{-2}.sec^{-1} below the wavelength of 700 nm, and of 8600 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm.

In both light qualities plants were exposed at 20 °C, to photoperiods of 540, 600, 660, 690, 720, 750, 780, 810, 840, 1000, 1200 or 1440 minutes per day. The experiment started 22-7-'69 and ended 18-9-'69, after 58 days.

As can be seen from fig. 7, shooting is faster in the mixed irradiation than in fluorescent light only. It is not likely that this is due to the lower intensity of photosynthetically active light in the mixture, since EL HATTAB (28) found the same for equal light intensities. He concluded that the acceleration of shooting is, at least in part, directly due to the admixture of incandescent light. From fig. 7, it is evident that for visible shooting the critical daylength is somewhat shorter (660–690 min.) under the mixed irradiation than under pure fluorescent

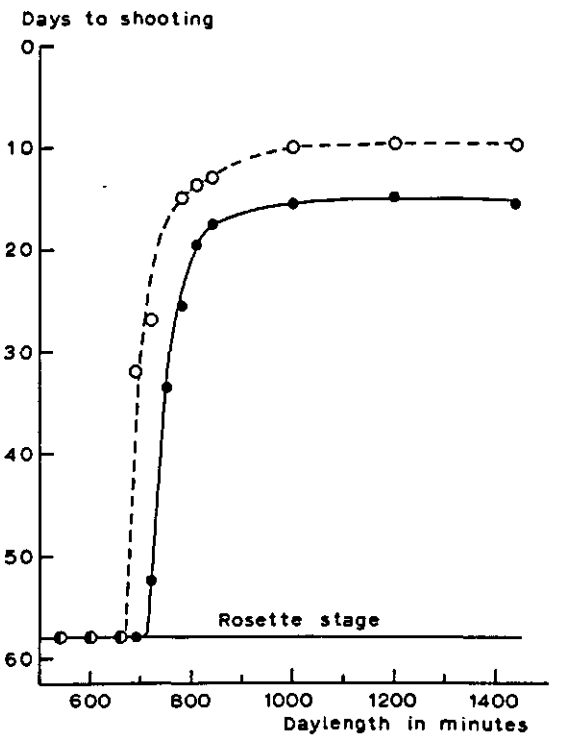


FIG. 7. Days to shooting in *Hyoscyamus* under various photoperiods with two sources of white light, viz., fluorescent light (●———●) at an intensity of 53,000 ergs.cm^{-2}.sec^{-1}, and a mixture of fluorescent and incandescent light (○- - - -○) at an intensity of 25,000 ergs.cm^{-2}.sec^{-1} < 700 nm and of 8600 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm. The experiment started 22-7-'69 and was finished after 58 days. Averages of 5 plants.

light (690–720 min.). This observation does not disagree with EL HATTAB'S results, since he did not discriminate between photoperiods in the range between 10 and 12 hours. Under both light qualities, the number of days to shooting reached a saturation level at about 960 minutes of light; longer daylengths did not or only slightly speed up shooting.

Microscopic examination of the growing point at the end of the experiment showed that under both light qualities all plants in photoperiods of 660 minutes or longer had initiated flower primordia. Apparently, the critical daylength for flower initiation lies between 600 and 660 minutes (Table 1), which is somewhat shorter than that for visible shooting.

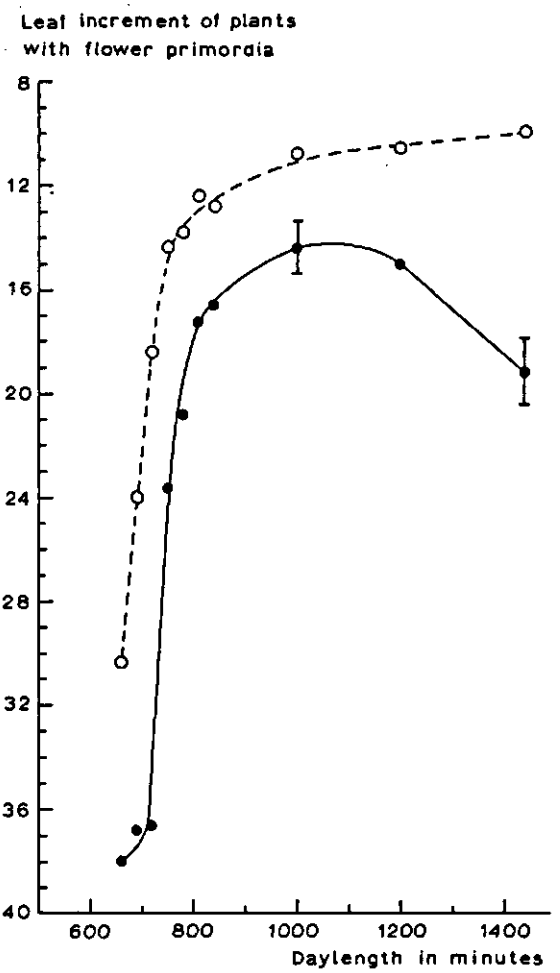


FIG. 8. Leaf increment till flower initiation in *Hyoscyamus* under various photoperiods with two sources of white light, viz., fluorescent light (●———●), and a mixture of fluorescent and incandescent light (○- - - -○). Standard deviations are indicated by bars. See also legend fig. 7.

TABLE 1. State of the growing point of *Hyoscyamus* under various photoperiods with two different light sources; v: vegative; f: flower primordia formed. Examination after 58 days. See also legend fig 7.

Daily light period (minutes)	540	600	660	690	720	750	780	810	840	1000	1200	1440
TL + Inc.	v	v	f	f	f	f	f	f	f	f	f	f
TL	v	v	f	f	f	f	f	f	f	f	f	f

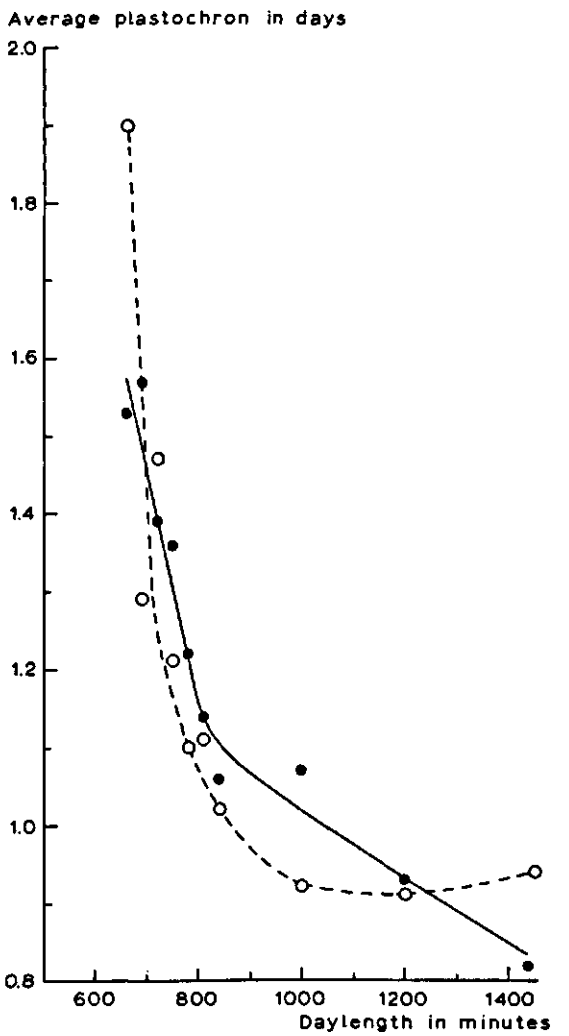


FIG. 9. Average plastochron in days in *Hyoscyamus* till flower initiation under various photoperiods of two sources of white light, viz., fluorescent light (●———●), and a mixture of fluorescent and incandescent light (○- - - -○). See also legend fig. 7, and text.

However, in this experiment, in all plants that had initiated flower primordia some 'shooting' had started, since a 'stem' of at least 2 mm could be observed upon microscopic examination. On the whole, our observations agree nicely with those of LANG and MELCHERS (82).

The graphs presenting leaf increment of the plants with flower primordia (fig. 8) show that, in the mixed irradiation, leaf increment values are lower than in fluorescent light; thus, not only flower initiation as determined from shooting, but also as determined from leaf increment values is faster in the mixture than in fluorescent irradiation alone. The higher values of leaf increment, indicating somewhat slower flower initiation under continuous fluorescent light (1440 min. photoperiod), as compared with 1000 and 1200 minutes, agree nicely with the 'BEST-curve' (cf. fig. 1) for LDP, where a slight increase in time to flower initiation in the longest photoperiods is assumed (5).

In fig. 9, the average plastochron is presented for the various treatments. A plastochron represents the time in days, necessary to initiate a leaf, and is obtained here by dividing the number of days to shooting by the corresponding leaf increment value. It is evident that leaf initiation is faster in longer days. The increase in leaf increment values under continuous fluorescent light, therefore, is mainly due to the increased rate of leaf initiation and not to retarded initiation of flower primordia. Since 'days to shooting' and 'leaf increment' both clearly depend on daylength, but not exactly in the same way (compare fig. 7 with fig. 8), they are not fully interchangable parameters for flower initiation.

3.3. EXPERIMENTAL FACTORS POSSIBLY INFLUENCING THE DAYLENGTH DEPENDENCE OF FLOWER INITIATION

3.3.1. *Introduction*

In a previous paper (72) some results have been presented from an experiment of which the schedule of treatment was: (2 SD + 3 TD) 4 times, with an after-treatment of 10 LD (TD = Treatment Day(s)). In the present paper, most experiments are carried out according to this type of schedule, and therefore, it seems worth-while to examine in some detail the influence on flower initiation of the various components involved.

In subsection 3.3.2., the alternation of 2 SD with 3 TD, which had been chosen rather arbitrary, will be compared with another one to see how the sequence influences the results.

In subsection 3.3.3., the effect of the duration of the LD after-treatment – which was not exactly the same in all experiments – will be discussed.

In subsection 3.3.4., we will try to analyse the possible interaction of the daylength effect of the SD cycles, intercalated to keep the plants in sufficient condition for prolonged experimental periods, with that of the TD cycles proper.

In subsection 3.3.5., the effect of the number of repetitions of each combination will be discussed.

In the previous paper (72), daylength curves have been presented with daylengths on a logarithmic scale as in (87). This presentation resulted in graphs

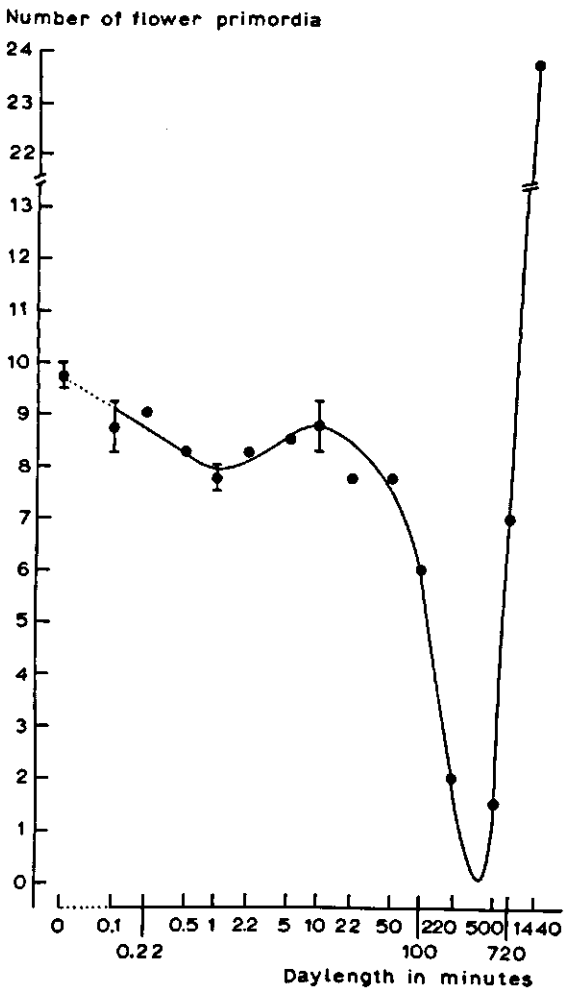


FIG. 10. Number of flower primordia in *Hyoscyamus*. Daylength treatment schedule: (2 SD + 3 TD) 4 times, followed by 10 LD. Light during TD as well as LD was given as mixed radiation from fluorescent and incandescent lamps (total radiation < 700 nm: 24,000 ergs.cm^{-2}.sec^{-1} and 7400 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm). SD was given as fluorescent light (52,000 ergs.cm^{-2}.sec^{-1}). The experiment started 14-5-'68. Averages of 4 plants. Standard deviations indicated by bars.

with a clear second maximum for inhibition of flower initiation around 1 minute (fig. 10). It appears, however, more suitable to present the same data with the daylength on a linear scale (fig. 11), as has mainly been done in the present paper, because the small maximum around 1 minute exposure may well receive too much stress in presentations on a logarithmic scale. The consequence of this is that data regarding extremely short photoperiods cannot always be recorded

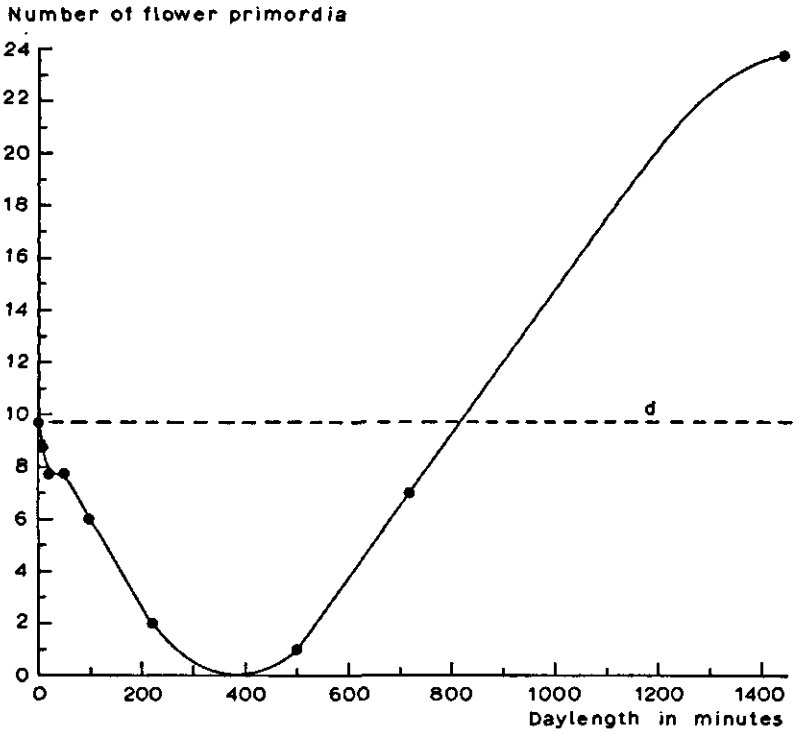


FIG. 11. Number of flower primordia in *Hyoscyamus* upon different daylength treatments. Line *d* marks the dark level, that is the number of flower primordia obtained with TD = 0 min. Other daylength treatments will always be compared with *d*. Daylength treatments resulting in values below the dark level are called inhibitive and those resulting in values above *d*, promotive for flower initiation. See also legend fig. 10.

properly in the graphs; they are, however, included, if they are especially relevant.

3.3.2. *Short days interrupting a period of daylength treatments*

The following experiment shows the difference in daylength sensitivity of flower initiation in treatments given according to the schedule (2 SD + 3 TD) 4 times or to (3 SD + 4 TD) 3 times, both with an after-treatment of 11 LD and carried out at 20 °C. The SD consisted of 495 minutes of fluorescent light. The TD had photoperiods of: 0, 0.1, 0.22, 0.5, 1, 2.2, 5, 10, 22, 50, 100, 220, 500, 720 or 960 minutes. Both the TD and the LD after-treatment were given as a mixture of fluorescent and incandescent light (see legend fig. 12*a*, cf. also subsection 2.2.2.). The counting of flower primordia has been carried out after 11 LD of after-treatment, at the time that all plants were shooting (fig. 12*a*).

In the daylength region between 100 and ca. 650 minutes, both treatments gave about the same results, but in the less inhibitive and in the promotive day-

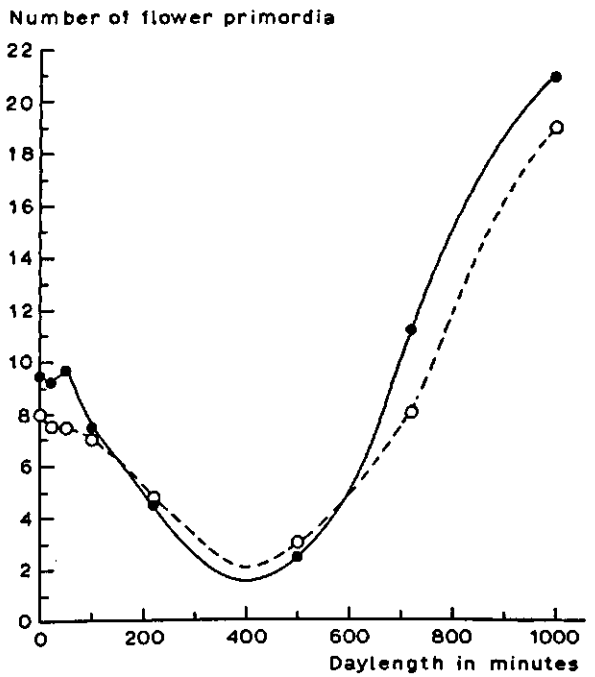


FIG. 12*a*. Number of flower primordia in *Hyoscyamus* upon daylength treatments according to the schedule (2 SD + 3 TD) 4 times (●———●), or (3 SD + 4 TD) 3 times (○- - - -○), both followed by 11 LD. Light intensities of the mixed radiation in LD and TD: 25,500 ergs.cm^{-2}.sec^{-1} < 700 nm and 9000 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm; SD was applied as fluorescent light (53,000 ergs.cm^{-2}.sec^{-1}). The experiments started at 13-7-'68 and 12-7-'68 respectively. Averages of 4 plants; only in the 1000 min. TD averages of 3 (●) or 2 plants (○).

lengths, viz., between 0 and 100 minutes, and longer than ca. 650 minutes, the first schedule, (2 SD + 3 TD) 4 times, resulted in slightly more flower primordia than the second one, (3 SD + 4 TD) 3 times.

Since the last group of TD was immediately followed by the LD after-treatment without intercalation of SD, in both schedules, the plants have received 12 TD, interrupted at different times by a total of 6 SD. Thus, the differences between the two effects obtained are due either to the different numbers of SD per interruption or to the different numbers of repetitions. For the sake of simplicity of the scheme, the last few SD of the precultivation period were considered to provide the start of the treatment.

When one of the curves is shifted so that the 0 minutes TD values coincide, it is evident that the effect of the most inhibitive daylengths is greater with the first schedule than with the second one, while the effect of the most promotive daylengths applied seems about the same in both schedules (fig. 12*b*). Thus, the type of schedule seems to influence differently the effect of inhibitive and promotive daylengths and it seems probable that different reactions are limiting the

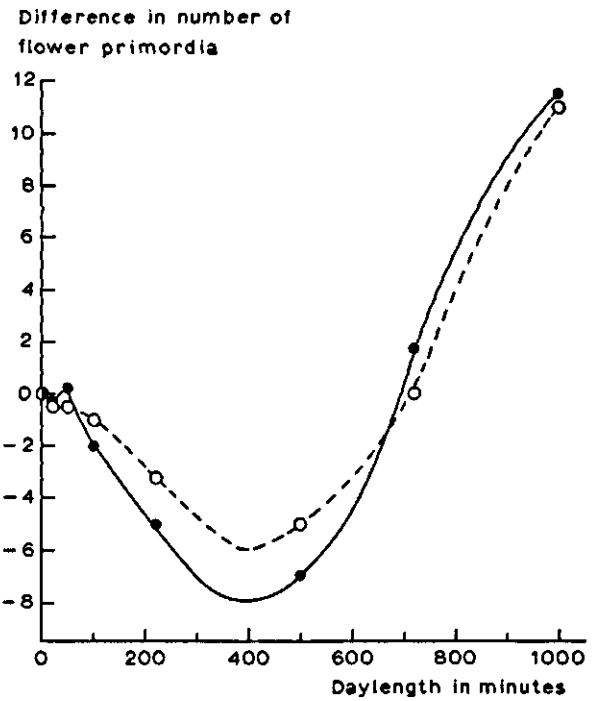


FIG. 12*b*. Difference in number of flower primordia in *Hyoscyamus* between values of the 0 min. TD and different TD-daylengths, applied according to the schedule (2 SD + 3 TD) 4 times (●———●) or (3 SD + 4 TD) 3 times (○- - - - -○), both followed by 11 LD. The 0 min. TD-value is zero for both schedules. See also legend fig. 12*a*.

overall process in the inhibitive daylengths and in the promotive ones.

However, on the whole, for flower initiation, with both schedules very similar daylength dependence curves are obtained.

3.3.3. *The duration of the LD after-treatment*

In this subsection, some data are presented about the effect of the duration of the LD after-treatment on the daylength dependence curves for flower initiation. The schedule of treatment in this experiment was: (4 SD + 3 TD) 4 times, with an after-treatment of 7, 10 or 12 LD. The experiment has been carried out at 20 °C. The SD consisted of 9 hours fluorescent light. The TD had photoperiods of: 0, 0.1, 0.3, 1, 3, 7, 15, 30, 100, 220, 500, 720 or 1000 minutes. The TD as well as the LD were given in a mixture of fluorescent and incandescent light. Harvests have been made after 7, 10, and 12 LD, and flower primordia of all plants were counted. Data are presented in fig. 13. Average plants from the last group (which had received 12 LD) are presented on Plate 3.

The curves in fig. 13 are sufficiently parallel to allow the conclusion that prolongation of the after-treatment does not essentially alter the effect of the TD on flower initiation.

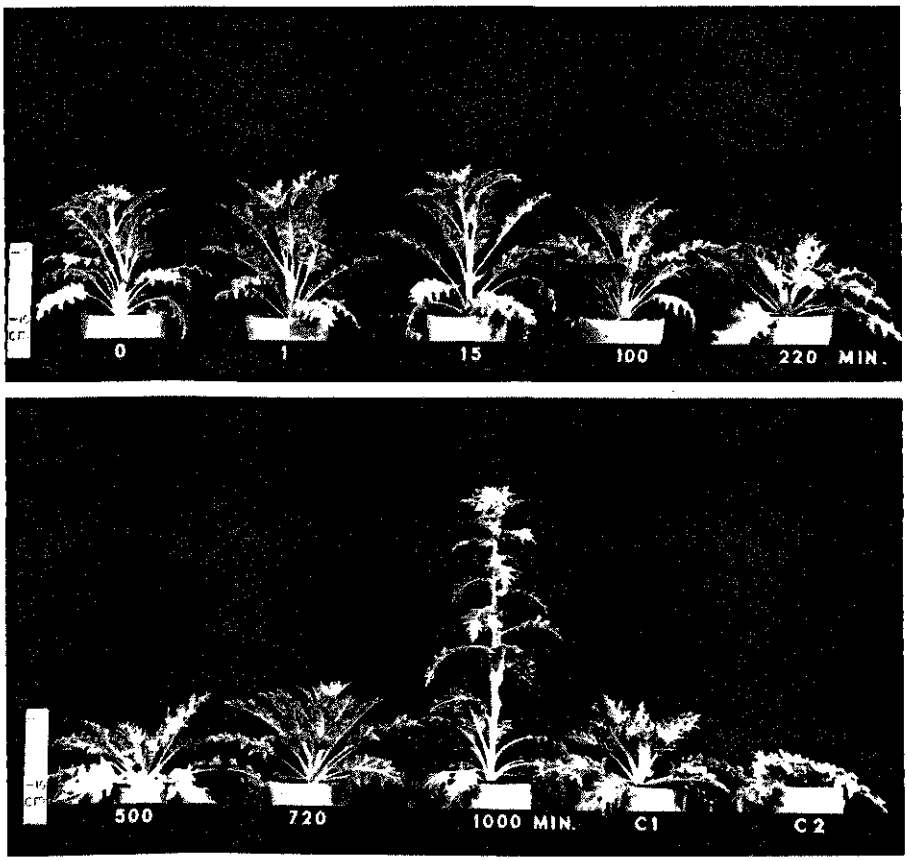


PLATE 3. *Hyoscyamus* as affected by different daylength treatments, applied according to the schedule (4 SD + 3 TD) 4 times, followed by 12 LD. C1 received 9 hours of fluorescent light during the TD, and the LD after-treatment, while C2 received SD in fluorescent light for the entire duration of the experiment. Photographed at the end of the after-treatment, at 12-2-'69. See also legend fig. 13.

3.3.4. *The photoperiod of the days interrupting the daylength treatments*

In this subsection, SD in the schedule formulae will be replaced by ID (Intercalated Days), since the duration of the photoperiod of the cycles interrupting the TD was varied.

In the first experiment, ID with photoperiods of 6 and 9 hours of fluorescent light were compared. The schedule was: (3 ID + 4 TD) 3 times, with an after-treatment of 12 LD. The TD had photoperiods of: 0, 0.1, 0.22, 0.5, 1, 2.2, 5, 10, 22, 50, 100, 220, 500, 720 or 960 minutes; LD and TD irradiations were applied as mixed fluorescent and incandescent light, all at 20°C.

Flower primordia of all plants have been counted at the end of the after-treatment; averages are presented in fig. 14.

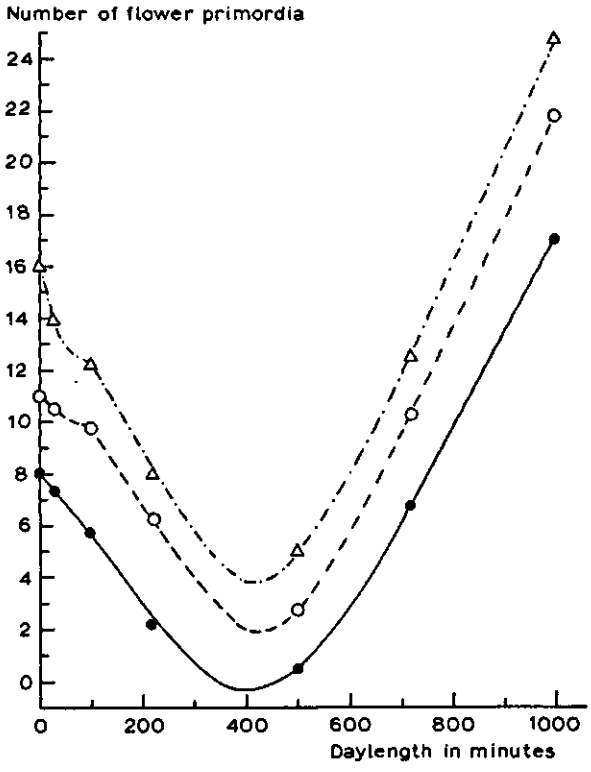


FIG. 13. Number of flower primordia in *Hyoscyamus* upon daylength treatments according to the schedule (4 SD + 3 TD) 4 times, followed by 7 LD (●———●), 10 LD (○- - - -○) or 12 LD (△-.-.-△). Light intensities of the mixed radiation in LD and TD: 24,000 ergs.cm^{-2}.sec^{-1} < 700 nm and 9000 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm; SD was applied as fluorescent light (52,000 ergs.cm^{-2}.sec^{-1}). The experiment started 3-1-'69. Averages of 4 plants (in the second series, in the 0 min. TD, 1 plant died).

Although the same type of curves is obtained for both ID, there are some differences. In the 6 hours series, some plants were rather weak or even dying by the end of the experiment; in particular, plants receiving less than 100 minutes of light per day during the TD.

For the region of TD with photoperiods longer than ca. 650 minutes, it is clear that the difference between the two treatments is practically nil: the slope of both curves is about the same. For the region of TD with photoperiods shorter than ca. 400 minutes, it is evident that with the 6 hours photoperiod ID, which daylength as such produces about maximum inhibition, the slope of the curve is less steep than that with 9 hours photoperiod ID. Thus, a strongly inhibitive ID (compare the 0 min. values in fig. 14) can reduce the inhibitive effect of the TD (as compared to their own zero min. value) in the daylength region shorter than ca. 400 minutes. However, in the region of daylength treatments

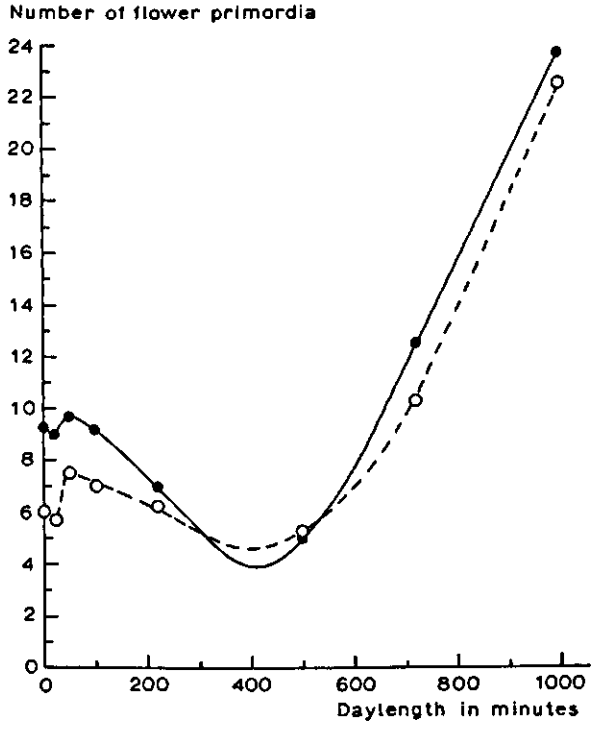


FIG. 14. Number of flower primordia in *Hyoscyamus* upon daylength treatments according to the schedule (3 ID + 4 TD) 3 times, followed by 12 LD. The ID had photoperiods of 9 hours (●———●) or of 6 hours (○- - - -○) of fluorescent light (53,000 ergs.cm^{-2}.sec^{-1}). Light intensities of the mixed radiation in LD and TD: 25,500 ergs.cm^{-2}.sec^{-1} < 700 nm and 9000 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm. The experiment started 2-8-'68. Averages of 4 plants (2 plants only in the 0, 50, and 960 min. TD, and 3 plants in the 22 min. TD of the 6 hours series; also 3 plants in the 960 min. TD of the 9 hours series).

longer than ca. 650 minutes, the increase with daylength of the promotive action is equal in both curves.

In the second experiment, daylengths of ID had 9 or 12 hours of fluorescent light. This time, the schedule of treatment was: (4 ID + 3 TD) 4 times. The photoperiods applied during the TD were the same as in the preceding experiment, except for the 960 minutes which was replaced by 1000 minutes. Again, a mixed irradiation was used for the photoperiods of TD and LD.

The duration of the LD after-treatment varied for both ID series, 10 LD being applied to the ID series with the 9 hours photoperiod and 6 LD to the series with the 12 hours photoperiod, since in this case flower formation was already more advanced. As shown in subsection 3.3.3., the duration of the LD after-treatment, though influencing their relative position in the graph, does not alter the shape of the daylength dependence curves, so that it is allowed to directly compare the two curves obtained, with respect to their shape.

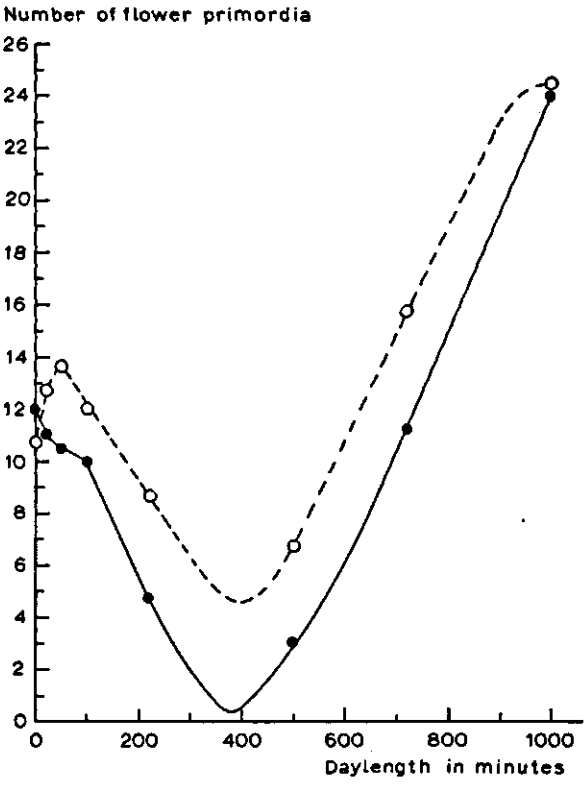


FIG. 15. Number of flower primordia in *Hyoscyamus* upon daylength treatments according to the schedule (4 ID + 3 TD) 4 times, followed by a certain number of LD. In series *a* (●———●) the ID had photoperiods of 9 hours and 10 LD were applied, and in series *b* (○- - - -○) the ID had photoperiods of 12 hours and 6 LD were applied. The intensity of the fluorescent radiation in the ID was 73,000 ergs.cm^{-2}.sec^{-1} and in the mixed radiation of the TD it was 24,000 ergs.cm^{-2}.sec^{-1} < 700 nm and 10,000 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm, and in the LD it was 27,000 ergs.cm^{-2}.sec^{-1} < 700 nm and 8200 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm. The experiment started 8-11-'68. Averages of 4 plants (except, series *a* 22 min. TD, 1 plant, and 50 min. TD, 2 plants; series *b* 50 and 220 min. TD, 3 plants).

Averages of the numbers of flower primordia are presented in fig. 15.

The curves of series *a* (ID = 9 hrs) and *b* (ID = 12 hrs) are very similar. Only at the shortest TD there is some difference, and near the 1000 minutes TD the curve of series *b* seems to reach some saturation level while that of series *a* does not.

From the data presented in this subsection it can be concluded that, in order to obtain the greatest differences between the effects of the different TD, ID should have a photoperiod of about 9 hours or slightly higher, viz. about the 'critical daylength'.

Preliminary data suggest, that ID with photoperiods of 16 hours tend to flatten the entire curve.

Taking into consideration the results reported in this subsection, in the following experiments described in this paper, we mostly used ID with photoperiods of 9 hours which, moreover, are the same as used in the SD precultivation period.

3.3.5. *The number of repetitions of the treatments*

In the experiments, described in the preceding sections, always 12 TD have been applied, interrupted by a certain number of, mostly, SD. In the following experiment, the effect of the number of repetitions of the combination (4 SD + 3 TD) on the shape of the daylength response curves for flower initiation is examined. The schedule of treatment was: (4 SD + 3 TD) which was repeated: 2 (series *a*), 4 (series *b*) or 6 (series *c*) times respectively, all with an after-treatment of 10 LD. The photoperiods of the SD consisted of 9 hours of fluorescent light. Photoperiods of the TD were: 0, 0.1, 0.22, 0.5, 1, 2.2, 5, 10, 22, 50, 100, 220, 500, 720 or 1000 minutes. The TD and the LD were given as a mixture of fluorescent and incandescent light. For series *a* and *c*, the experiment started on 4-10-1968, and for series *b* on 18-10-1968. At the end of the experiment, on 28-10-1968 for series *a*, and on 25-11-1968 for series *b* and *c*, flower primordia were counted (primordia in the 1000 min. TD of series *b* and *c* were estimated; in particular in series *c* only a rather rough estimation could be made). Averages are presented in fig. 16.

It is evident that the three curves have essentially the same shape with a minimum at about the same daylength (ca. 385 min.). With more repetitions, the minimum is lower relative to the 0 minutes TD value. The effect of the third plus fourth week of treatment is considerably less for the extreme short photoperiods than that of the fifth plus sixth week of treatment. For the long photoperiods this is not so. This finding may point to the existence of a sequence or combination of reactions which are different for very short daylengths and long ones.

3.4. The effect of light intensity on the daylength dependence of flower initiation

In 1953, de Zeeuw (161) found that the SDP *Perilla crispa* could be brought to initiate flowers in LD and even in continuous light, provided the light intensity was low. On the other hand, 8 hours of high light intensity followed by 8 hours of low light intensity produced the normal long day effect in *Perilla*. For *Perilla*, the light intensity range allowing flowering, decreases with increasing daylength (162). De Lint (87) compared the effect of two intensities of red light (9500 and 4250 ergs.cm^{-2}.sec^{-1}) for several daylengths on shooting of *Hyoscyamus*, and observed a weaker SD-effect and a somewhat stronger LD-effect with *Hyoscyamus* at the lower light intensity.

In order to obtain some more information about the effect of light intensity in several daylengths, the following experiment was set up.

Three different light intensities, viz., 24,000, 2400, and 400 ergs.cm^{-2}.sec^{-1}

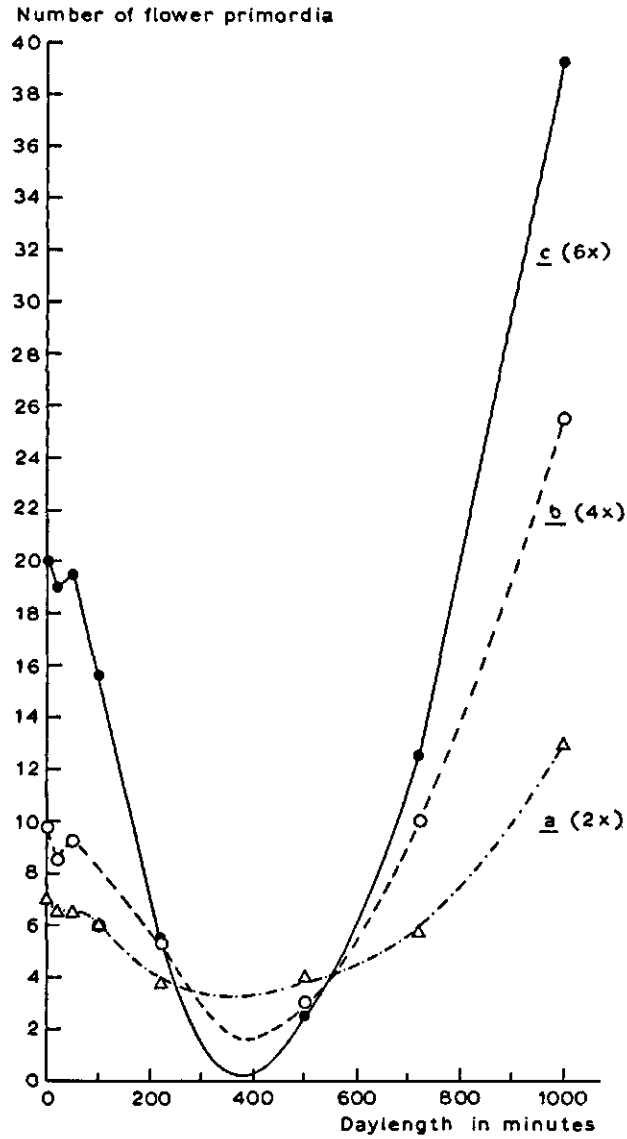


FIG. 16. Number of flower primordia in *Hyoscyamus* upon different daylength treatments according to the schedule (4 SD + 3 TD) *n* times, followed by 10 LD; *n* being, *a*: 2 (△-.-. △), *b*: 4 (○- - - - -○), and *c*: 6 (●——●). The light intensity of the mixed radiation in the TD was 24,000 ergs.cm^{-2}.sec^{-1} < 700 nm and 10,000 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm; in the LD: 27,000 ergs.cm^{-2}.sec^{-1} < 700 nm and 8200 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm, and in the SD (fluorescent light) 66,000 ergs.cm^{-2}.sec^{-1}. The experiment started for series *a* and *c* at 4-10-'68, and for series *b* at 18-10-'68. Averages of 4 plants (3 plants in the 22 min. TD of series *a*).

(radiation wavelengths $<$ 700 nm) were applied. The value of 24,000 ergs.cm^{-2}.sec^{-1} was chosen since it had been used also in several other experiments. With respect to photosynthesis, this intensity certainly was far above the compensation point for the leaves, which is around 4000 ergs.cm^{-2}.sec^{-1} at 20°C; the intensity of 2400 ergs.cm^{-2}.sec^{-1} is just below and that of 400 ergs.cm^{-2}.sec^{-1} is far below the compensation point.

The schedule of the treatment again was: (4 SD + 3 TD) 4 times, with an after-treatment of 10 LD. During the SD, the plants received photoperiods of 9 hours of fluorescent light, and during the TD, photoperiods of 0, 0.1, 0.22, 0.5, 1, 2.2, 5, 10, 22, 50, 100, 220, 320, 500, 720, 840 or 1000 minutes. TD as well as LD were applied as a mixture of fluorescent and incandescent light. Flower primordia have been counted at the end of the experiment; averages are presented in fig. 17. Average plants of the three series, photographed at the beginning and at the end of the after-treatment, are shown on Plates 4 and 5.

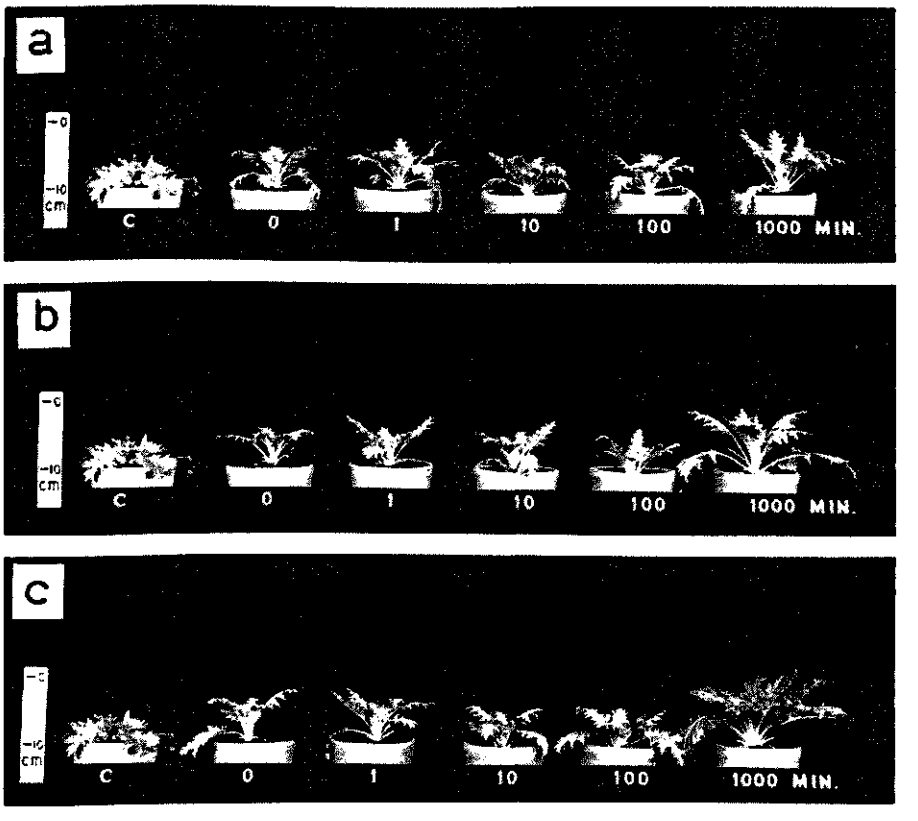


PLATE 4. *Hyoscyamus* as affected by different daylength treatments in three light intensities: *a*: 400, *b*: 2400, and *c*: 24,000 ergs.cm^{-2}.sec^{-1} ($<$ 700 nm.) Schedule of treatment: (4 SD + 3 TD) 4 times, followed by 10 LD. C is a control plant (SD fluorescent light). Photographed at the beginning of the LD after-treatment, 9-5-'69. See also legend fig. 17.

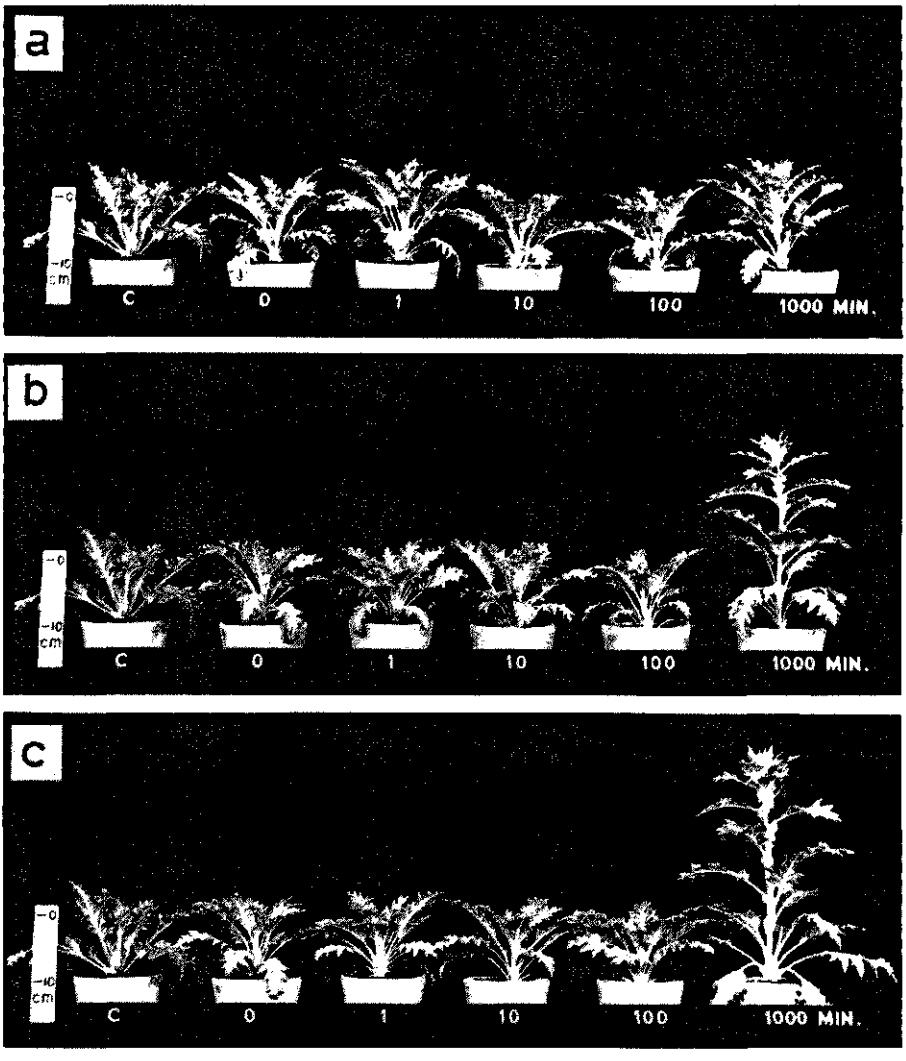


PLATE 5. *Hyoscyamus* as affected by different daylength treatments in three light intensities. Control C was in SD till the after-treatment started, then in LD as the other plants. Photographed at the end of the LD after-treatment, 19-5-'69. See also Plate 4 and legend fig. 17.

It is evident that the inhibition of initiation of flower primordia between 300 and 600 minutes daylengths at 24,000 ergs.cm^{-2}.sec^{-1}, is nearly lost at 2400 ergs.cm^{-2}.sec^{-1} and is wholly absent at 400 ergs.cm^{-2}.sec^{-1}.

The LD-effect is weaker with decreasing light intensities, but certainly not completely lost at 400 ergs.cm^{-2}.sec^{-1}.

Average numbers of days to shooting have been determined as well, and are presented in fig. 18. The zero time mark indicates the beginning of the LD after-treatment. Negative values in 'days to shooting' mean that these plants started shooting already before the beginning of the after-treatment.

Again, only in the highest light intensity there is an appreciable inhibition in the region between 300 and 600 minutes daylength, viz., in that of the normal short days. In the region above 800 minutes light per day the promotive action of the lowest light intensity is somewhat less in 'days to shooting' than in 'number of flower primordia', but in general the results obtained with these two parameters show great similarity.

Another parameter that has been recorded in this experiment is that of 'leaf increment', averages of which are presented in fig. 19. Although the variation

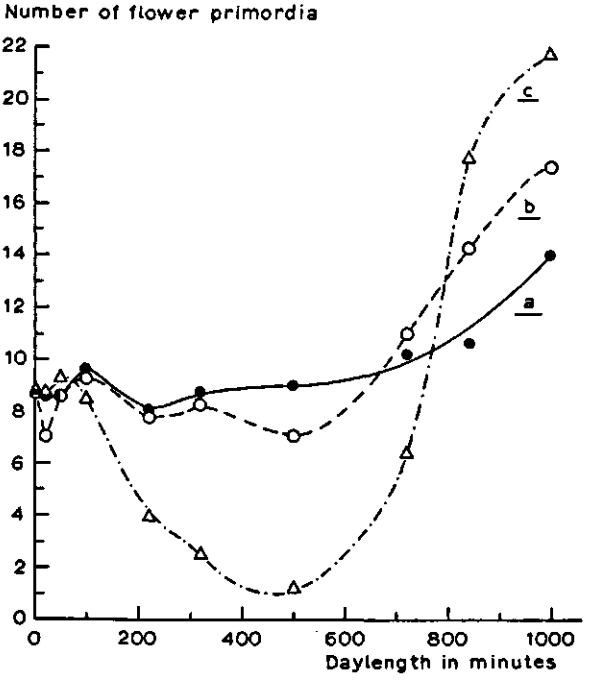


FIG. 17. Number of flower primordia in *Hyoscyamus* upon different daylength treatments according to the schedule (4 SD + 3 TD) 4 times, followed by 10 LD. Three intensities of the mixed radiation of fluorescent and incandescent lamps were used in the TD, *a* (●———●): 400 ergs.cm^{-2}.sec^{-1} < 700 nm and 150 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm, *b* (○- - -○): 2400 ergs.cm^{-2}.sec^{-1} < 700 nm and 1000 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm, and *c* (△- . - △): 24,000 ergs.cm^{-2}.sec^{-1} < 700 nm and 10,000 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm. Light intensity in LD was the same as that during the TD of series *c*, and 58,000 ergs.cm^{-2}.sec^{-1} in SD (fluorescent light). The experiment started 11-4-'69. Averages of 4 plants (3 plants only in the 100 and 840 min. TD of series *a*, in the 1000 min. TD of series *b*, and in the 22 and 50 min. TD of series *c*).

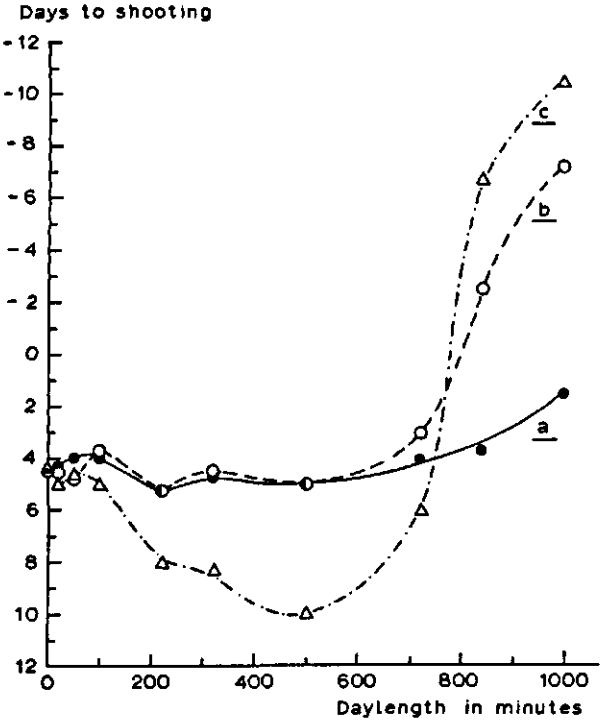


FIG. 18. Days to shooting in *Hyoscyamus* upon different daylength treatments in three light intensities: *a* (●———●): 400, *b* (○- - - -○): 2400, and *c* (△-.-. △): 24,000 ergs.cm^{-2}. sec^{-1} (< 700 nm). Schedule of treatment: (4 SD + 3 TD) 4 times, followed by 10 LD. Zero time marks the beginning of the LD after-treatment. Averages of 4 plants (3 plants only in 100 and 840 min. TD of series *a*, and in 50 min. TD of series *c*). See also legend fig. 17.

is rather great, it is evident, that again only the highest light intensity shows an appreciable increase in leaf increment, thus an inhibition of initiation of flowering, in the normal short-day region. In long days, the decrease in leaf increment, i.e. the promotion of flower initiation, for higher light intensities is not as evident as it is with the other two parameters; with photoperiods longer than ca. 800 minutes, in all three light intensities the same results are obtained.

From the foregoing, it can be concluded that the three parameters, 'number of flower primordia', 'days to shooting', and 'leaf increment' show a fairly good similarity in the region of photoperiods below ca. 800 minutes, while in longer photoperiods data obtained for 'leaf increment' clearly deviate from those obtained with the other two parameters. Evidently, as soon as conditions for flower initiation are favourable, leaf increment does not decrease further, and attains a similar low value at all light intensities.

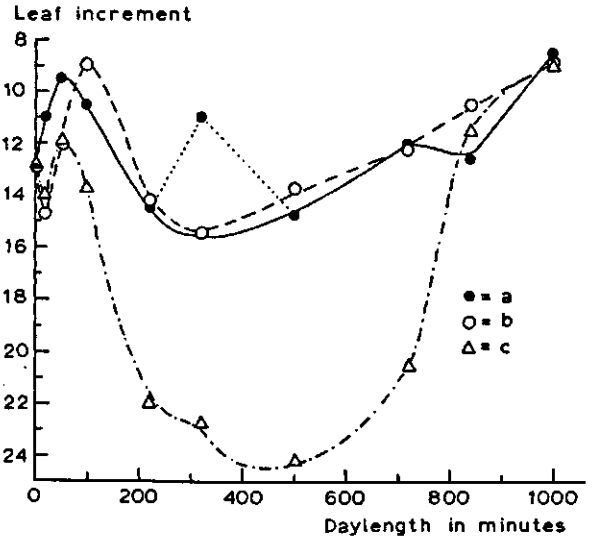


FIG. 19. Leaf increment in *Hyoscyamus* upon different daylength treatments in three light intensities: *a* (●———●): 400, *b* (○- - - -○): 2400, and *c* (△-.-. △): 24,000 ergs.cm^{-2}. sec^{-1} (< 700 nm). See also legend fig. 17.

3.5. DISCUSSION

In this chapter, daylength dependence of flower initiation in *Hyoscyamus* in white light was studied. First, an uninterrupted period of daylength treatments was applied, in order to establish the 'critical daylength' under two white light sources (section 3.2.). Furthermore, a period of daylength treatments was applied, interrupted at regular intervals by SD and followed by inductive conditions so that flower initiation was finally obtained in all treatments (sections 3.3. and 3.4.).

EL HATTAB'S observation (28) that the daylength reaction of *Hyoscyamus* is identical under fluorescent light and under a mixture of fluorescent and incandescent light, only with a slightly faster response under the last quality, has been confirmed, using the parameter 'days to shooting' (fig. 7). Concerning the parameter 'leaf increment', it was found that continuous fluorescent light resulted in an increase of 'leaf increment' and not in a slight decrease as might have been expected, and was indeed found in the mixed irradiation (fig. 8). This effect, however, was shown to be due to an increased rate of leaf initiation at longer daylengths (figs. 8 and 9). For flower initiation, no difference of critical daylengths between the two white light sources was observed (Table 1).

In experiments in which a period of daylength treatments was interrupted at regular intervals by a few SD for reasons of energy balance, some of the schedule components that could possibly alter the daylength dependence of flower initiation, were separately studied, e.g. the influence of the number of short days in-

terrupting a period of daylength treatments (subsection 3.3.2.), the duration of the LD after-treatment (subsection 3.3.3.), the photoperiod of the intercalated days (subsection 3.3.4.), and the number of repetitions of the treatment (subsection 3.3.5.). None of these factors seemed to interfere essentially with the type of response curve obtained, except that different slopes were observed in many cases when a factor was changed. Most likely, below and above a certain daylength, different light sensitive systems or different combinations of systems determine the daylength response.

The daylength response curve for white light, as obtained with the method of a period of TD interrupted at regular intervals by SD and followed by LD (e.g. fig. 11), may be analyzed as follows. Apart from an apparently extra phenomenon at extreme short photoperiods (below ca. 50 min.), the response curve may

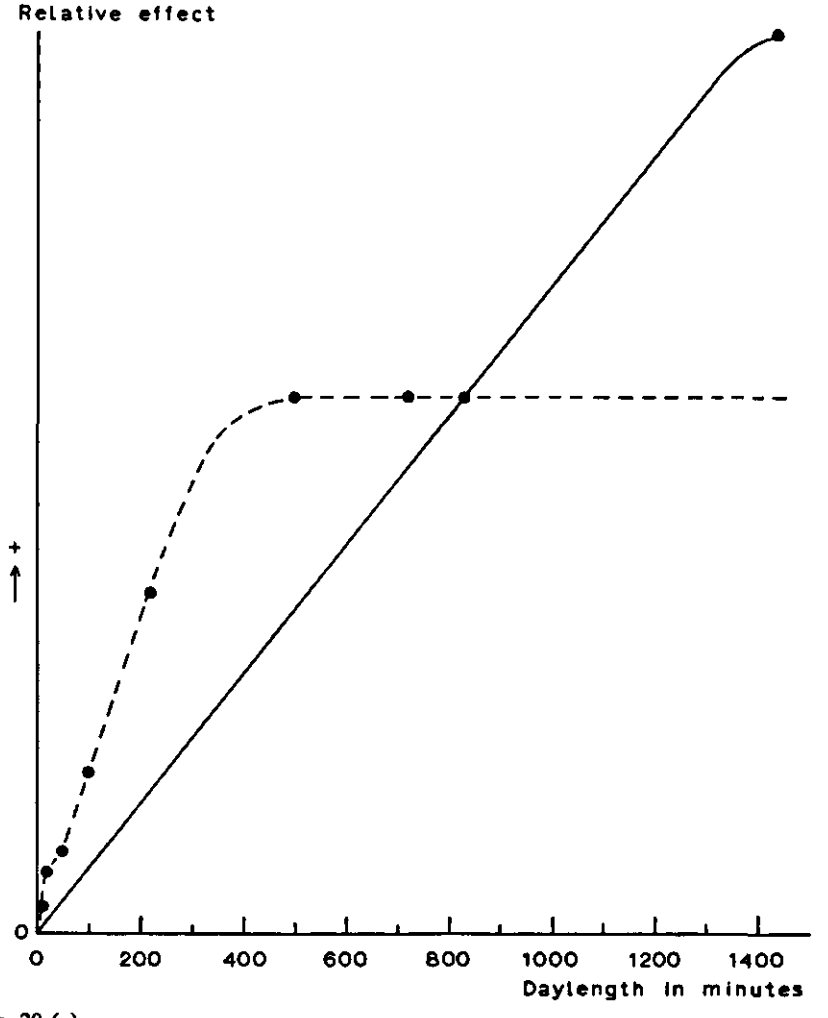


Fig. 20 (*a*)

be split into a saturation curve, representing an inhibitive action, and a curve reaching saturation at much longer photoperiods which represents an action, promotive for flower initiation. Since in continuous darkness, no light inhibition or promotion can be induced, both actions are zero. For the general scheme, it may be assumed that the promotive action, as manifest in longer photoperiods, either is operative over the whole daylength region and thus starts at zero minutes (cf. fig. 20*a*), or that it only starts from the photoperiod where inhibition has reached its maximum (cf. fig. 20*b*). In both assumptions the slope of the promotion curve is assumed to be the same as that of the part of the response curve beyond the inhibition maximum.

Both assumptions include the idea that the realization of a final effect requires specific pool values of products built up in cooperation of inhibitive and promotive processes. The balance of this cooperation decides whether the final effect is inhibitive or promotive. For the analysis of curves obtained in white

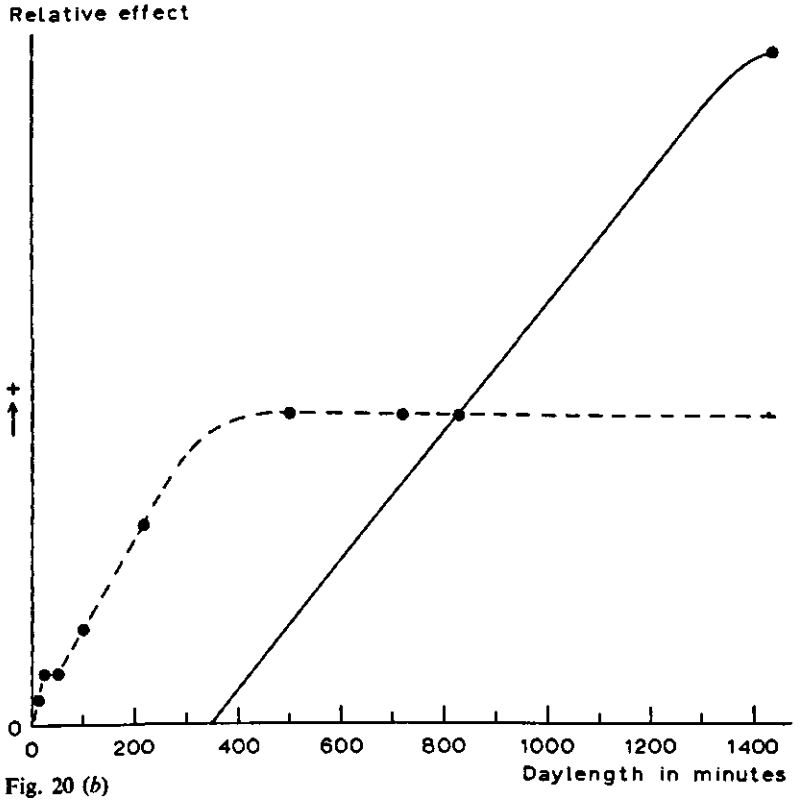


Fig. 20 (*b*)

FIG. 20 *a, b*. Relative effect at various daylengths of light induced inhibition (– – –) and of light induced promotion (———) of flower initiation in *Hyoscyamus*. Based on data of fig. 11. *a*. Inhibition and promotion assumed to increase as soon as light is switched on. *b*. Promotion assumed to increase at the photoperiod where inhibition becomes saturated; inhibition assumed to increase when light is switched on. See also text.

light at normal (high) light intensity, a choise between both possible assumptions appears difficult. The first possibility has the benefit that it seems logical to assume that all light reactions start as soon as light is switched on. However, in attempting the analysis of experimental curves obtained at lower intensities of white light, and still more so for curves obtained in specific spectral regions (Chapter 4), we have felt preference for adopting the second assumption as basis for our analysis. It should be mentioned that the second assumption operates not so much with the velocities of elementary processes, but more with the accumulation of essential products leading to inhibition or promotion. Some further considerations regarding possible mechanisms for producing inhibitive or promotive products will be given on pp. 39 and 40, and in the Chapters 4 and 5.

Based on data of fig. 11, the inhibition and promotion curves according to the first assumption are plotted in fig. 20*a*, and in fig. 20*b* according to the second one. In fig. 20*a* as well as in fig. 20*b* we plotted first the promotion curve, and then the inhibition curve by adding the measured number of inhibition units (indicated by the decrease in number of flower primordia) to the number of units obtained from the promotion curve for each photoperiod. From these totals, the inhibition curve is plotted up to a photoperiod of about 830 minutes, at which in fig. 11 the response curve has come back to level *d*, so that there the light induced inhibition and promotion again are equal, like in total darkness, though either of them evidently is not any more zero at this point. From that photoperiod on, the light induced inhibition is assumed to remain saturated and, thus, for longer photoperiods, the light induced promotion can be calculated from the established inhibition level and the measured data.

We will now see in how far literature contains suggestions for possible mechanisms of these inhibitive and promotive reaction systems, for the existence of which the evidence, as shown above, was derived exclusively from our experimental results.

As far as inhibition is concerned, LANG (80) also concluded that under SD the leaves exert an inhibition on flowering, because defoliated plants of *Hyoscyamus* flower in SD while intact plants do not (79).

The short-day effect, i.e. flowering in SDP and non-flowering in LDP, was shown to be greatly depressed by factors limiting photosynthesis. Already 1940, it was shown by HAMNER (53) and by PARKER and BORTHWICK (110), that for SDP the SD-effect decreased at low light intensities. The last mentioned investigators, furthermore, showed that when CO_2-supply or the duration of the high-intensity light period was decreased, the SD-effect in Biloxi Soybean disappeared. They concluded that initiation of flower primordia was limited by photosynthesis during induction. For another SDP, *Pharbitis nil*, it was found that photoperiodic sensitivity became manifest only under conditions of positive net photosynthesis (94).

The data, presented in section 3.4. agree nicely with this concept. From preliminary experiments, we have evidence that the compensation point for *Hyoscyamus*, grown at 20 °C, is at a light intensity of about 4000 ergs.cm^{-2}.sec^{-1}.

It was shown that at a light intensity far above the compensation point, the SD-effect was clear, while at a light intensity just below this point the effect was almost absent and at one far below compensation no inhibition of flower initiation was observed at all (cf. figs. 17, 18, 19).

Since 400 ergs.cm^{-2}.sec^{-1}, applied during 5 to 10 hours will certainly be sufficient to bring the two forms of phytochrome, P_{fr} and P_r, into a photostationary state, the disappearance of the inhibition of flower initiation can hardly be explained in terms of phytochrome only. Therefore, an effect connected with photosynthesis may well play a role.

The role of photosynthesis in the SD-effect may consist only in a supply of substrate for respiration and other processes which take place during the following dark period, for in *Xanthium* the application of sucrose or other sugars and KREBS cycle intermediates can replace the high-intensity light requirement (90), but HARDER et al. (56) and FREDERICQ (42) found that not in every SDP photosynthesis could be replaced by a supply of sugars. SALISBURY (124) also concluded that light, preceding the dark period, clearly exerts more functions than just mediating photosynthetic substrate production. For the photoperiodic tuberization of *Begonia evansiana* it was shown by ESASHI (33) that during the main light period CO_2 was required only under aerobic conditions; the effect of the main light period was not reduced under nearly anaerobic conditions, not even in an atmosphere, free from CO_2. He suggested 'that the indispensable reaction in the main light period will be no photosynthetic fixation of CO_2 itself, but the photophosphorylation and the photoreduction of NADP if be involved in it', and his conclusion was that photosynthesis in the main light process may not be essential to photoperiodism. In view of the fact that our experiments were aerobic, it should be remarked that photosynthesis is capable of producing reduced substances as well as several phosphorylated products, also under aerobic conditions.

Concerning flower initiation in *Hyoscyamus* it is concluded that, 'in connection with the evidence just discussed, under our experimental conditions photosynthesis (as mediated essentially by the photosynthetic 'system II') is involved in the realization of the SD-effect, which consists of the inhibition of flower initiation (figs. 17, 18, 19).

The LD-effect, i.e. non-flowering in SDP and flowering in LDP, has been taken up in the studies in this chapter by including the application of long periods of white light also. As already mentioned in section 3.4., DE ZEEUW (161) was able to induce the SDP *Perilla crispa* to flower initiation in LD, provided the light intensity was sufficiently low. The LD-effect thus appeared clearly intensity sensitive. Also in experiments presented in section 3.4., the LD-effect was smaller in weaker light, at least with the parameters 'number of flower primordia' and 'days to shooting' (figs. 17, 18). In 1958, FREDERICQ (41) found for *Hyoscyamus* that, when plants were fed with sucrose through the leaves, flower initiation could be obtained in a CO_2-free LD. Also, when after a short day in normal air, supplementary light was given in CO_2-free air, he obtained flower initiation in *Hyoscyamus* as well as in another LDP, *Sinapis alba*. Thus, reduced

CO_2-assimilation does not inhibit flower initiation, as was suggested by LANG and MELCHERS (82); they supposed that in SD, during the long night, assimilates necessary for the synthesis of florigen are destroyed by dissimilatory processes. Also, the behaviour of *Hyoscyamus* in extremely short days (this Chapter), in which inhibition evidently is decreased, suggests that inhibition of flowering in SD cannot merely be due to dissimilatory processes during a long dark period.

The whole of evidence discussed above suggests that promotion of flower initiation in *Hyoscyamus* by a supplementary light period is not directly dependent on photosynthesis in the sense of CO_2-fixation.

The promotion of flower initiation by light is not merely inactivation of the inhibition, but light has also a direct promotive function on flower induction. The effect of this direct promotive function was shown to be dependent on light intensity (figs. 17 and 18). Since the light quality in the three series (section 3.4.) is practically the same, phytochrome obviously could only be a main factor in promotion of flower initiation in case the HER, as interpreted by HARTMANN (58), is involved. He suggested, that many 'high energy phenomena mainly or merely result from oscillation excitation of P_{fr} under conditions of competitive selfinhibition'; or as recently interpreted by HENDRICKS et al. (64), who envisaged a form of phytochrome with an absorption maximum near 720 nm, the wavelength of maximum effectiveness in the HER.

Still another explanation for a promotive action of light, viz., via an unknown pigment, directing the HER, has been suggested by MOHR (102, 103), ENGELSMA (30, 31) and others (133, 127).

Recently, KANDELER (73, 74) demonstrated that, at least for the LDP *Lemna gibba*, an increased level of ATP or dATP markedly promoted flowering. He presented strong evidence that photophosphorylation via 'system I' of the photosynthetic apparatus is involved in the direct promotion of flower induction in this LDP.

The data presented in this section do not contain evidence which would appear specifically in favour of one of the above suggestions as far as *Hyoscyamus* is concerned, but neither oppose these views.

In the next chapter, the influence of light of different spectral regions on flower induction will be described, after which the effects of light on flower initiation will be discussed in further detail.

4. DAYLENGTH DEPENDENCE OF FLOWER INITIATION IN LIGHT OF RESTRICTED SPECTRAL REGIONS

4.1. INTRODUCTION

Effects of coloured light on flowering often gave rise to apparently conflicting results. In 1960, NAKAYAMA et al. (109), with seedlings of *Pharbitis nil*, obtained inhibition of flowering by both red and far-red irradiations in the middle of 16-hour dark periods. However, with older plants, night interruptions in red light inhibited flowering which could be repromoted by a successive irradiation with far-red, as was also found for *Xanthium* and several other plant species (25). Some years later, FREDERICQ (44) showed that, also with seedlings of *Pharbitis*, far-red reversal of the red effect is obtainable, however, only when the red and far-red irradiances do not last longer than 30 seconds and are not separated by darkness.

This example may show that minor modifications should be very carefully considered when resulting in different reactions. In this section, the more general observations from the literature and only a limited amount of more detailed information will be reported.

Much of the earlier work on the effects of coloured light in photoperiodicity and photomorphogenesis, and on flower initiation in particular, has been carried out in The Netherlands in this laboratory by WASSINK, STOLWIJK, DE LINT, and others (e.g., 146, 149, 131, 133, 23, 85, 87), and in the PHILIPS laboratories by MEYER et al. (e.g., 99, 101, 100).

In both long and short-day plants, normal short day will give the SD-effect, irrespective of the light colour applied during the photoperiod. For the LDP *Hyoscyamus* this was shown in this laboratory by STOLWIJK and ZEEVAART (133), and for SDP by MEYER and VAN DER VEEN (101). However, in general this is true only when just one colour is used and when the light intensity is sufficiently high. When different light qualities are applied in succession, the SD-effect is not guaranteed. For *Hyoscyamus* this was shown by DE LINT (85), who obtained stem elongation in 10-hour photoperiods consisting of 8 hours of strong fluorescent light followed by 2 hours of weak far-red.

Whether a normal long day will cause the LD-effect depends on light quality and intensity. Violet, blue, and far-red are active, red light is hardly active, and green light is virtually inactive in producing the LD-effect (133, 23, 101, 100, 87).

As was already observed some 20 years ago in this laboratory by WASSINK et al. (146, 149), flower initiation in *Brassica* (LD-effect) can also be obtained by extending a short photoperiod with low intensity coloured light, violet, blue, and far-red being most active, and yellow, green, and red apparently antagonizing when applied together with them, e.g., in white light. This assumption could also be applied for the elongation of the stem in *Cosmos* and *Lactuca* (149). Low intensities of supplementary far-red especially, had a pronounced elongating effect. The existence of such an antagonism was supported by data of

STOLWIJK (131), obtained for flowering and petiole elongation in *Spinacia*, and for internode elongation in *Cosmos*.

It was recognized later that the violet and blue effects in these early experiments with *Brassica* and *Cosmos* were mainly or wholly due to the presence of small amounts of far-red (145). However, it was found as well that high intensities of blue light in *Hyoscyamus* present a true promoting effect on flower initiation, also in complete absence of far-red. Results of STOLWIJK and ZEEVAART (133), that flowering in *Hyoscyamus* occurs under long-day conditions in the presence of blue and far-red radiation, but not in red and green, were confirmed by CURRY and WASSINK (23), who furthermore demonstrated clearly that in these cases the blue response cannot be attributed to a far-red contamination.

Growing *Cosmos* or tomato plants in a high intensity of monochromatic light only, WASSINK and STOLWIJK (147), however, observed that red, yellow, and green light may have a strongly elongating influence, whereas blue suppresses stem elongation as compared with the white control. WASSINK et al. (145a) observed with *Hyoscyamus* in coloured light only, a well-developed red/far-red antagonism, and indications for the existence of a red-blue antagonism. For blue (applied together with red) a relatively higher light intensity was necessary for obtaining an LD-effect than for far-red (applied together with red).

As supplementary light, far-red, or a mixture or combination of red and far-red, may often also be more effective than blue light (100, 36, 45, 3). In SDP, sometimes the effect of a far-red extension can repeatedly be reversed by red light and vice versa (108, 109, 9, 44), depending on the duration, the light intensity, and the light quality of the main photoperiod (100, 9, 45). In *Hyoscyamus* and other LDP, this far-red/red reversibility has not yet been observed. On the contrary, sometimes a red exposure after a daylength extension with far-red (36, 28) or a brief exposure to far-red following a prolonged extension with red light (36) enhanced the LD-effect. Similarly, DE LINT (87) found for *Hyoscyamus*, that after a basic 8-hour day in white light, the LD-effect of subsequent far-red was enhanced by intercalating an exposure to red light between the white light and the exposure to far-red. This effect of red light was the more pronounced, the longer the period of red light was (from 0 to 8 hrs). This may be the same effect as that obtained by EL HATTAB (28), also with *Hyoscyamus*, where stem elongation occurred in plants receiving 10 hours red (8000 ergs.cm^{-2}.sec^{-1}) followed by 10 minutes red plus 10 minutes far-red (both 3800 ergs.cm^{-2}.sec^{-1}) at different moments in the first half of the dark period. Controls receiving 10 minutes of red or far-red alone, at the same time of the dark period did not show shooting.

When a long night, following a short photoperiod which alone would produce the SD-effect, is interrupted, in particular with red light, plants may show the LD-effect (112). An action spectrum for the photoperiodic control of floral initiation of *Hyoscyamus* has been determined by PARKER et al. (111). DOWNS (25) showed for *Hyoscyamus* that the effect of a night break with red light could be repeatedly reversed with far-red. Many other plants have been shown to react to a night break according to the phytochrome system (11, 100, 44). How-

ever, like in the case of daylength extension, it was shown by MEYER (99, 100) and others (45, 32, 65) that the effect of a night break with red and the reversibility of the red-effect by far-red often is highly dependent on the duration, the light intensity, and the light quality of the main light period (99, 100, 45, 32, 65). In connection to this, it should be mentioned that mostly the short night break with red light causes an LD-effect only when the main light period contains blue or far-red irradiation (100). However, EL HATTAB (28) found for *Hyoscyamus*, that in combination with a main light period in red, a night break with a relatively high intensity of red light (3800 ergs.cm $^{-2}$.sec^{-1}) did produce an LD-effect, whereas a low intensity (600 ergs.cm^{-2}.sec^{-1}) did not.

Sometimes, no red/far-red reversal can be found in the night break reaction; even a rather long exposure to far-red then seems to act as red light (100, 32). FREDERICQ (45), by varying the duration of the main light period in *Kalanchoë*, could increase the capacity for red/far-red reversal for the daylength extension and decrease it for the night break, or just the opposite.

Both forms of phytochrome, being the pigment mediating the red/far-red reversal, have, besides the main maxima in red or far-red, a relatively low absorption maximum in the blue region of the spectrum (58) which may partly account for the sometimes complex reaction of photomorphogenesis in blue light, as observed in this laboratory and elsewhere (e.g. 132, 133, 23, 100, 28). In the following sections, the main attention will be paid to the effects of red and far-red light in order to avoid these complications.

In general, SDP as well as LDP are assumed to flower, though not always in an optimal way, if a moderate level of P_{fr} is constantly maintained (21, 78, 66, 28). Most reactions fit the assumption that SDP, after a short day for flower induction, need a high P_{fr} level in the beginning of the long night and a low one later in the dark period (66, 37), while LDP for flower induction need a low level in the beginning of the long night or a high one later in the night (78, 28). For *Hyoscyamus*, this was clearly demonstrated by EL HATTAB (28). He interrupted 14-hour dark periods, following 10-hour periods in red light (8000 ergs. cm^{-2}.sec^{-1}), with 2 hours of far-red, red or blue light (all 3800 ergs.cm^{-2}.sec^{-1}) at various moments. He found that the promotive effect of far-red, from the beginning of darkness decreased to zero after 6 hours. A promotive effect of red light was only detectable when given after 6, 8 or 10 hours of darkness. A red light interruption given earlier or later in such a long night did not cause shooting. Blue light acted as red light. Short breaks of only 10 minutes were ineffective for blue and far-red; for red they were only effective after 8 and 10 hours of darkness.

Besides the phytochrome reaction, a second photoreaction often seems to be involved in photomorphogenesis. The pigment mediating this photoreaction shows an action spectrum with a maximum in the far-red (710–720 nm) and often also one in the blue region (99, 102, 106, 104). While the normal phytochrome reaction seems saturated at relatively low energies, this photoreaction needs much higher energies for saturation and was therefore called the 'High Energy Reaction' (HER). As already mentioned in the preceding section, the

nature of the pigment controlling the HER is not yet clear (127, 3), although HARTMANN (58) presented evidence that also in this case phytochrome is the controlling pigment. Recently, HENDRICKS et al. (64) tried to explain the HER on the basis of an intermediate form of phytochrome, absorbing at about 720 nm.

DOWNS et al. (26), for anthocyanin synthesis in apple skin, found that the photosynthetic system was the photoreceptor for the HER involved in this synthesis. As mentioned in the preceding section, KANDELER (73) pointed to the photosynthetic 'system I' as being involved in flower induction of *Lemna gibba*.

It is difficult, at this moment, to explain all HER phenomena on the basis of one and the same pigment system. If the HER is not mediated by phytochrome, it may be necessary to assume the existence of several types of HER, controlled by different pigment systems, for how could the HER in etiolated plants without a properly developed photosynthetic system (4a, 58), be controlled by photosynthetic pigments?

Extensive daylength response curves for flower initiation are very rare, in particular with coloured light. For *Hyoscyamus*, DE LINT (87) in this laboratory, established such daylength response curves with red and blue light, both at an intensity of 9500 ergs.cm^{-2}.sec^{-1}. In the short-day region, red light was more inhibitive than blue, and in the long-day region only blue light promoted shooting, while continuous red light had the same effect as darkness.

In the following sections, the influence of light of various colours, especially red and far-red, on flower initiation in *Hyoscyamus niger*, will be discussed further. The same experimental methods as in the preceding chapter have been used. It should be emphasized here, as SCHNEIDER et al. (127) did for their data, that comparison of specific numerical values is valid only within, rather than between experiments, because of certain differences in vigour of growth and development that may exist between plants of the various lots.

4.2. DAYLENGTH RESPONSE CURVES FOR FLOWER INITIATION IN FAR-RED, RED, GREEN, AND BLUE LIGHT

In this section, an experiment is described, resulting in daylength response curves for flower initiation in far-red, red, green, and blue light. Precultivation was in SD white fluorescent light (see section 2.2.). Coloured light in this experiment has been given in the 'low intensity cabinets', in which far-red is produced by a filter combination including water to eliminate radiation wavelengths above 1000 nm. TD were given according to the scheme (4 SD + 3 TD) 4 times, with an after-treatment of 6 LD for the series that received far-red or blue light, and of 10 LD for those that received red or green light during the TD. An SD supplied during the experiment consisted of 9 hours of white fluorescent light, and the TD had photoperiods in coloured light of: 0, 1, 7, 100, 300, 720 or 1000 minutes respectively; the LD was given as a mixture of white fluorescent and incandescent light. As in general, the light intensity values are given in the legends of the figures. Flower primordia were counted at the end of the after-treatment. Averages are presented in fig. 21. In this figure, the values for

the 0 minutes TD are all put at zero. Actually, the dark value for the plants from far-red and blue (harvested first, because of rapid development for long TD photoperiods) was 6.9. flower primordia, and 11.1 flower primordia for the plants from red and green (harvested later because of slow development for long TD photoperiods). Units on the ordinate are numbers of primordia expressed as differences from the dark value in the series.

In all four colours, a very short photoperiod already brings about relatively large effects; in far-red and blue, the shortest daylengths are promotive and in

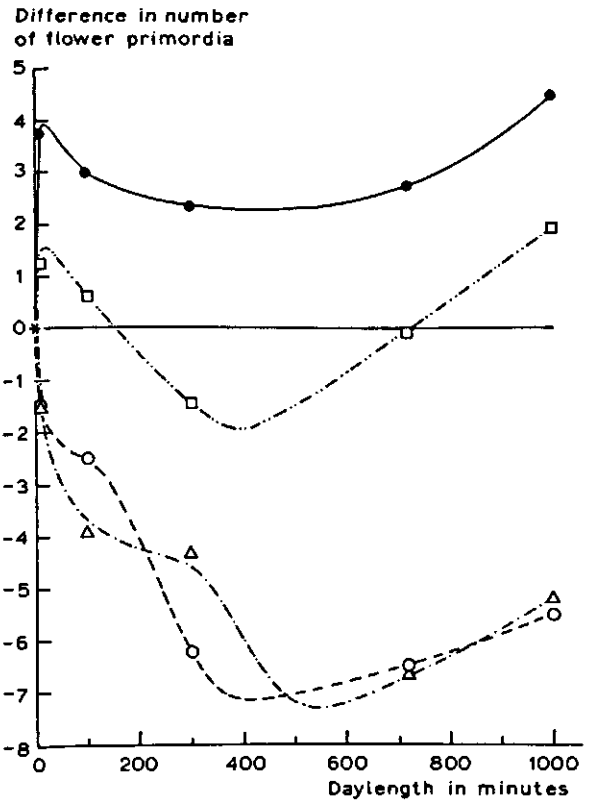


FIG. 21. Difference in number of flower primordia in *Hyoscyamus niger* between different daylength treatments in 4 light colours and continuous darkness during TD according to the schedule (4 SD + 3 TD) 4 times, followed by 6 LD for far-red (●———●) and blue light (□-. . -. . □), and by 10 LD for red (○- - - - -○) and green light (△-.-. △). ★ : Zero value for all colours. Light intensities in far-red (700 to 1000 nm), red, green, and blue light were 5000, 5700, 2700, and 4100 ergs.cm^{-2}.sec^{-1} respectively. In SD (fluorescent light), the light intensity was 52,000 ergs.cm^{-2}.sec^{-1} and in LD (mixed irradiation): 26,000 < 700 nm and 9500 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm. The experiment started 3-1-'69. Averages of 4 plants (8 in 0 min. TD; 3 in 300 min. TD of far-red; 3 in 300 min., 1 in 720 min. and 2 in 1000 min. TD of green, and 2 in 7 min., 3 in 100, 300, 720 and 1000 min. TD of blue).

red and green they are inhibitive. These very short irradiations seem to be either promotive or inhibitive for flower initiation, depending on their colour; these effects will be further referred to as 'reaction 1'. They may correspond to the 'irregularities' observed already in the curves obtained with white light at photoperiods shorter than 60 minutes (e.g. fig. 10, p. 21, fig. 15, p. 28 and fig. 17, p. 33).

Lengthening of the photoperiod in all four colours seems to start another process which inhibits flowering; red (and green) being strongly, blue moderately, and far-red hardly inhibitive. In this experiment, the inhibition was at its maximum at a photoperiod of about 450 minutes. With still longer photoperiods, a process promotive for flowering seems to become effective, also somewhat dependent on the colour of the light.

It seems that the effect of 'reaction 1' attains a certain level, but it is not clear whether this determines the rate of the following processes.

The other two processes mentioned above, seem to be mainly responsible for the curves obtained in white light; the first of them being responsible for the SD-effect and the second for the LD-effect. In the following sections, the effects of far-red and red light on the shape of the curves will be examined in greater detail.

4.3. Experiments on red/far-red reversal

In LDP, involvement of phytochrome in the photoperiodical response has thus far only been found for night interruptions (25), not for daylength extensions (28). In SDP it was demonstrated in both types of experiments (100, 45).

On the basis of the data of the preceding section, it seemed interesting to examine a possible involvement of phytochrome in the reactions upon different TD photoperiods. So far, only short TD photoperiods were examined in this type of experiment.

In these experiments, coloured light was applied in the new cabinets (far-red filter combination without water). Because red and far-red were applied in separate cabinets, 15 seconds of darkness between two different illuminations could not be avoided.

For comparison, a series of plants receiving total darkness during the TD, was included; all light effects have been evaluated in comparison with this treatment.

After illuminations of 5 or 420 minutes of red light respectively, the red/far-red reversibility was examined with alternate doses of about 3 minutes (165 sec.) of far-red and red light up to two doses of each.

After 5 or 420 minutes of far-red radiation, the red/far-red reversibility was examined in the same way as indicated above.

Since these treatments may be considered as special daylength treatments, the same schedule as in the preceding section has been used: (4 SD + 3 TD) 4 times, with an after-treatment of 7 LD in a mixture of white fluorescent and incandescent light. As before, light in SD was applied as 9 hours of white fluorescent light. Flower primordia were counted, the values of which are presented in fig. 22*a*. Furthermore, the values of two other parameters, 'days to shooting' and

'leaf increment', have been recorded; data are presented in figs. 22*b* and 22*c*. Average plants, photographed on the first LD, are shown on Plates 6 and 7.

From the photographs, it is evident that the position of the leaves is strongly controlled by phytochrome, as was also observed by SCHNEIDER et al. (126). When the last illumination was far-red, leaves were strongly upright, with red they were in a much more horizontal position, as in total darkness. This phenomenon was still apparent in white light on the first LD, as is shown on Plates 6 and 7. The effect was strongly masked when 7 hours of far-red preceded the brief red and far-red illuminations.

In fig. 22*a*, for flower primordia, hardly any effect of 5 minutes red light is demonstrated, however, a weak red/far-red reversibility may be operative; in figs. 22*b* and 22*c*, with the other two parameters, the reversibility is more evident; the effect of 5 minutes red in 'days to shooting' indicates moderate inhibition of flower induction, and is reversed by far-red. In 'leaf increment' 5 minutes red has no effect as compared to the dark value, but 3 minutes far-red following red has a moderately inhibitive effect which is reversible by red.

It can be concluded, therefore, that after 5 minutes of red light, whether or not effective by its own, the phytochrome system is active in flower initiation.

PLATE 6. *Hyoscyamus* as affected by different illuminations with red (R) and far-red (FR) light during TD given according to the schedule: (4 SD + 3 TD) 4 times, followed by 7 LD.

A: Total darkness
B: 5'R
C: 5'R + 3'FR
D: 5'R + 3'FR + 3'R
E: 5'R + 3'FR + 3'R + 3'FR
F: 5'R + 3'FR + 3'R + 3'FR + 3'R
G: 420'R
H: 420'R + 3'FR
K: 420'R + 3'FR + 3'R
L: 420'R + 3'FR + 3'R + 3'FR
M: 420'R + 3'FR + 3'R + 3'FR + 3'R

Photographed at the first LD, 10-10-'69. See also legend fig. 22.

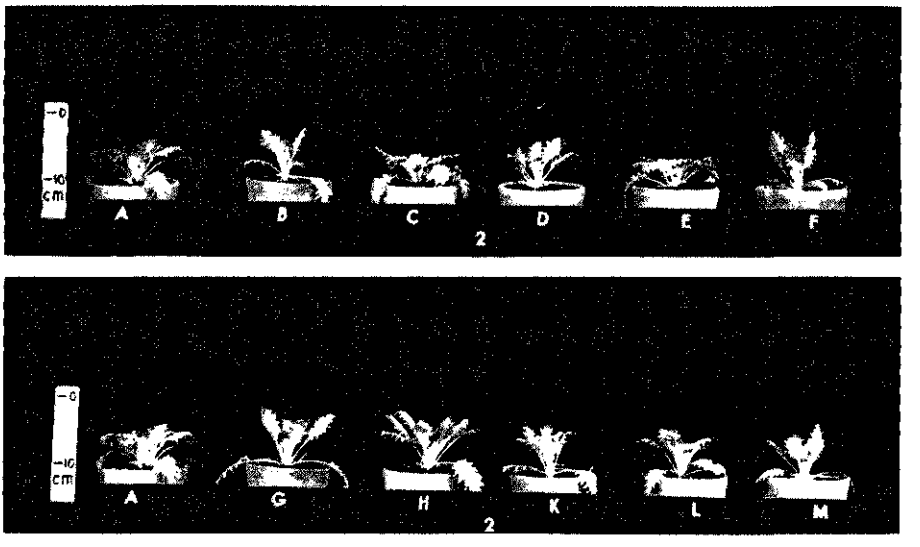


PLATE 7. *Hyoscyamus* as affected by different illuminations with far-red (FR) and red (R) light during TD given according to the schedule: (4 SD + 3 TD) 4 times, followed by 7 LD.

A: Total darkness
B: 5'FR
C: 5'FR + 3'R
D: 5'FR + 3'R + 3'FR
E: 5'FR + 3'R + 3'FR + 3'R
F: 5'FR + 3'R + 3'FR + 3'R + 3'FR
G: 420'FR
H: 420'FR + 3'R
K: 420'FR + 3'R + 3'FR
L: 420'FR + 3'R + 3'FR + 3'R
M: 420'FR + 3'R + 3'FR + 3'R + 3'FR

Photographed 10-10-'69. See also legend fig. 22.

After 420 minutes of red light, a strong phytochrome effect is evident for all three parameters; the strongly inhibitive effect of 420 minutes red is completely reversed by 3 minutes far-red and can be fully re-established by 3 minutes red following the far-red irradiation. Obviously, the inhibition of flower initiation has not yet become irreversible during the illumination period of 420 minutes of red light and it is concluded, therefore, that, at least under these conditions, the reactions effectuating the inhibition of flower initiation occur in the dark period following the illumination period only when phytochrome is predominantly in the P_{fr} form.

FIG. 22 (see p. 49). *a.* Number of flower primordia, *b.* days to shooting, and *c.* leaf increment in *Hyoscyamus* upon different treatments with far-red and red light during TD given according to the schedule (4 SD + 3 TD) 4 times, followed by 7 LD. Light intensity in far-red (between 700 and 1000 nm): 15,000 ergs.cm^{-2}.sec^{-1}; in red: 6800 ergs.cm^{-2}.sec^{-1}; in SD (fluorescent light): 53,000 ergs.cm^{-2}.sec^{-1}, and in LD (mixed irradiation): 23,000 ergs.cm^{-2}.sec^{-1} < 700 nm and 8600 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm. The experiment started 12-9-'69. Averages of 4 plants ('total darkness'-TD 10 plants, and (420'R + 3'FR + 3'R)-TD 3 plants).

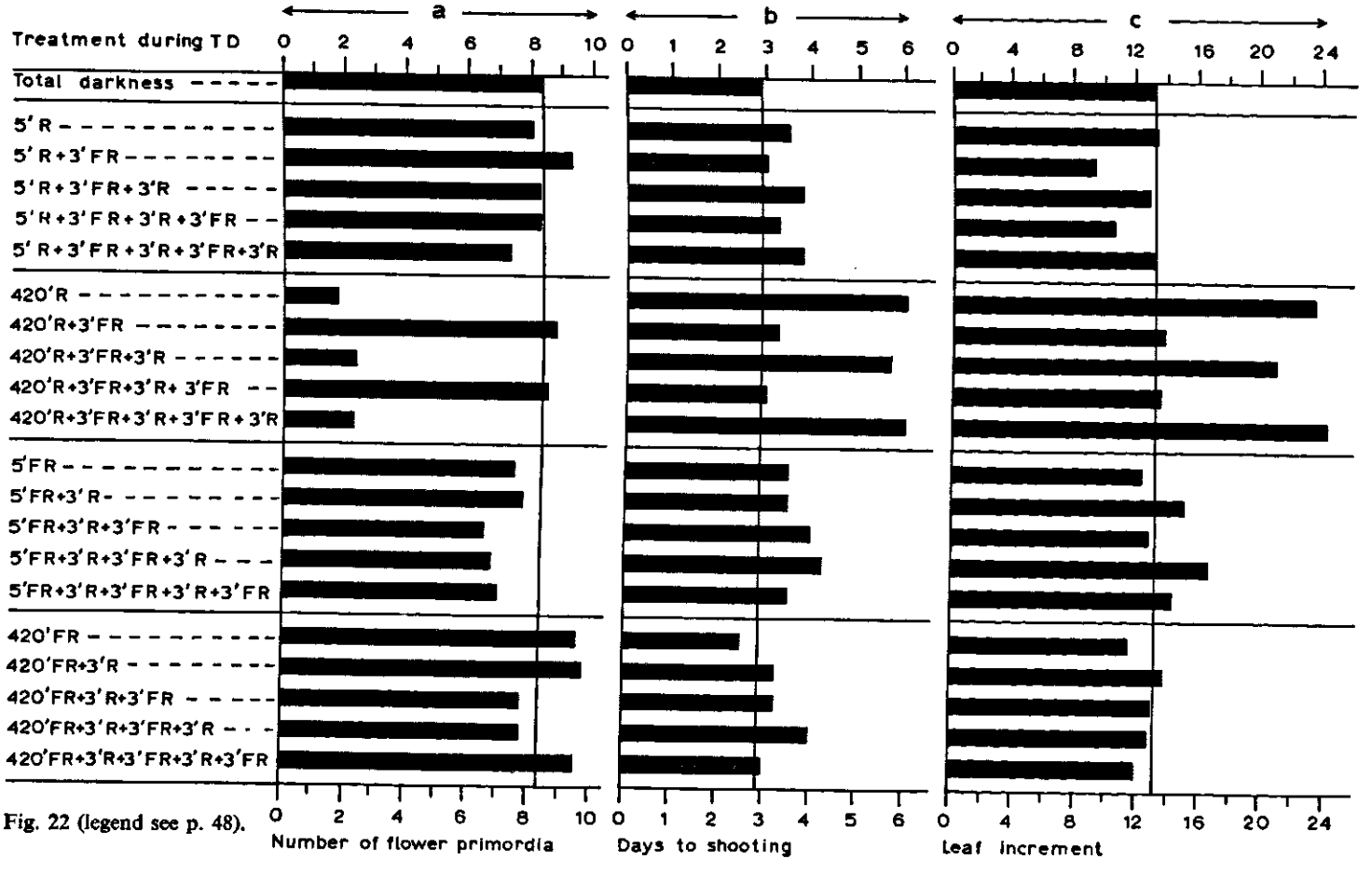


Fig. 22 (legend see p. 48).

After 5 minutes of far-red, only 'leaf increment' shows a moderate effect of phytochrome; red extensions increase leaf increment, and thus delay flower initiation. This effect is reversed by far-red (fig. 22*c*). With the other two parameters, no red/far-red reversibility could be observed.

After 420 minutes of far-red, a slight phytochrome effect may be seen with the parameter 'days to shooting' (fig. 22*b*), but with the other two parameters no reversibility was observed (figs. 22*a* and 22*c*).

4.4. Some further effects of daylength extensions with far-red and red light

In an attempt to further analyse the three reactions, distinguished in section 4.2., experiments were made (at 20 °C), in which 20 minutes of red light were given as extensions to different daylengths in far-red. Transfer of plants from one light quality to the other took some 15 seconds (darkness).

In order to study the effect of the red light extensions, TD were given according to the schedule: (4 SD + 3 TD) 4 times, with an after-treatment of 7 LD in mixed radiation of white fluorescent and incandescent lamps. Like always, SD was given in white fluorescent light only. The photoperiods of the TD in far-

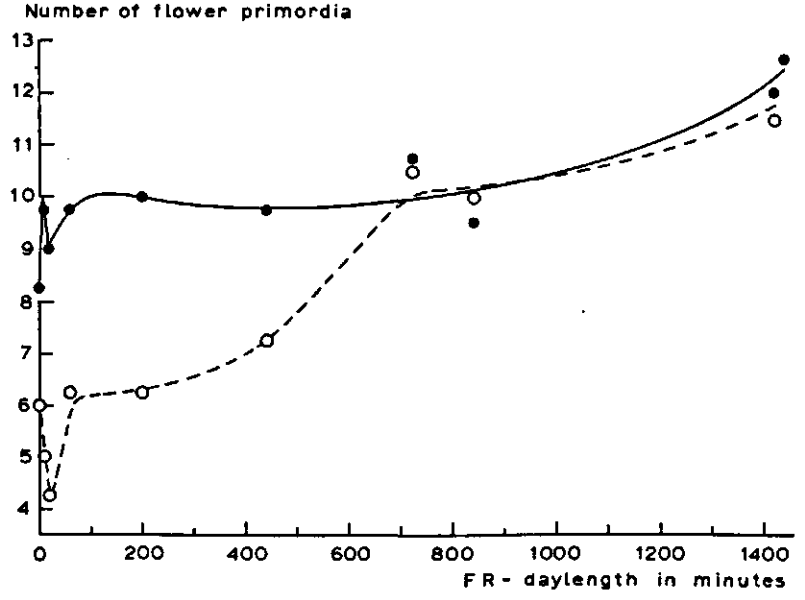


Fig. 23. Number of flower primordia in *Hyoscyamus* upon different daylength treatments in far-red (FR) (radiation between 700 and 1000 nm: 12,600 ergs.cm^{-2}.sec^{-1}), followed either by darkness (●———●) or by 20 min. red light (6000 ergs.cm^{-2}.sec^{-1}) and then darkness (○---○). TD were given according to the schedule (4 SD + 3 TD) 4 times, followed by 7 LD. Intensity of fluorescent light during the SD: 53,000 ergs.cm^{-2}.sec^{-1}; of the mixed irradiation during LD: 23,000 ergs.cm^{-2}.sec^{-1} < 700 nm and 8600 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm. The experiment started 18-5-'69. Averages of 4 plants.

red were: 0, 0.1, 0.3, 1, 3, 10, 20, 60, 200, 440, 720, 840, 1420 or 1440 minutes; each of these was followed either directly by darkness or first by 20 minutes of red light and then darkness, except, of course, that with 1440 minutes far-red. Averages of the numbers of flower primordia are presented in fig. 23.

The far-red curve is essentially the same as the one presented in section 4.2. (fig. 21, p. 45); slight differences may be due to the higher light intensity (cf. legends figs. 21 and 23) given in the present experiment and to the fact that this experiment was carried out in the new, movable cabinets, in which the far-red contains also radiation above 1000 nm. The response to photoperiods shorter than 20 minutes far-red cannot be clearly shown in this figure and will be presented in some more detail in the last part of this section (fig. 29).

The effect of the red light extension as such (in number of flower primordia) is shown in fig. 24. It is again evident that the daylength response curve can be divided into at least three parts, the first one up to daylengths of 20 to 50 minutes ('reaction 1'), the second one from daylengths of 20 to 50 minutes to those of about 750 minutes and the third one from daylengths of about 750 minutes to continuous light.

In fig. 25 the difference curve for 'leaf increment' is presented. The same three parts as in fig. 24 can be distinguished, be it that 'reaction 1' seems to prevail up to photoperiods of 100 to 200 minutes.

As far as 'reaction 1' is concerned, the inhibitive effect of 20 minutes red light appears enhanced by a short pre-illumination with far-red; in the case of flower primordia this enhancement is at its maximum at 18 seconds far-red. The same far-red illumination alone, however, had a promoting effect, and the discussed effect thus represents more than a simple reversion. Obviously, the pre-illumination with far-red has rendered the plant more sensitive to the red light.

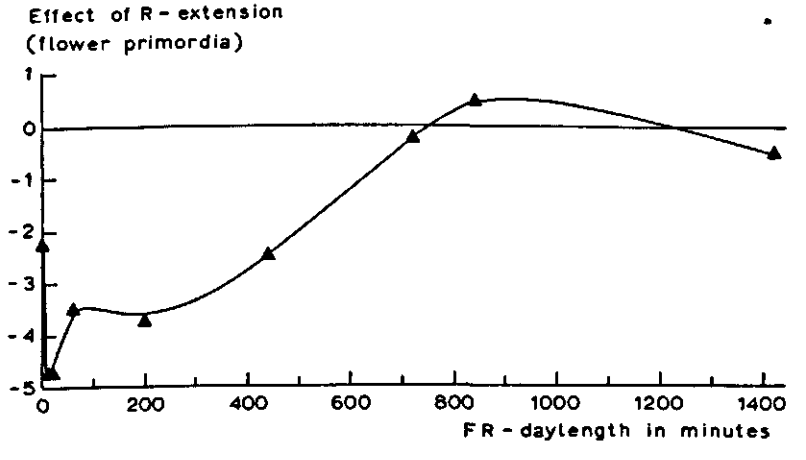


FIG. 24. Effect on flower initiation in *Hyoscyamus* of extensions of 20 min. red light (R), applied after different daylength treatments in far-red (FR), given according to the schedule (4 SD + 3 TD) 4 times, followed by 7 LD. The effect is measured as difference in numbers of flower primordia. Derived from data of fig. 23.

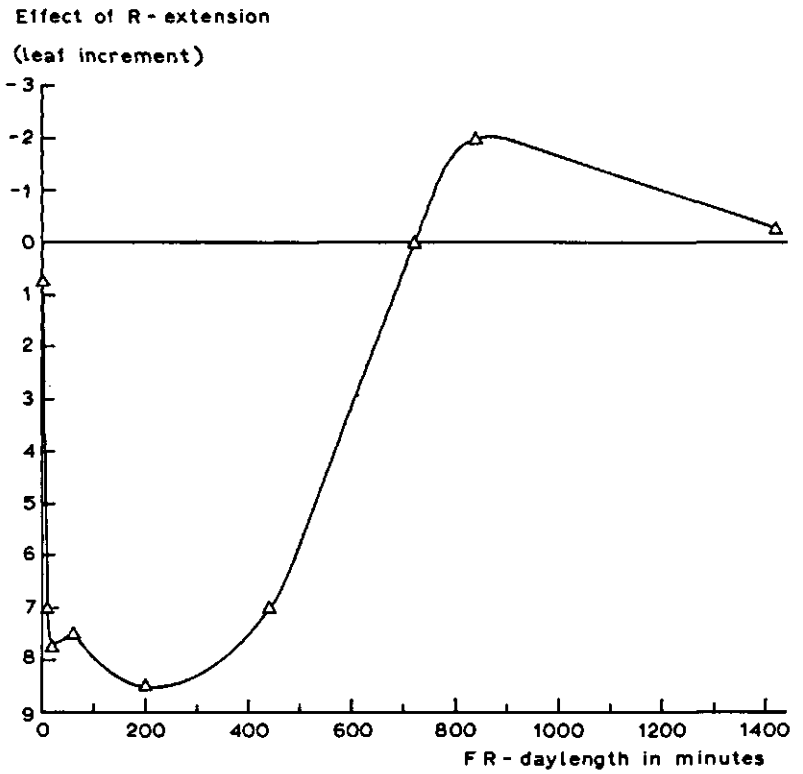


FIG. 25. Effect on flower initiation in *Hyoscyamus* of extensions of 20 min. red light (R) applied after different daylength treatments in far-red (FR), given according to the schedule (4 SD + 3 TD) 4 times, followed by 7 LD. The effect is measured as difference of leaf increments. See also legend fig. 23 and text.

In the second part, the discussed enhancement of the red effect, as well as the red light effect itself, are gradually lost with increasing photoperiods of far-red. Obviously, the effect of a far-red irradiation gradually escapes reversion by red light.

In the third part, red reversibility is essentially lost after these long far-red illuminations.

In the following experiment, the effect of a far-red extension after a pre-illumination in red light was studied. TD were again given according to the schedule (4 SD + 3 TD) 4 times, followed by 10 LD in mixed irradiation; SD was again given in fluorescent light. The same photoperiods as in the preceding experiment were applied during the TD except that instead of 20 and 60 minutes, 30 and 100 minutes were applied respectively. The photoperiods in red light were either directly followed by darkness or first by 20 minutes far-red radiation and then darkness (except again for the 1440 minutes TD). Averages of the numbers of flower primordia are presented in fig. 26. In general, the curve for red light only is the same as the one presented in fig. 21 (p. 45).

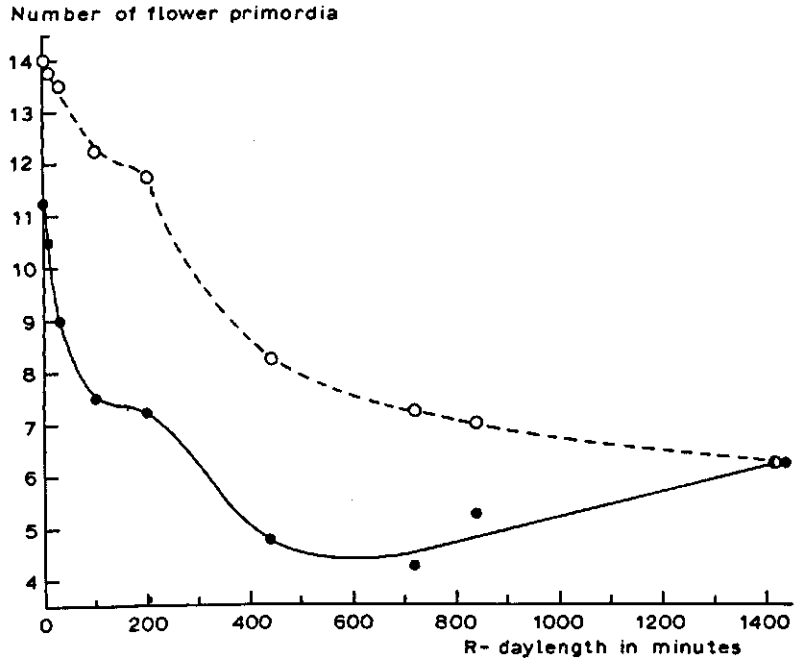


FIG. 26. Number of flower primordia in *Hyoscyamus* upon different daylength treatments in red light (R) (6500 ergs.cm^{-2}.sec^{-1}), followed either by darkness (●———●) or by 20 min. far-red (radiation between 700 and 1000 nm: 13,000 ergs.cm^{-2}.sec^{-1}) and then darkness (○- - -○). TD were given according to the schedule (4 SD + 3 TD) 4 times, followed by 10 LD. Intensity of fluorescent light during SD: 53,000 ergs.cm^{-2}.sec^{-1}; of the mixed irradiation during LD: 23,000 ergs.cm^{-2}.sec^{-1} < 700 nm and 9000 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm. The experiment started 18-7-'69. Averages of 4 plants.

The effect of the extensions with far-red light as such, is presented in fig. 27. This curve seems to have only two parts, the first one extending again up to daylengths of 50 to 100 minutes and the second one from 50 to 100 minutes up to continuous light. The first part resembles very much the reverse of that of the preceding experiment. The promotive effect of 20 minutes far-red is enhanced by a short pre-illumination with red light. The red illumination alone, however, had an inhibitive effect and thus again the effect is more than a simple reversion. Obviously, the pre-illumination with red light has rendered the plant more sensitive to far-red, a striking parallel with the effect of far-red upon the effects of red illumination, as shown in fig. 24.

In the second part, the enhancement as well as the effect of the far-red illumination itself, gradually decrease with increasing photoperiods in red light. However, in contrast to the preceding experiment, there is no clear segregation to the third part.

Comparing these results with those of section 4.3. (fig. 22, p. 49), in which it was found that 3 minutes far-red after 7 hours of red light removed the inhibi-

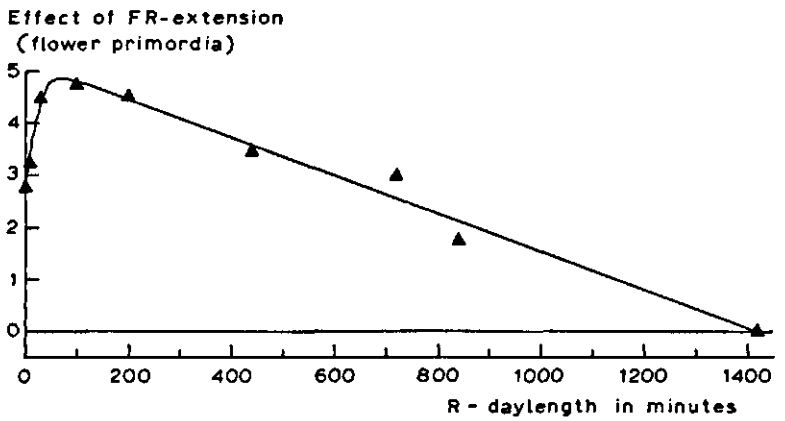


FIG. 27. Effect on flower initiation in *Hyoscyamus* of extensions of 20 min. far-red (FR), applied after different daylength treatments in red light (R), given according to the schedule (4 SD + 3 TD) 4 times, followed by 10 LD. The effect is measured as difference of numbers of flower primordia. Derived from data of fig. 26.

tion of flower initiation completely, it appears strange that in the present experiment, with 20 minutes far-red after 7 hours red, a marked inhibition still remained, resulting in fewer flower primordia than in the dark treatment. However, in comparing results of separate experiments it must be taken into account that these may diverge somewhat.

It appears more conclusive to compare different far-red extensions following a fixed photoperiod in red light within one experiment. This has been the object of the next experiment.

After photoperiods of 390 minutes red light, extensions with far-red have been given during: 0, 1, 3, 10, 20, 30, 60 or 150 minutes. The experiment includes also the same far-red irradiations applied to plants not receiving any red light. The coloured light treatments were applied during TD, given according to the schedule: (4 SD + 3 TD) 4 times, followed by 6 LD in mixed irradiation of fluorescent and incandescent light; SD was, as usually in fluorescent light. The temperature was 20°C, and the relative humidity 65%. Averages of the numbers of flower primordia are presented in fig. 28.

The curve of the treatments in far-red only of fig. 28 resembles quite well the one of fig. 29 which is an enlargement of the first part of fig. 23. The promotion in the first part of the curve (fig. 29) seems to be enhanced with another promotive action on a still shorter time scale. After 390 minutes of red light, the same far-red illuminations show much the same type of curve (cf. fig. 28). The level of promotion with respect to the dark value is slightly lower, but the promotive effect of extremely short extensions is much greater after the red preillumination, in that it fairly completely removes the red inhibition. This is a typical phytochrome effect. (cf. section 4.3.); it cannot yet be decided whether the smaller effect of extremely short far-red illuminations alone is of the same nature.

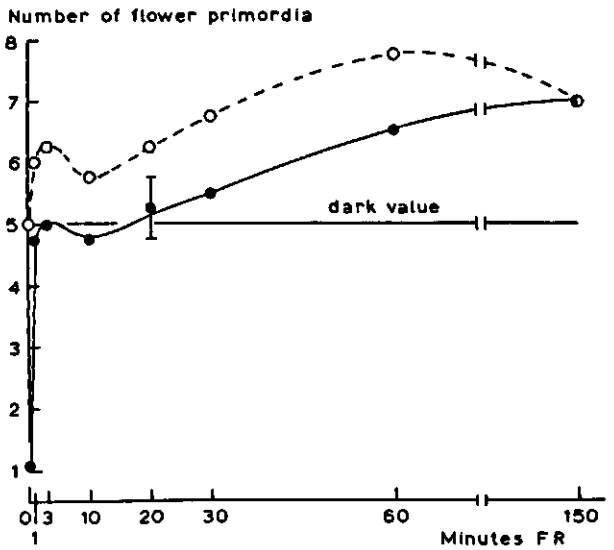


FIG. 28. Number of flower primordia in *Hyoscyamus* upon daylength treatments in far-red (FR) (radiation between 700 and 1000 nm: 13,500 ergs.cm^{-2}.sec^{-1}), preceded either by 390 min. of red light (7600 ergs.cm^{-2}.sec^{-1}) (●———●) or by just darkness (○- - - -○). TD were given according to the schedule (4 SD + 3 TD) 4 times, followed by 6 LD. Light intensity during SD (fluorescent light): 56,000 ergs.cm^{-2}.sec^{-1} and during LD (mixed irradiation): 21,000 ergs. cm^{-2}.sec^{-1} < 700 nm and 9000 ergs.cm^{-2}.sec^{-1} between 700 and 1000 nm. The experiment started 19-12-'69. Averages of 4 plants (in both 0 min. far-red treatments 10 plants).

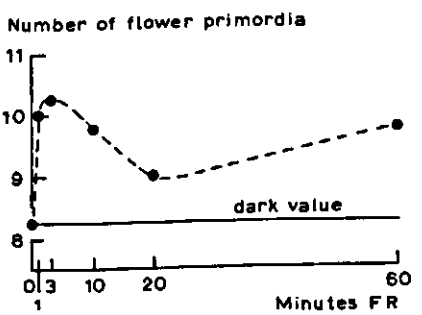


FIG. 29. Number of flower primordia in *Hyoscyamus* upon daylength treatments in far-red (FR). Enlargement of the first part of fig. 23.

In fig. 30, the difference curve derived from fig. 28 is presented, clearly showing the decrease of the red inhibition by a far-red extension already of 1 minute. Between 1 and 60 minutes, no further decrease is evident, and only with an extension of 150 minutes far-red, the red-effect is reduced to zero.

It can be concluded, that the response to very short far-red irradiations after a short day in red light, is mediated by a 'normal' phytochrome-reaction type. Far-red irradiations longer than 10 to 30 minutes seem to be mediated by a second reaction type, the same that mediates the effects of photoperiods in far-red alone.

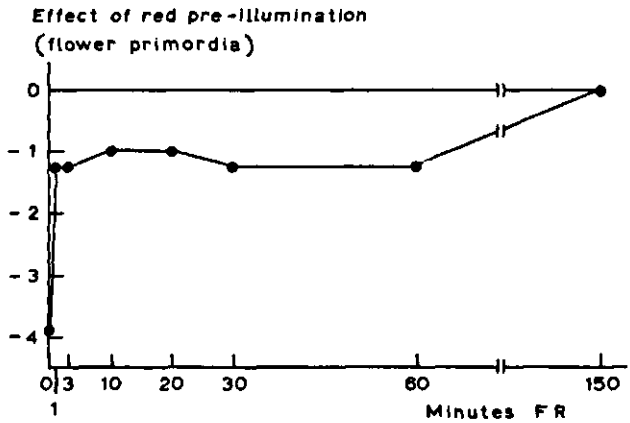


FIG. 30. Effect on flower initiation in *Hyoscyamus* of pre-illumination with 390 min. red light before different daylengths in far-red (FR). Derived from data of fig. 28.

4.5. DISCUSSION

The effects of photoperiods in coloured light, presented in this chapter, have been investigated with the method also adapted previously, viz., of series of daylength treatments, interrupted by SD, and finally followed by inductive LD. Attention was paid to effects of far-red and red light (in section 4.2. green and blue also, fig. 21, p. 45). Though different light intensities were applied, it is evident, that when compared to the 0 minutes TD (dark value) red and green light are inhibitive, while far-red is promotive for flower initiation in all daylengths, and blue light is promotive only in extremely short and long photoperiods and is inhibitive in the region of normal short daylengths.

For red and blue light there are discrepancies with some data presented by DE LINT (87). This may be due to different experimental procedures or to differences in light intensity, the daylength response being shown to be rather sensitive to this factor (section 3.4.). Certainly, a complete comparison between different colours should include several light intensities for each colour. Therefore, our discussion will necessarily be limited to the results of the experiments described in this chapter.

The daylength response curves as obtained for coloured light (e.g. those of fig. 21) can be split into separate curves for inhibition and promotion, just like those for white light, i.e. according to the 2nd assumption of section 3.5., cf. fig. 20*b*.

For red and green light, the inhibition clearly dominates; the light induced promotion reaches only a low level. In the first part of the inhibiton curve, the extra phenomenon ('reaction 1'), as already observed for white light, is of much greater importance here. The inhibition- and promotion curves for red light are presented in fig. 31; for green light they are rather similar. The extra inhibition in the first part is presented in this figure also separately

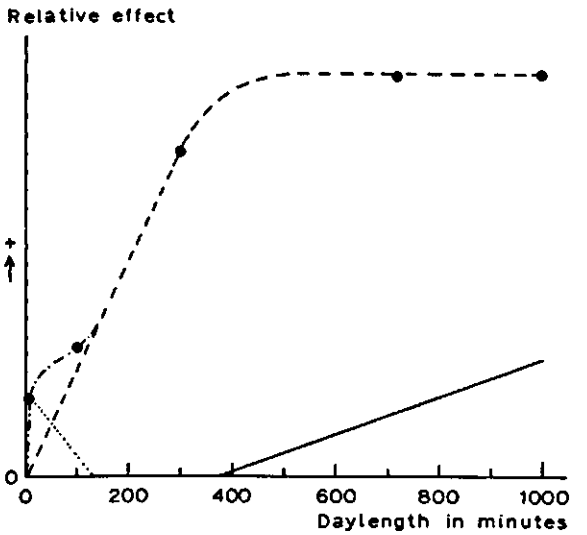


FIG. 31. Relative effect at various daylengths of light induced inhibition (– – – –) and light induced promotion (———) of flower initiation in *Hyoscyamus* in red light. The relative effect of 'reaction 1' is plotted separately (. . . . . .) and as an extra on the curve for light induced inhibition (– . – . – .). Based on data of fig. 21. See also text.

from the main inhibition curve, so that three processes are clearly distinguished, two inhibitive and one promotive for flower induction.

For blue light, the extra effect in the first part is promotive instead of inhibitive for flower initiation. Now, two of the three processes are promotive and one is inhibitive for flower initiation, the inhibiting one only dominating between

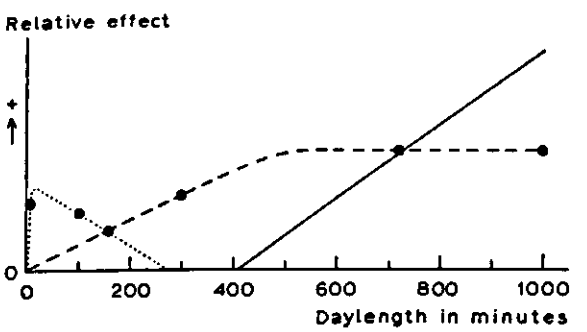


FIG. 32. Relative effect at various daylengths of light induced inhibition (– – – –) and of light induced promotion (———) of flower initiation in *Hyoscyamus* in blue light. The relative effect of 'reaction 1' is only plotted separately (. . . . . .), because it is promotive for flower initiation and thus cannot be presented as an extra on the inhibition curve. Based on data of fig. 21. See also text.

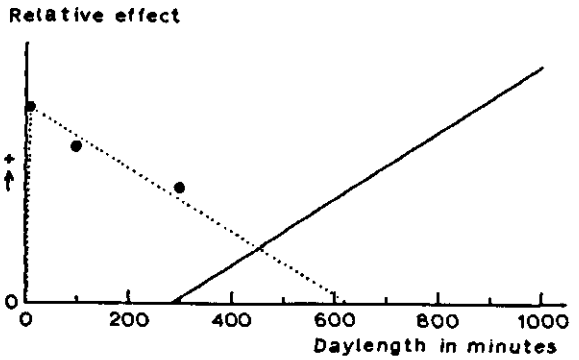


FIG. 33. Relative effect at various daylengths of two actions of far-red radiation, both promotive for flower initiation in *Hyoscyamus*. Based on data of fig. 21. See also text.

photoperiods of ca. 160 and ca. 730 minutes. In fig. 32, the inhibition- and promotion curves for blue light are presented.

For far-red, the extra effect in the first part also is clearly promotive for flower initiation. Light induced inhibition is not necessarily involved in far-red. Splitting of the far-red curve (fig. 21) into two curves, each representing a promotive action, seems possible (fig. 33), although the detailed shape of each of these curves cannot yet be given with certainty.

Our observations (figs. 21 and 26) indicate that brief red illuminations, producing a high P_{fr}/P_{total} ratio, are inhibitive for flower induction in LDP. This would correspond with the findings of FREDERICQ (43) with *Kalanchoë*, that 5 minutes red – and even 1 second of daylight (55) – per day could induce flowering in that SDP; FREDERICQ could show this to be a phytochrome effect. Although in section 4.3. hardly any effect of 5 minutes red was observed, still phytochrome could be shown to be involved to a certain degree (fig. 22).

In the following discussion of our coloured light experiments we will tentatively depart from the same sort of reasoning we have adopted in the discussion of our white light experiments. We will recall in mind that it was assumed, in connection with data in literature, that photosynthetic reactions may influence the photoperiodic behaviour, in that substrates formed in photosynthesis (especially by the action of 'system II') tend to inhibit flowering, while ATP, especially produced in cyclic photophosphorylation (mediated by 'system I'), may promote flowering.

In radiation wavelengths above 700 nm (far-red), the photosynthetic 'system II' is practically inactive, while 'system I' still works. Therefore, the ATP level gradually increases, while in red light the ATP level becomes rather soon constant at a much lower level, due to ATP consumption by CO_2 reduction (159, 118).

Extremely brief far-red irradiations establish a low P_{fr}/P_{total} ratio, and, at the same time, the ATP level is increased via the photosynthetic 'system I'. How-

ever, P_{fr}, be it only present at a low level, may still be the main factor for the promotion of flowering during the very first minutes in FR; later, the increased ATP level may take over the promotive action (figs. 28 and 29). This would mean that the first reaction is promotive for flower initiation when the P_{fr}/P_{total} ratio is low and, according to figs. 21 and 26, is inhibitive when that ratio is high.

Since in darkness P_{fr} is broken down or reverted to P_r, it may be assumed that in the dark controls (0 min. TD) no P_{fr} will be available and, therefore, no light induced inhibition or promotion will occur. This is consistent with the theory that P_{fr} is the physiologically active form of phytochrome (148, 25).

In prolonged far-red irradiations of the order of the normal short day region (420 min.), flowering will not be inhibited, since hardly any photosynthesis takes place. However, when daylength is increased further, a promotive action again becomes manifest owing to available P_{fr} and synthesized ATP.

In prolonged red irradiations (420 min.), normal photosynthesis takes place, which means that the inhibitive factor produced in photosynthesis (cf. sections 3.4. and 3.5.) is available and inhibition of flower initiation occurs, but, as is shown in section 4.3. (fig. 22), only when phytochrome at the beginning of the dark period is predominantly in the P_{fr} form. Thus, it appears that conditions for the occurrence of inhibition are that photosynthesis has taken place and that the P_{fr}/P_{total} ratio at the beginning of the dark period is high.

It cannot yet be decided which of two possibilities exists, viz., 1. whether for the realization of inhibition a high P_{fr}/P_{total} ratio at the beginning of darkness is needed or, 2. whether only a certain amount of P_{fr} has to be available. Terminating an illumination period with a brief exposure to red light instead of far-red means that in the dark P_{fr} is available during a longer time, so that then more inhibition can be realized. However, if the illumination is terminated with a prolonged exposure to far-red (e.g. 2 hrs), given after a short day in red light, one would expect that, in case of the second possibility mentioned above, during the far-red illumination the inhibition reaction can proceed. After the inhibitive reaction is saturated, the promotive process may start to express itself. Because a brief red light irradiation, applied after such an extension with far-red, enhances the LD-effect (36), obviously all substrate of photosynthesis (of the main light period), which is assumed to be necessary for obtaining inhibitor, has disappeared during the far-red irradiation. We suppose that this disappearance has taken place in the reaction in which the inhibitor is produced, and, since during the far-red exposure only a low P_{fr}/P_{total} ratio was maintained, the second possibility mentioned above is preferred.

In section 4.4., some more red and far-red extensions have been examined. Since already very brief pre-illuminations with far-red (e.g. already 18 sec.) enhance the effect of 20 minutes red light, a phytochrome effect on cell membranes could be involved, as with the fast closing movements of the pinnae of *Mimosa pudica* (39). Because of the analogy of the effect of 20 minutes far-red after brief pre-illuminations in red with the effect of red after far-red (cf. figs. 27 and 24), phytochrome could be responsible also in this case. The reversal of

the effect of about 400 minutes red by some 3 minutes far-red may well be less simple than might be concluded from fig. 22, since the promotive effect of 3 minutes far-red alone is not reached in experiments in which far-red is applied after a prolonged exposure to red light (fig. 28). Obviously, during the red exposure some irreversible reaction has taken place, which prevents far-red to exert its promotive action.

HAUPT (61) made similar observations with growth of internodes and petioles of pea seedlings. Red effects could be annihilated by far-red, but, even with saturating far-red following red, the response was not the same as in far-red alone. He also explained this phenomenon by assuming fast irreversible reactions occurring during or immediately after photoconversion of P_r to P_{fr}.

Leaf position was shown (Plates 6 and 7) to be strongly affected by phytochrome, which confirms observations of SCHNEIDER et al. (126) who refer this to nastic leaf movements. In other plants also, movements of leaves or leaflets were shown to be influenced by phytochrome (39, 47). However, after prolonged far-red irradiations (420 min.), no red/far-red reversal effect on leaf position could be observed any more (Plate 7). Possibly, the cyclic photophosphorylation had generated so much ATP that the action of P_{fr} was nullified.

5. GENERAL DISCUSSION

Working with the 'qualitative' long-day plant *Hyoscyamus niger*, the daylength dependence of flower initiation has been investigated. Several parameters of flower initiation were used; number of flower primordia on the main flower stalk was found to be the most reliable one. 'Days to shooting' also may be used in most experiments on flower initiation, but 'leaf increment' was found to be not completely correlated with flower initiation, in particular was this evident in the region of very long daylengths (sections 2.3.2. and 3.2.).

Most of our experiments on daylength dependence have been carried out by interrupting a period of treatment days (TD) with short days (SD) in white fluorescent light in order to improve the condition of the plants. To obtain flower initiation in all treatments, after-treatment in long days (LD) was applied. With this procedure it was possible to investigate the influence of daylength on flower initiation over the whole daylength region from zero to 24 hours. In section 3.3., it was shown that none of the components of the experimental scheme, except TD, affected essentially the shape of the daylength dependence curve.

It was possible to split the experimental curve (e.g. fig. 11) into separate curves for (light induced) inhibition and for (light induced) promotion (section 3.5., fig. 20). In the analysis, the behaviour in very short light periods appeared as an extra phenomenon either of an inhibitive or of a promotive nature, dependent on the spectral composition of the light (cf. figs. 31 and 32). This phenomenon has been referred to as 'reaction 1'.

Curves, obtained with red light (cf. figs. 21 and 26) approximate a curve for light induced inhibition (cf. figs. 20 and 31), though in the extreme short photoperiods and in the long ones, other actions ('reaction 1' and light induced promotion) seem to interfere. Evidence derived from experiments with different light intensities, as well as from literature suggests that photosynthesis, in the sense of system II, may be a factor involved in the inhibition of flower initiation in *Hyoscyamus* (figs. 17, 18 and 19). BAVRINA et al. (4) recently concluded that flowering of long-day species is more dependent both on the photosynthetic reduction of CO_2 and on photophosphorylation than that of short-day species. Most of the literature (53, 110, 56, 90, 42, 94), as well as data presented in section 3.4. suggest that photosynthetic reduction of CO_2, as mediated essentially by the photosynthetic 'system II', is an important requirement for SD-effects. Another factor involved in the SD-effect is phytochrome (section 4.3.). Inhibition of flower initiation was obtained when, at the end of an SD, phytochrome was predominantly in the far-red absorbing form (fig. 22). The effect of a brief far-red irradiation (20 min.) appears enhanced by brief pre-irradiations with red light ($<$ 50 min.). This effect of red light decreased when its duration was increased beyond 50 minutes, as well as the effect of the 20 minutes far-red when following the red, (figs. 26 and 27). Obviously, the inhibition becomes irreversible not only in a long dark period, but also during an illumination period, at least in red light.

The concept of the photoperiodic reaction system as given by DE LINT (87), can, in part, be applied for the light induced inhibition. The inhibitor-precursor may be identical with the factor derived from photosynthesis. However, this precursor then should not only be converted into an inhibitor during a subsequent dark period as long as P_{fr} is present, but also during a prolonged illumination.

The light induced inhibition is supposed to become saturated when light induced promotion becomes evident (cf. section 3.5.). This implies that, when for some reason promotion is suppressed, maximum inhibition will be reached at longer photoperiods. According to a recent observation of JONES (71), ATP levels in plants vary inversely with temperature; so if the ATP level is a factor in determining promotion, then at higher temperatures maximum inhibition should also shift to longer photoperiods. This indeed has been observed in preliminary experiments. The observation that the critical daylength for *Hyoscyamus* is longer at higher temperatures (82) is in good agreement with the assumptions made above.

In the curves obtained in far-red light (cf. figs. 21 and 23) it is somewhat difficult to recognize the curve for light induced promotion (cf. figs. 20 and 33), owing to the strong effect of 'reaction 1'. During the far-red light period, ATP should become available mainly via cyclic or pseudo-cyclic photophosphorylation, but during the dark period, ATP, generated in dissimilation, might also be promotive for flower initiation, provided that some P_{fr} is available.

This may explain why a short interruption of a long night with red light, converting phytochrome into the P_{fr} form, causes an LD-effect. The inhibitor- precursor, produced in photosynthesis during the short main light period, is no longer available after a certain period of darkness (converted into inhibitor and for the rest broken down in dissimilation or transported away), so that no inhibition occurs. On the other hand, for promotion of flower initiation the required factors are available (P_{fr}) or become so (ATP).

The observations of section 4.1. that a red exposure after a short day in white light extended with far-red, may enhance the LD-effect (36, 28), can also be explained. During the far-red extension, light induced inhibition has become saturated and the ATP level is increased, so that light induced promotion can proceed. By ending the illumination period with a red exposure, P_{fr} will be available in darkness during a longer period of time than by ending with far-red so that the promotive action can continue for a longer period of time.

The promotive action of a brief exposure of far-red, following a prolonged extension of a short day in white light with red (36), may be explained by assuming that during the red light extension not all precursor is converted into inhibitor, and that because of the transfer of most of the phytochrome into the P_r form the production of the inhibitor will soon stop.

It appears difficult to explain the promotive effect of long photoperiods by assuming a HER directed by phytochrome, as was advanced by HARTMANN (58) for seed germination and hypocotyl lengthening of lettuce. For the opening of the leaflets of *Mimosa pudica*, phytochrome was not the photoreceptor, though this response had an action spectrum (40) with characteristics of HER action

spectra as determined e.g. for the control of flowering in *Hyoscyamus niger* (127).

It may well turn out that promotion of flower initiation in *Hyoscyamus niger*, as well as several other HER phenomena (40) simultaneously require the presence of P_{fr} and the production of energy-rich phosphorylated compounds, either produced via cyclic or pseudo-cyclic photophosphorylation or in dissimilation. Indeed, SCHNEIDER and STIMSON (127a) recently pointed to the photosynthetic system I for being involved in a HER. They attributed the far-red HER response of anthocyanin synthesis in turnip seedlings to the photostimulation of two pigments, i.e., chlorophyll-a and phytochrome.

Not much is yet known about the nature of the substances which play a role in the subsequent phases of the reaction chain leading to inhibition or promotion of flower initiation. However, it has been shown in recent years that some growth regulators may be important. So, e.g. in the leaves of several plants the content of natural gibberellins is higher in LD than in SD. In continuous darkness, destruction of gibberellins has been observed (17). Some observations on rosette plants indicate, however, that gibberellins are not directly involved in flower initiation of LDP, but that they do play a direct role in bolting (95, 134, 18). EVANS (35), with *Lolium temulentum*, concluded that endogenous gibberellins play no direct role in floral induction, but that compounds sharing early steps in the biosynthetic pathway to gibberellins may do so, and that their action can be enhanced by applied gibberellins.

In *Hyoscyamus*, gibberellins applied under otherwise non-inductive conditions cause shooting and flower initiation (23), but possibly, like in *Lolium*, gibberellins act on flower initiation only in an indirect way.

Inhibition of gibberellin synthesis by the endogenous growth inhibitor 'dormin' or 'abscisic acid' (ABA) was suggested by WAREING et al. (144). They argued that the failure of LDP to form flowers under SD is due to the low level of gibberellins in combination with high levels of endogenous inhibitors occurring under those conditions. Under LD conditions, application of ABA to LDP may inhibit flower induction (34, 27, 144).

In this connection an observation of TAYLOR and SMITH (139a) may be of interest, viz., that a growth inhibitor with ABA-like properties, recently tentatively called xanthonin (139), is produced by photo-oxidation of certain xanthophylls. From all xanthophylls of *Urtica dioica* L., violaxanthin was found to produce the greatest inhibitory effect when illuminated, although it was only one of the minor xanthophylls in the leaf extract. A recent observation of HAGER (52) may present a connection between such an appearance of ABA-like substances and the availibility of ATP. He was able to show that de-epoxidation of violaxanthin to zeaxanthin occurs under conditions under which photophosphorylation takes place; addition of ATP to chloroplasts could trigger the conversion without illumination. The light activation of the responsible enzyme, de-epoxidase, was assumed to be the result of a light induced decrease of pH in the chloroplast compartment containing the enzyme. According to DAVIES (23a) molecular oxygen is required for the conversion of zeaxanthin into violaxanthin.

It can be questioned whether or not under all circumstances in the plant enough molecular oxygen is available for this process.

ADDICOTT and LYON, in their review (1), suggested that promotion of flowering in certain SDP by ABA may be due to restriction of vegetative growth rather than to a specific stimulation of flowering.

According to RUSSELL and GALSTON (120), gibberellins may be involved in auxin responses, since they may block phytochrome controlled flavonoid synthesis and thus inhibit IAA-oxidase systems.

Auxin application causes a reduction of the effect of SD treatment in SDP; this effect seems reversible by antiauxins (7, 81). However, CLAES (19) was not able to promote or inhibit flower initiation in *Hyoscyamus* by application of auxin, not even when the daylength applied was close to the critical one. Surprisingly, shooting without any flower initiation was obtained in SD in *Hyoscyamus* plants treated with an antiauxin (19). Only, the duration of the experiment was rather short (about 1 month), so that, ultimately, flower initiation might have been obtained also.

Working with the LDP *Hyoscyamus niger* and *Silene armeria*, LIVERMAN and LANG, in 1956 (91), found that, under SD extended with light intensities not sufficient for optimal flowering response, auxin application increased the response. However, in 1965, LANG (81) questioned the significance of this effect, since a similar response was also obtained with several antiauxins.

ALLEWELDT (2), discussing the role of auxin in the flowering process, stated in summary that there is no clear-cut effect of auxins in flower initiation in LDP. The findings show quantitative differences and seem to indicate the possibility of modification of the photo-induction.

In recent years, TANADA (135, 136) presented evidence for participation of 3-indoleacetic acid (IAA), together with some other substances (e.g. ATP and K^+) in a peculiar phytochrome response, viz., the attachment of root tips to wet glass surfaces under the influence of P_{fr}. He, and also JAFFE (69), attributed this phenomenon to changes in electric charges in the membrane, induced by alterations in the molecular structure of phytochrome in its response to red or far-red irradiation. Recently, WAGNER and CUMMING (143) postulated that endogenous rhythmicity (13, 22) is due to the spatial separation of production and use of energy in different cell particulates, with phytochrome acting as a membrane operator, possibly in cooperation with plant hormones. LÜTTGE and PALLAGHY (93), however, found that the transient change of the trans-membrane electric potential of green cells of higher plants, subjected to a sudden change in the intensity of illumination, is linked to electron transport by photosystem II, although they did not completely rule out direct effects of light on membrane permeability via phytochrome. Recently, SIDAWAY (129), in an experiment on germination of lettuce seeds could, to a limited extent, substitute electrostatic treatment for red light in raising the dark germination level. He assumed interaction with the phytochrome system. Also redox chemicals may be substituted for radiation in some phytochrome-mediated responses (70).

Involvement of phytochrome in permeability changes (63) is not surprising,

since evidence was obtained for phytochrome being associated with membranous structures (e.g. 60, 119).

TANADA (136) already discussed the possibility that a change in membrane charge might affect the formation of polyribosomes on the membrane. Indeed, WILLIAMS and NOVELLI (158), examining ribosomes in vitro, found that the percentage of polyribosomes was increased following exposure of dark grown seedlings to low levels of light, red light being most effective in promoting this response.

We may assume that differential gene activation and repression through P_{fr}, as proposed by MOHR (105), may be controlled by the action of P_{fr} on membrane potentials. In the 'positive photoresponses', reported by MOHR, the polyribosomes still have to be built up before the gene-regulated response can start, which may explain the lag-phase in the response. For nitrate reductase it was indeed observed (140a) that the ability of etiolated corn leaves to form the active enzyme in the light, was correlated with the time course of polyribosome development. In the 'negative photoresponses', the polyribosomes may be suddenly disturbed in their action by a sudden change in membrane potential, so that the response will show hardly any lag-phase. Consequently, one may assume that fast leaf movements that are mediated by changes in membrane permeability (40, 70) as well as differential gene activation (105), are regulated by phytochrome via its control on membrane potentials. However, a more direct activation or repression of genes certainly is not ruled out.

In movements of leaves or leaflets, not only P_{fr} but also an ATP-dependent process appears to be involved (40, 47); normally, leaves close in the dark in the presence of a high P_{fr}/P_{total} ratio. The supposed participation of ATP may explain why in light a leaf opening response is obtained in *Mimosa pudica* in spite of a high percentage of P_{fr}; FONDEVILLE et al. (40) suggest that the HER may well be involved in ATP formation effective in maintaining the membrane function. We have explained in the same way the masking of nastic leaf movements by 420 minutes far-red, as shown on Plate 7 (cf. section 4.5.). GALSTON (47) reported that leaflet movements in *Albizzia julibrissin* were correlated with K^+ transport, so that closure was effectively antagonized by high concentrations of external K^+, and recently RAVEN (116) concluded, among other things, that either cyclic or pseudo-cyclic photophosphorylation may accellerate K^+ influx in the light; thus, in the green plant, activation of 'system I' should be able to effectuate K^+ influx.

Differences in K^+ availability may cause discrepancies between experiments, and thus a suitable control of plant nurtition may be extremely important, just as control of the water supply, since WRIGHT and HIRON (160) found that ABA could be induced in (detached) wheat leaves by a period of wilting. Changes in turgor in the primary pulvini of *Mimosa pudica* were observed to lead to petiole dropping (40).

In section 4.5., the effects of extremely short photoperiods have been explained, as a phytochrome effect on cell membranes. Probably, a change in membrane permeability is the main factor. This might not only be important in extremely

short photoperiods, but always when another illumination starts, so that in experiments with subsequent illuminations in different colours or different light intensities not only light induced inhibition and promotion, but also the above effect may play a role.

In conclusion, evidence is presented that during illumination of *Hyoscyamus* separate processes are active, causing either inhibition or promotion of flower initiation, and depending on two (or more) factors, phytochrome being one of these in both types of processes. The other factors are suggested to depend on aspects of photosynthesis, including photophosphorylation. In extremely short photoperiods, a direct phytochrome action on cell membranes may well be the main effect, which may be promotive or inhibitive for flower initiation in *Hyoscyamus*, depending on the spectral region of the light.

SUMMARY

Investigations have been carried out on the photoperiodic control of flower initiation in the annual strain of the long-day plant *Hyoscyamus niger* L. Plants were grown in soil; precultivation (in short day (SD) fluorescent light) as well as experimental treatments were applied at 20 °C. Coloured radiation was obtained by combining special coloured fluorescent lamps with 'plexiglas' filters (subsection 2.2.2.).

In white light no absolute juvenile phase could be observed, but with increasing age, up to 30 to 40 days, plants were more sensitive to inductive treatment (subsection 2.3.2.). For most experiments, the precultivation period was 3 to 4 months.

As a parameter of flower initiation 'number of flower primordia' was found to be more generally applicable than 'days to shooting' and 'leaf increment' (sections 2.4. and 3.2.). For 'days to shooting', plants in a mixed radiation of fluorescent and incandescent lamps had a slightly shorter critical daylength than plants in fluorescent light only. For initiation of flower primordia there was no difference in critical daylength between the two light qualities (section 3.2.).

In most experiments various daylengths, from zero to 1000 minutes or to continuous light, were applied during a period of treatment days (TD) interrupted at regular intervals by a few SD in high intensity white fluorescent light, for energy supply. After-treatment was given in long days (LD) in a mixed irradiation of fluorescent and incandescent light, in order to ultimately obtain flower initiation in all treatments. Flower primordia for all treatments were counted at the end of the LD after-treatment. None of the components of the experimental scheme (except, of course, TD) was found to affect essentially the type of response curve obtained (section 3.3.).

The effect of light intensity in a mixture of fluorescent and incandescent light on daylength dependence of flower initiation was investigated in section 3.4.. Inhibition of flower initiation (SD-effect), as observed at high light intensity (24,000 ergs.cm^{-2}.sec^{-1} $<$ 700 nm) for daylengths between about 300 and 600 minutes, was lost at low light intensities (2400 or 400 ergs.cm^{-2}.sec^{-1} $<$ 700 nm) for all three parameters mentioned above. With photoperiods longer than ca. 800 minutes, promotion of flower initiation (LD-effect), determined as 'number of flower primordia' or 'days to shooting', was weaker at low light intensities. For 'leaf increment', in all three light intensities a similar, weak LD-effect was observed.

The daylength response curve for white light could be split into a saturation curve, representing the inhibitive light action, and a curve reaching saturation at much longer photoperiods, representing a light action, promotive for flower initiation. At extremely short photoperiods (below 50 min.), a weak extra inhibition was apparent (cf. section 3.5., fig. 20).

In the experiments of Chapter 4, in which coloured light (far-red, red, green, and blue) was applied during the TD, it was observed that the extra effect at

short photoperiods ('reaction 1') could be either inhibitive (in red and green) or promotive (in far-red and blue) for flower initiation. With red and green light, inhibition of flower initiation was observed at all daylengths (cf. section 4.2., fig. 21); the postulated light induced promotion attained only a low level in LD (cf. section 4.5., fig. 31). With far-red, promotion of flower initiation was observed at all daylengths (cf. section 4.2., fig. 21); light induced inhibition was not necessarily involved (cf. section 4.5., fig. 33). With blue light, inhibition was only observed at daylengths between 160 and 730 minutes (cf. section 4.2., fig. 21); at other daylengths promotive actions dominated (cf. section 4.5., fig. 32).

Inhibition of flower initiation by 420 minutes red light could be annihilated by 3 minutes far-red; this was shown to be a phytochrome effect (section 4.3., fig. 22). The promotive effect, observed with 3 minutes far-red alone, was not obtained when far-red was applied after exposures to red light of the order of 400 minutes (section 4.4., fig. 28).

A brief exposure to far-red, preceding 20 minutes red light, enhanced the inhibiting effect of the red, while a brief exposure to red light, preceding 20 minutes far-red enhanced the promoting effect of the far-red (cf. section 4.4.).

Leaf position was strongly affected by phytochrome; however, after 420 minutes far-red, no red/far-red reversal effect on leaf position was any more observed (cf. section 4.3., Plates 6 and 7).

The above data, together with suggestions from literature, give rise to the following interpretation.

The effect occurring at extremely short daylengths ('reaction 1') most likely is a phytochrome effect on cell membranes.

The inhibitive effect (light induced inhibition), responsible for the normal SD-effect, is assumed to require some factor from photosynthesis ('system II') for its realization and, besides this, phytochrome in the far-red absorbing form (P_{fr}).

The promotive effect (light induced promotion), responsible for the normal LD-effect, is assumed to require ATP for its realization (produced via the photosynthetic 'system I' or via dissimilation), and also P_{fr}.

It has been demonstrated that it is possible to split the experimental curves into these three components which shows the daylength dependence of these effects separately (cf. section 4.5.).

Occasional masking of the phytochrome control of leaf position may well be attributed to a high ATP level, nullifying the action of P_{fr}.

In chapter 5, the nature of the substances which play a role in the subsequent phases of the reaction chain leading to inhibition or promotion of flower initiation, has been discussed. Special attention was paid to effects of gibberellins, auxins and abscisic acid.

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SAMENVATTING

Het onderzoek van de daglengte-afhankelijkheid van de bloemaanleg is uitgevoerd met behulp van een eenjarige vorm van de lange-dag plant *Hyoscyamus niger* L. (Bilzenkruid). De planten werden gekweekt in aarde; de opkweek (in korte dag (KD) fluorescentielicht) en de proeven geschiedden bij 20°C. Gekleurd licht werd verkregen met behulp van speciale, gekleurde fluorescerende buizen, tezamen met geschikte 'plexiglas' filters (subsectie 2.2.2.).

In wit licht werd geen absolute jeugdfase waargenomen; wel waren oudere planten (30 à 40 dagen oud) gevoeliger voor inductieve behandelingen dan jongere (subsectie 2.3.2.). Voor de meeste proeven duurde de opkweekperiode 3 à 4 maanden.

Als criterium voor de beoordeling van de bloemaanleg bleek 'aantal bloemprimordia' meer algemeen toepasselijk te zijn dan 'dagen tot schieten' en 'bladtoename' (secties 2.4. en 3.2.). In een gemengde belichting van fluorescentiebuizen en gloeilampen bleek de kritische daglengte iets korter te zijn dan in fluorescentielicht alleen. Voor bloemaanleg zelf werd geen verschil in kritische daglengte in deze twee lichtsoorten waargenomen (sectie 3.2.).

In de meeste proeven werden daglengten, varierend van nul tot 1000 minuten of tot continu licht, gegeven gedurende een periode van behandelingsdagen (BD), welke regelmatig werd onderbroken door enkele KD met hoge intensiteit wit fluorescentielicht, om te grote verzwakking van de planten te voorkomen. Teneinde uiteindelijk bloemaanleg in alle behandelingen te krijgen, werd aan alle planten een nabehandeling in lange dag (LD) wit licht gegeven. Aan het eind van de LD-nabehandeling werd van alle planten het aantal bloemprimordia geteld. Gebleken is, dat geen van de componenten van het proefschema de vorm van de daglengte-afhankelijkheidscurve wezenlijk veranderde (sectie 3.3.).

In sectie 3.4. werd de invloed van de intensiteit van wit licht op de daglengte-afhankelijkheid van de bloemaanleg bestudeerd. Remming van de bloemaanleg (KD-effect), zoals die werd waargenomen onder hoge lichtintensiteit (24.000 $erg.cm^{-2}.sec^{-1} < \lambda$-700 nm) voor daglengten tussen 300 en 600 minuten, bleek onder lage lichtintensiteit (2400 of 400 $erg.cm^{-2}.sec^{-1} < \lambda$-700 nm) voor geen van de drie genoemde criteria meer te bestaan. Bevordering van bloemaanleg (LD-effect), zoals waargenomen onder daglengten langer dan ca. 800 minuten, bleek, gemeten via de criteria 'aantal bloemprimordia' of 'dagen tot schieten', bij lage lichtintensiteiten af te nemen. Met 'blad toename' werd in alle drie lichtintensiteiten eenzelfde, zeer zwak LD-effect waargenomen.

De daglengte-afhankelijkheidscurve voor wit licht bleek te kunnen worden samengesteld uit een verzadigingscurve, voorstellende de remmende werking van licht en een curve, welke eerst bij veel grotere daglengte verzadiging bereikt en de bevorderende werking van licht op bloemaanleg voorstelt. Onder extreem korte daglengten (korter dan 50 min.) bleek onmiskenbaar een zwakke extra remming voor te komen (zie fig. 20).

Dit extra effect bij korte daglengten ('reactie 1') bleek in de proeven, vermeld

in hoofdstuk 4, in welke gekleurd licht (donker-rood, rood, groen en blauw) werd gegeven tijdens de BD, hetzij remmend (in rood en groen), hetzij bevorderend (in donker-rood en blauw) voor bloemaanleg te zijn.

In rood en groen licht werd bij alle daglengten remming van de bloemaanleg waargenomen (zie sectie 4.2., fig. 21); de gepostuleerde, door licht geïnduceerde bevordering bereikte hier in LD dan ook slechts een laag niveau (zie sectie 4.5., fig. 31). In donkerrood werd bij alle daglengten een bevordering van de bloemaanleg waargenomen (zie sectie 4.2., fig. 21); een door licht geïnduceerde remming behoefde hier niet te worden verondersteld (zie sectie 4.5., fig. 33). In blauw licht werd alleen bij daglengten tussen 160 en 730 minuten een remming waargenomen (zie sectie 4.2., fig. 21); bij andere daglengten overheersten bevorderende werkingen (zie sectie 4.5., fig. 32).

Drie minuten donker-rood kon de remming van de bloemaanleg door 420 minuten rood licht opheffen; dit bleek een fytochroom-effect te zijn (zie sectie 4.3., fig. 22). Het bevorderend effect van 3 minuten donker-rood alleen werd niet volledig bereikt als het na ca. 400 minuten rood werd gegeven (zie sectie 4.4., fig. 28).

Het remmend effect van 20 minuten rood licht werd versterkt door een voorafgaande, korte belichting met donker-rood, terwijl een korte rood-belichting, voorafgaande aan 20 minuten donker-rood, het bevorderend effect van donker-rood versterkte (zie sectie 4.4.).

De bladstand bleek onder invloed van het fytochroom-systeem te staan; na 420 minuten donker-rood echter, kon 3 minuten rood licht geen invloed meer op de bladstand uitoefenen (zie sectie 4.3., Plaat 6 en 7).

Bovenstaande resultaten, tezamen met literatuurgegevens, hebben aanleiding tot de volgende beschouwingen gegeven.

Het effect van zeer korte daglengten ('reactie I') wordt hoogstwaarschijnlijk bepaald door een fytochroom-effect op celmembranen.

Voor het tot stand komen van het remmend effect dat verantwoordelijk is voor het normale KD-effect, zou een bepaalde factor uit het fotosyntheseproces, gevormd via 'systeem II', tezamen met fytochroom in de donker-rood absorberende vorm (P_{fr}) aanwezig moeten zijn.

Voor het tot stand komen van het bevorderend effect, verantwoordelijk voor het normale LD-effect, zou ATP (gevormd via 'fotosynthesesysteem I' of via de ademhaling) en ook P_{fr} nodig zijn.

Door de experimentele curven in deze drie componenten te splitsen, kon de daglengte-afhankelijkheid van deze afzonderlijke effecten worden weergegeven (zie sectie 4.5.).

Het verdwijnen van de fytochroom-controle op de bladstand na 420 minuten donker-rood, kan wellicht worden toegeschreven aan een hoog ATP niveau, dat de werking van P_{fr} in deze teniet zou doen.

De aard van de stoffen, die een rol spelen in latere fasen van de reactie-ketens en leiden tot remming of bevordering van bloemaanleg, is besproken in hoofdstuk 5. Vooral aan effecten van gibberellinen, auxinen en abscisinezuur is daarbij aandacht besteed.

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