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DAYLENGTH DEPENDENCE OF FLOWER INITIATION IN *HYOSCYAMUS NIGER* L.

Preliminary communication

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1. INTRODUCTION

In 1960, BEST (1) presented daylength response curves for flowering of both short-day and long-day plants (fig. 1). The critical daylengths between vegetative and generative growth were characterized by a level *a*, the position of which depends, according to DE LINT (9), upon experimental conditions and duration of treatment. In daylengths in which the response curves rise above *a*, plants remain vegetative.

Also in 1960, DE LINT (9) confirmed the 'BEST curve' for long-day plants with the 'qualitative' long-day plant *Hyoscyamus niger*. He managed his plants to survive even the shortest daylengths by giving these treatments during 6 days

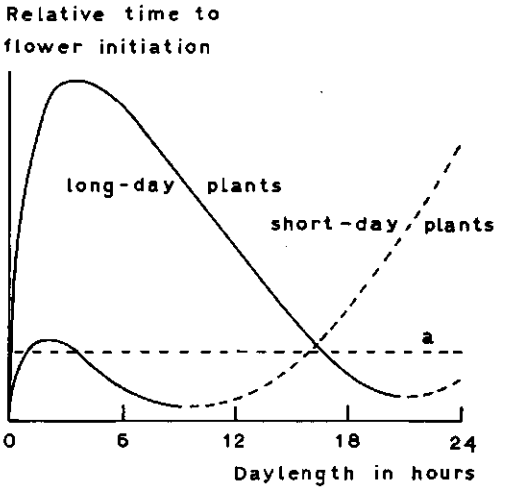


FIG. 1. Schematic daylength-dependence curves of flowering in long-day and short-day plants, as proposed by BEST (1). (Line *a* added by DE LINT (9), see text).

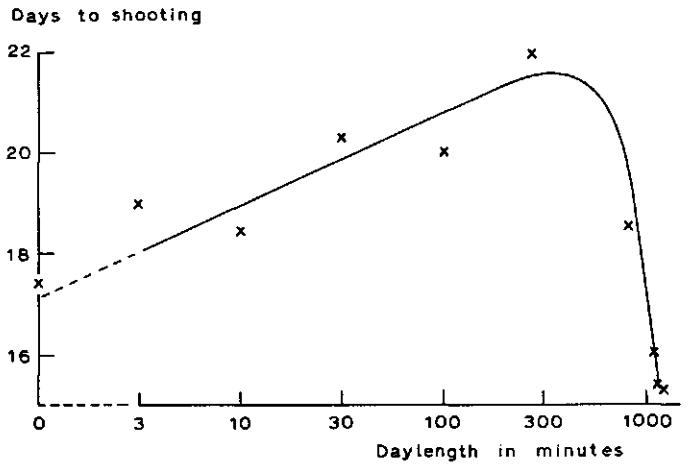


FIG. 2. Days to shooting upon a daylength-treatment in white light (± 20,000 ergs $cm^{-2}sec^{-1}$) during 6 days, following short days in the greenhouse. After-treatment in long summer days in the greenhouse. (As given by DE LINT (9)).

only, after which all plants were exposed to flower induction in long days (fig. 2).

We reinvestigated the 'DE LINT-curve' in an attempt to obtain more detailed information about the influence of these very short daylengths on flower initiation.

2. PARAMETERS OF FLOWER-INITIATION

Determination of the earliest stages of flower initiation, and thus of the moment of beginning of floral differentiation, often is impossible without microscopic examination of the meristem. Determination of the moment of flower initiation, therefore, is mostly made by measuring later stages of flowering or by estimating the extent of preceding vegetative growth.

Several parameters, such as the appearance of visible flower buds or of open flowers, or the number of flowers or flower primordia, or even the developmental condition of a particular flower after a definite experimental period, all have, as LANG (6) mentiones, the same potential error, i.e. they may reflect differences in the developmental rate of the initiated flowers rather than their initiation.

The extent of the preceding vegetative growth can be suitably expressed as the number of leaves or nodes preceding the first flower or inflorescence. The leaf or node index is probably, as LANG (6) states, the most reliable parameter of flower initiation, reflecting differences in the relation between vegetative growth and flower initiation, regardless of environmental effects on developmental rates of later stages of flower development.

For *Hyoscyamus*, several parameters have been used in the past. FINN (4) compared some of them and he found that in his experiments it made no difference whether he used the number of flower primordia or the differences in

shooting date or those in stem length at a certain day. Also DE LINT (9) compared stem length and days to shooting and found a close correspondence.

Thus, in *Hyoscyamus*, stem elongation and flower initiation generally appear together, so that parameters for shooting and flowering may be interchanged. However, in 1967, SEIDLOVA et al. (11) found experimental conditions in which they could have *Hyoscyamus* plants with normal stems, that were entirely vegetative. Moreover, we found that under sterile conditions with sugar feeding it was possible to obtain considerable stem elongation without generative development (unpublished).

In experiments, described in this paper, the following parameters were used: the number of flower primordia on the main flower-stalk, the number of unexpanded leaves and, for comparison with literature, the number of days to shooting which turned out to be the least accurate one. Leaves longer than $2\frac{1}{2}$ cm were called 'expanded leaves' and leaves shorter than or equal to $2\frac{1}{2}$ cm 'unexpanded leaves'. In 'Days to shooting', zero-time marks the beginning of the long day after-treatment, except in fig. 4, where zero-time marks the start of the experiment.

3. MATERIALS AND METHODS

In this investigation, we used the annual, yellow flowering strain *Hyoscyamus niger* var. *pallidus*. Seeds were originally obtained from DR. A. LANG (12). In this laboratory many others (13, 12, 2, 15, 7, 8, 14, 9, 10, 3) have used the same species.

Plants were raised during 3–4 months in the phytotron at 20 °C in a 9-hour day. During this period they were transplanted twice (into larger pots). The light intensity was kept somewhat lower directly after transplanting ($\pm$ 30,000 ergs $cm^{-2}sec^{-1}$) and thereafter raised to 50,000–60,000 ergs $cm^{-2}sec^{-1}$. The light was obtained from fluorescent lamps (PHILIPS TL 33/120 W or TL 33/40 W). During the raising period as well as during the experimental period air humidity was around 65%.

At the start of an experiment, plants were selected on uniformity and some plants were examined on numbers of expanded and unexpanded leaves; the growing points at this moment always were vegetative as expected. Counting of unexpanded leaves and of flower primordia and examination of the growing points was carried out under a binocular microscope. During long-day after-treatment and sometimes during the daylength treatments, plants received a mixed irradiation, consisting of PHILIPS TL 33/120 W fluorescent tubes and incandescent lamps (PHILIPS 75 W/240 V). Short days during the experimental period were always given in fluorescent light only, at an intensity of $\pm$ 60,000 ergs $cm^{-2}sec^{-1}$, the same as during the last part of the raising period.

Light intensities have been measured on the level of the vegetative plants, in ergs $cm^{-2}sec^{-1}$ (1 erg = 10^{-7} W.sec) with a cosine corrected photocell (5). This cell was calibrated for the wavelength combinations used with the aid of a standardized thermopile. The intensities of mixed irradiations were determined by separate measurement of the components.

Abbreviations and terms to be used in this article are:
SD: Short Day(s), 9 hours of light per day;
LD: Long Day(s), 16 hours of light per day;
DD: Day(s) of total Darkness;
unexpanded leaves: leaves shorter than 2½ cm (counted up to the 1st flower);
d.t.: Daylength-treatment(s).

4. Results and discussion

In order to reinvestigate the 'DE LINT-curve', plants of 4 months old were brought into total darkness at the beginning of the experiment. The number of unexpanded leaves was counted in 10 plants. During 6 days, the remaining plants received photoperiods of 0, 0.5, 1.0, 1.5, 3.0, 5.0, 10, 15, 30, 50, 100, 150, 300, 485, 1000 or 1440 mins daily, in fluorescent light only. After the 6 daylength treatments the after-treatment in long days in mixed fluorescent and incandescent light started. The experiments (i.e. the daylength treatments) started 8-2-'67. At 27-2-'67, when the after -treatment had lasted 13 days, all plants were bolting, and the leaves that expanded during the experimental period as well as the unexpanded leaves and the flower primordia were counted. The day of bolting of each individual plant has also been recorded. Averages are given in fig. 3.

The differences between the 0.0 and 0.5 mins treatments and between the 0.5 and 10 mins treatments have, for all 3 parameters, a reliability of 99.9%. In curve 3.3 the difference between the 1 and 10 mins treatment has a reliability of 99%.

It is quite clear that in the region of very short photoperiods our graphs deviate from the one presented by DE LINT (9) (compare fig. 2), in that our shortest photoperiod was 0.5 instead of 3 mins; and the number of observation points in our curve is twice as high. DE LINT's data are confirmed, but his extrapolation to 0 min daylength appears unjustified. From 10 mins down to 0.5 min photoperiods, floral inhibition increases again to a second peak, the exact position of which is determined in a subsequent experiment, presented in this paper.

Six days of darkness, however, is still a rather long period, and at the end of this period plants usually are very weak. In order to avoid this as much as possible we interrupted a sequence of very short photoperiods with pairs of short days (9 hrs). In a preliminary experiment we combined: *1.*: 2 DD with 2 SD; *2.*: 2 SD with 2 LD; *3.*: 2 LD with 2 DD; *4.*: 2 DD with 2 LD; *5.*: continuous LD (cf. legend table 1). The SD-control remained vegetative till the end of the experiment, and the continuous dark control died, still being vegetative, far before the end of the experiment. These 2 treatments will not be discussed. Treatments *3* and *4* differ in that *3* started with 2 LD and *4* with 2 DD. The experiment started 8-5-'67. SD as well as LD have been given in fluorescent light (PHILIPS TL 33/120 W), both at an intensity of 60,000–65,000 ergs $cm^{-2}sec^{-1}$. Each combination was observed for shooting, data of which are given in table 1 and in fig. 4. Representative plants, photographed 38 days after the start of the experiment are shown on plate 1.

At the end of the experiment, all plants of the above 5 groups had developed

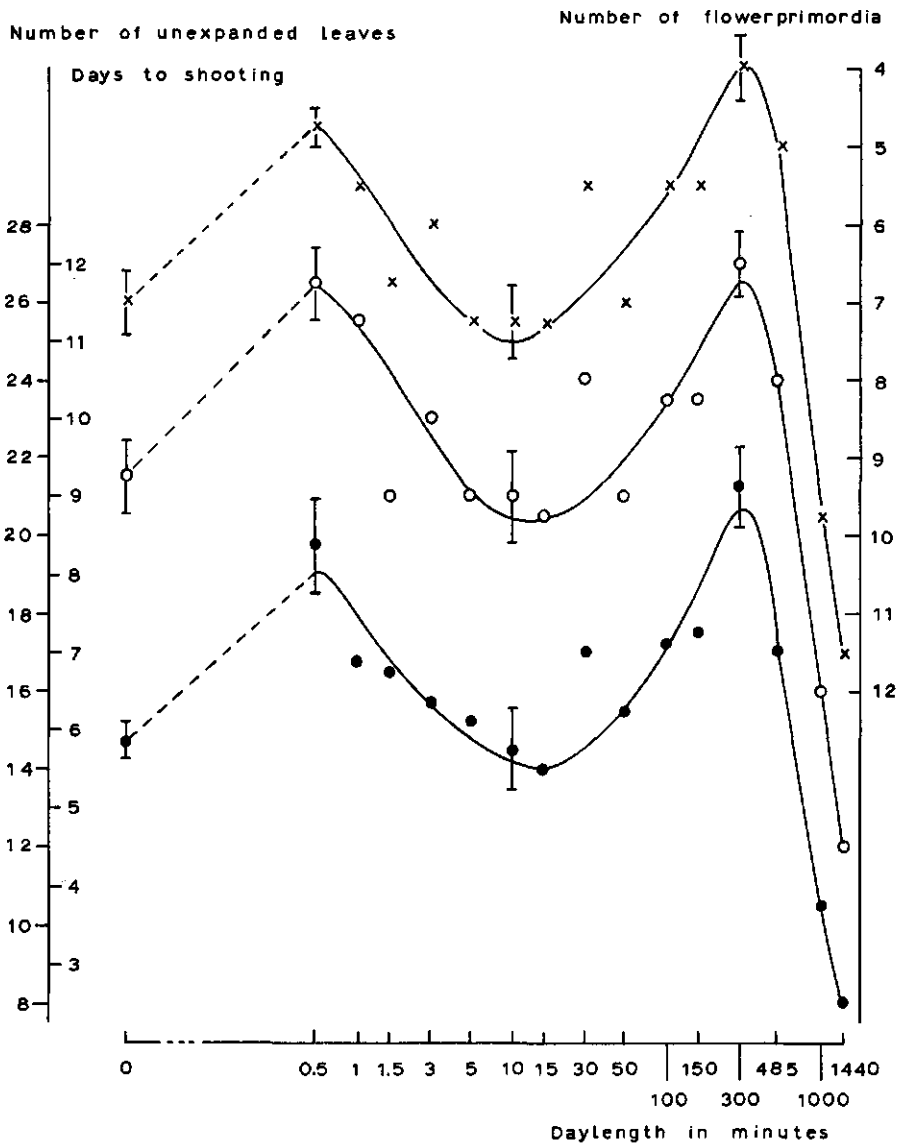


FIG. 3. Daylength sensitivity of: 1. number of flowerprimordia (×), 2. days to shooting from beginning of after-treatment (○), 3. number of unexpanded leaves below the 1st flower (31.4± 0.9 at the start of the experiment) (●) in *Hyoscyamus*. Daylength treatment in fluorescent light (± 21,000 ergs $cm^{-2}sec^{-1}$) during 6 days, followed by 13 long days in mixed irradiation of fluorescent and incandescent lamps (total irradiation < 700 nm.: ± 22,500 ergs $cm^{-2}sec^{-1}$ and between 700 and 1000 nm.: ± 12,500 ergs cm^{-2} sec^{-1}). Experiment started 8-2-'67. Counting of flowerprimordia and of unexpanded leaves at the end of the long-day after treatment, on 27-2-'67. Averages of 4 plants. Standard deviations are indicated with bars.

TABLE 1. Dependence of shooting of *Hyoscyamus* upon repeated treatment of 2 SD or 2 LD alternated with 2 DD or 2 LD. Light intensity in LD and SD: 60,000–65,000 ergs $cm^{-2}sec^{-1}$. Treatment started 8-5-'67 and ended for groups *2–5* after 38 days, for group *1* after 73 days. Averages of 5 plants.

group	treatment	days to shooting	
		total	split up over the two treatments
1.	(2 DD – 2 SD)m ×	58,8	30,0 DD + 28,8 SD
2.	(2 SD – 2 LD)n ×	25,6	12,0 LD + 13,6 SD
3.	(2 LD – 2 DD)n ×	16.6	8,6 LD + 8,0 DD
4.	(2 DD – 2 LD)n ×	17,4	8,2 LD + 9,2 DD
5.	(2 LD – 2 LD)n ×	15,4	15,4 LD

flower buds and the groups *2–5* even had open flowers.

From this experiment it is clear that DD in combination with LD inhibit shooting less than SD in combination with LD, compared with continuous LD. Thus, DD in combination with SD can cause shooting and flower initiation, a treatment fully without the regularly promotive long day. This finding agrees with the curve of DE LINT (fig. 2); also here DD are less inhibitive for shooting and for flowering than are normal SD.

Therefore, it seems justified to further examine the daylength sensitivity, in particular with respect to very short daylengths, by interrupting the daylength-treatments with pairs of short days, several times during each experiment. In order to also bring into the generative state the plants that had received the most inhibitive treatment, all plants were given a LD-after-treatment.

The schedule of treatment for the following experiment was: (2 SD–3 d.t.) 4 times, with an after-treatment of 10 LD. The SD consisted of 495 mins of fluorescent light. The d.t. consisted of photoperiods of: 0, 0.1, 0.22, 0.5, 1.0,

FIG. 4. Days to shooting of *Hyoscyamus* upon repeated treatment of 2 SD or 2 LD alternated with 2 DD or 2 LD. See also table 1.

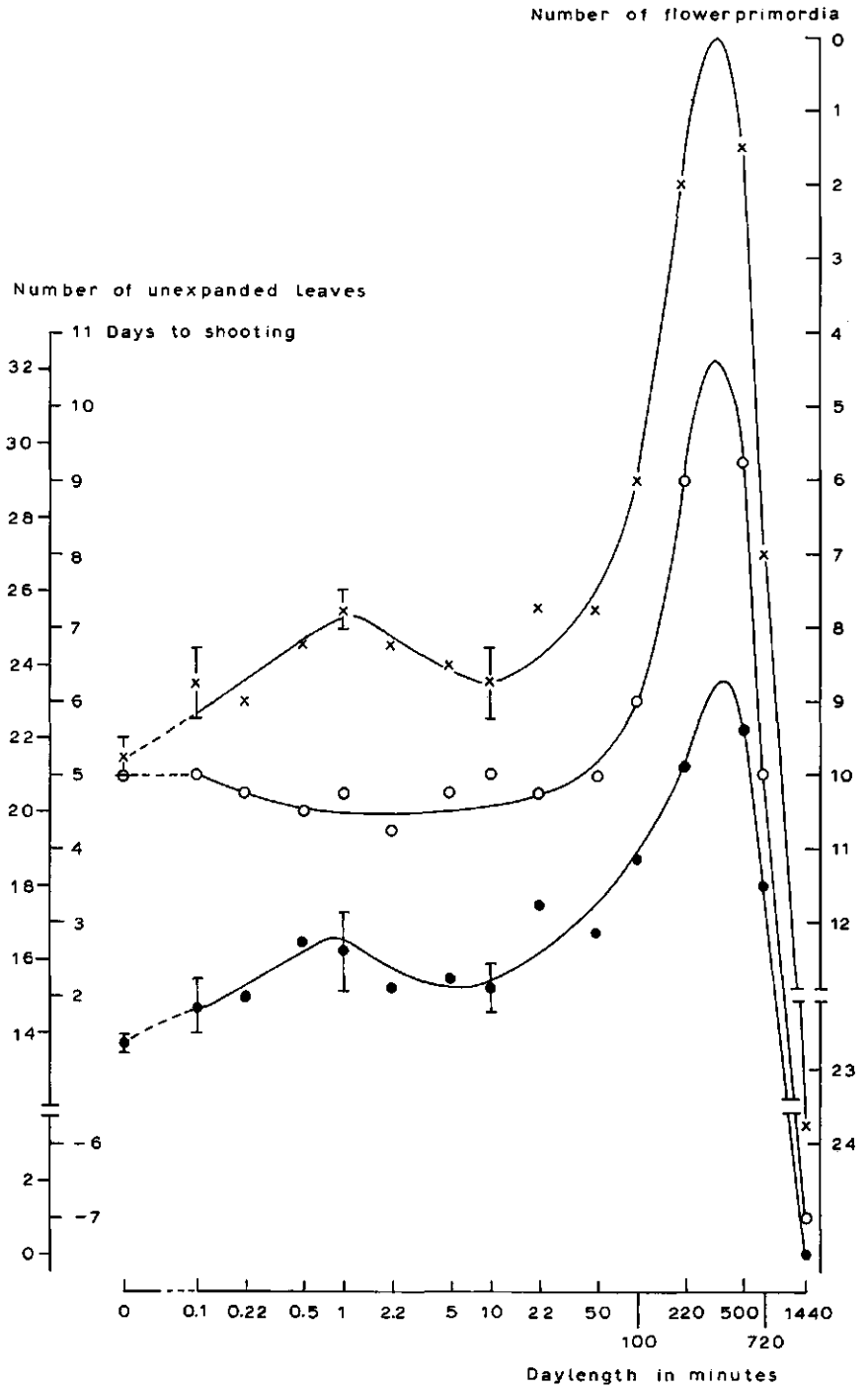
PLATE 1. Repeated treatment of *Hyoscyamus* with combinations of: *1.* 2 DD with 2 SD; *2.* 2 SD with 2 LD; *3.* 2 LD with 2 DD; *4.* 2 DD with 2 LD; *5.* 2 LD with 2 LD. See Fig. 4. Photographed 15-6-'67, after 38 days.

2.2, 5.0, 10, 22, 50, 100, 220, 500, 720, or 1440 mins. The d.t. as well as the LD-after-treatment was given as a mixture of fluorescent and incandescent light. During the experiment, we have recorded the data of visible shooting and at the end of the after-treatment, when all of the plants were shooting, the number of flowers and flower primordia, and the number of unexpanded leaves below the 1st flower at each plant. Data are given in fig. 5. The negative values in 'Days to shooting' in fig. 5 mean that those plants started shooting before the after treatment began.

With all 3 parameters we find a clear inhibition maximum at $\pm$ 300 mins photoperiods, just like DE LINT (fig. 2) found. But again, as in the first experiment, now with only 2 of the parameters – those for flower primordia and for unexpanded leaves – a second maximum somewhere between 0.5 and 1 min. photoperiod was found. It is, however, this time considerably lower than the one at 300 mins.

The graph for 'Days to shooting' does not show this second maximum, which may be due to some incongruencies in the judgement of shooting. This may show the need for a more objective parameter, like the number of flower primordia or the number of unexpanded leaves.

In general, these results confirm those of DE LINT (9). However, in the region of the very short daylengths, the present graphs show an unexpected extra, viz. a second, smaller maximum. An explanation of this second maximum of inhibition of flowering cannot yet be given, but is seems that the interpretation of daylength-dependence, given by DE LINT (9) does not explain this phenomenon. Further research on this subject is in progress.


Number of flowerprimordia
Number of unexpanded leaves
11 Days to shooting
32
30
28
26
24
22
20
18
16
14
2
0
10
9
8
7
6
5
4
3
2
-6
-7
0
1
2
3
4
5
6
7
8
9
10
11
12
23
24
0
0.1
0.22
0.5
1
2.2
5
10
22
50
100
220
500
720
1440
Daylength in minutes

5. Summary

Daylength-dependence of flowering in *Hyoscyamus niger* has been examined by exposing the plants during a period of several days to different (e.g. very brief) daylength-exposures, which may be interrupted by short days in order to keep the plants in better condition, so that more daylength-treatments with very short photoperiods could be given than without these interruptions.

In general, we could confirm the daylength-response curves for flowering found by DE LINT (9) in 1960. Inhibition of flowering decreased with photoperiods shortening below ± 300 mins. However, besides this, in the daylength-region below 10 mins a second maximum for flower inhibition is found, which seems hard to combine with the hypothesis for flowering presented by DE LINT (9). An alternative explanation for the findings presented here could not yet be given.

6. Acknowledgements

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FIG. 5. Daylength sensitivity of: 1. number of flowerprimordia (×), 2. days to shooting from beginning of after-treatment (○), 3. number of unexpanded leaves below the 1st flower (26.4 ± 1.2 at the start of the experiment) (●), in *Hyoscyamus*. Daylength treatments alternating with short days: (2 SD–3 d.t.) 4 ×, followed by 10 long days. D.t. and LD were given as mixed irradiation of fluorescent and incandescent lamps (total irradiation < 700 nm.: ± 24,000 ergs $cm^{-2}\,sec^{-1}$ and between 700 and 1000 nm.: ± 7400 ergs $cm^{-2}sec^{-1}$); SD has been given in fluorescent light only (± 52,000 ergs $cm^{-2}sec^{-1}$). Experiment started 14-5-'68. Counting of flowerprimordia and unexpanded leaves at the end of the long-day after-treatment. Averages of 4 plants. Standard deviations are indicated with bars.

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