

The Flower Inducing Mechanism of *Silene armeria* L.

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN
DE LANDBOUWWETENSCHAPPEN OP GEZAG VAN DE
RECTOR MAGNIFICUS, MR. J. M. POLAK, HOGLERAAR
IN DE RECHTS- EN STAATSWETENSCHAPPEN VAN DE
WESTERSE GEBIEDEN, TE VERDEDIGEN TEGEN DE
BEDENKINGEN VAN EEN COMMISSIE UIT DE SENAAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG 5 FEBRUARI 1971 TE 16.00 UUR

JAN VAN DE VOOREN

1970

LABORATORIUM VOOR TUINBOUWPLANTENTEELT,
LANDBOUWHOGESCHOOL, WAGENINGEN, NEDERLAND
Publikatie 347

CONTENTS

	Abbreviations	6
1	Summary	7
2	Samenvatting: Het mechanisme van de bloei-inductie in <i>Silene armeria</i> L.	9
3	Introduction	11
4	Materials and Methods	14
4.1	Plant material	14
4.2	Equipment	14
4.3	Measurement of flowering	14
4.3.1	General	14
4.3.2	<i>Silene armeria</i>	15
5	Experimental	17
5.1	Flowering habits	17
5.2	Sensitive period for 32° C	18
5.3	Photoperiodic response curves	18
5.4	Introduction to experiments on light	20
5.5	Light intensity during SD _{20°}	20
5.6	Light intensity during CL _{20°}	21
5.7	Different photoperiods followed by 32° SD	22
6	The mechanism of flower induction	24
6.1	High temperature	24
6.2	Light	25
6.3	Hypothesis	25
6.4	Models	25
6.4.1	LD _{20°} – high intensity light	26
6.4.2	LD _{20°} – high intensity + supplementary light	27
6.4.3	32° SD	27
6.5	Photoperiodic response curves	27
6.6	Discussion	28
7	Literature	29

Abbreviations

SD	Short day, 8 h light and 16 h darkness.
SD _{20°}	SD at about 20° C.
32° SD	SD at 32° C.
5° SD	SD at 5° C.
LD	Long day, 16 h light and 8 h darkness.
LD _{20°}	LD at about 20° C.
CL	Continuous light, 24 h light.
CL _{20°}	CL at about 20° C.
GA ₃	Gibberellic acid.
E ₁	Early strain 1 of <i>Silene armeria</i> .
L ₁	Late strain 1 of <i>Silene armeria</i> .
I	VOOREN, J. VAN DE (1969a)
II	VOOREN, J. VAN DE (1969b)
III	VOOREN, J. VAN DE (1971a)
IV	VOOREN, J. VAN DE (1971b)

1 SUMMARY

Investigations on the flower induction were carried out with *Silene armeria* L., a plant species flowering under all conditions after a treatment at LD, 5° C or 32° C. Two strains E₁ and L₁, differing in their photoperiodic and temperature requirements, were used in the present research, which completes former investigations (VAN DE VOOREN 1969a, b, 1971a, b).

High temperature

Plants under short day conditions at 32° C are induced to flower bud formation. A quantitative juvenility for 32° SD, interaction between 32° SD and LD_{20°}, partial induction by 32° SD and desinduction of this partial induction in SD_{20°} exist in both strains, but E₁ is more sensitive than L₁.

High temperature action on flower induction is maximal during the middle of a 16 h dark period for both E₁ and L₁. Maximal action in E₁ is more precisely 7 h after the onset of darkness, independent on the length of the dark period for 8–16 h photoperiods. There is no action for 20 and 24 h photoperiod.

No influence of temperatures on flower induction during SD is apparent in the range 10°–30° C and an increasing response in the range 35°–50° C.

Light

Light intensity is an important factor in flower induction. Increasing light intensities during SD or CL increase the flowering response. The optimal light intensity during CL is rather low in E₁ and has not been reached in L₁ in our experiments.

Photoperiodic response curves at 20° and 32° C for E₁ and L₁

For photoperiods from 8 to 18 h more numbers of cycles necessary for 50% flowering are needed for 20° C than for 32° C. The curves run parallel for both E₁ and L₁ at photoperiods from 18 to 24 h. In this range E₁ needs an equal number and L₁ decreasing numbers of cycles.

Hypothesis

The mechanism of flower induction is a balance between two processes:

1. A deblocking process in high intensity light, increasing light intensities accelerating it with an optimum, temperature insensitive.

2. A reblocking process in darkness, starting about 4 h after the onset of darkness' reaching its maximum after 6–7 h and decreasing for about 5 h, temperature sensitive, temperatures higher than 30°–35° C slowing it down increasingly. Incandescent light postpones the onset of darkness and hence the reblocking.

When these 2 processes are out of balance and more deblocking than reblocking occurs, a critical level or disappearance of the block is reached and flower induction is completed.

Models

Models, based on the foregoing hypothesis, are presented for induction by LD₂₀° – high intensity light, LD₂₀° – high + low intensity incandescent light, 32° SD. These models fit well and can be used to predict time of flowering under specific conditions.

2 SAMENVATTING

HET MECHANISME VAN DE BLOEI-INDUCTIE IN *SILENE ARMERIA L.*

Onderzoek op het gebied van bloei-inductie werd verricht met *Silene armeria L.*, een plant die onder iedere conditie bloeit na een behandeling bij LD, 5° C of 32° C. In dit onderzoek, dat een afsluiting vormt van vroegere publikaties (VAN DE VOOREN, 1969a, b, 1971a, b), worden de lijnen E_1 en L_1 , welke in hun fotoperiodieke en temperatuur-behoefte verschillen, gebruikt.

Hoge temperatuur

Planten worden geïnduceerd tot bloemknopvorming bij 32° C onder korte-dag condities. Een kwantitatieve jeugdfase voor 32° SD, interactie tussen 32° SD en LD_{20°}, partiële inductie door 32° SD en desinductie in SD_{20°} van deze partiële inductie komt in beide lijnen voor. E_1 is echter gevoeliger dan L_1 .

De werking van hoge temperatuur op de bloei-inductie is maximaal gedurende het midden van een 16 uur donkere periode voor zowel E_1 als L_1 . De maximale werking voor E_1 is nauwkeuriger vastgesteld op 7 uur na het begin van de donkere periode voor fotoperiodes van 8 tot 16 uur, terwijl er geen werking is voor 20 en 24 uur fotoperiode.

Temperaturen van 10° tot 30° C hebben geen invloed op de bloei-inductie gedurende SD_{20°}. Temperaturen van 35° tot 50° C hebben een toenemende invloed.

Licht

Een belangrijke factor bij bloei-inductie is de lichtintensiteit. Toenemende lichtintensiteiten gedurende SD of CL hebben een toenemende invloed op de bloei-inductie. De optimale lichtintensiteit gedurende CL is betrekkelijk laag in E_1 en wordt in onze proeven niet bereikt voor L_1 .

Fotoperiodieke responscurves bij 20° en 32° C voor E_1 en L_1

Het aantal cycli, benodigd voor 50% bloei, is hoger voor 20° dan voor 32° C voor fotoperiodes van 8 tot 18 uur. De curves lopen parallel voor fotoperiodes van 18 tot 24 uur bij zowel E_1 als L_1 . Het aantal cycli blijft gelijk voor E_1 , terwijl L_1 een afnemend aantal cycli nodig heeft.

Hypothese

Het mechanisme van bloei-inductie wordt voorgesteld als een evenwicht tussen twee processen:

1. Een ontremmend proces in licht van hoge intensiteit; toenemende lichtintensiteiten met een optimum bevorderen dit proces; ongevoelig voor temperatuur.
2. Een herremmend proces in het donker, dat ongeveer 4 uur na het begin van het donker inzet, een maximum bereikt na 6 à 7 uur en gedurende 5 uur afneemt; een temperatuur hoger dan 30°–35° C remt dit proces af. Gloeilamplicht stelt het begin van het donker en dus de herremming uit.

Als deze twee processen uit balans zijn en er meer ontremming dan herremming plaats vindt, zal een kritiek niveau bereikt worden, waarbij bloei-inductie voltooid is.

Modellen

Modellen werden ontwikkeld voor de inductie door LD_{20°} – licht van hoge intensiteit, LD_{20°} – licht van hoge intensiteit en aanvullend gloeilamplicht, 32° SD. Met deze modellen zal het mogelijk zijn de tijd van bloei-inductie te voorspellen voor bepaalde condities.

3 INTRODUCTION

Several plant species need a specific treatment for flower bud formation. Some time after this treatment the flower buds are formed, hence the treatment is called inductive. The induction of flowering usually takes place in the leaves. The realization of this induction is observed at the apex of the plant as a flower bud.

In this paper induction is the exclusive object of research. However, as this induction becomes visible in its realization, the measurement of flowering is of great importance. *Silene armeria* L., family *Caryophyllaceae*, is a very suitable plant for this research, since it is a qualitative long day plant at "ordinary" temperatures and no reversion of generative plants to the vegetative state occurs. For a literature survey see WELLENSIEK (1969a).

Temperatures above 30° C are a very important inductive factor under SD conditions, as was found by LIVERMAN (1952) and WELLENSIEK (1966a), who established that the high temperature action is restricted to the dark phase. Further details were worked out by the present author in four papers which will be referred to as I, II, III, IV:

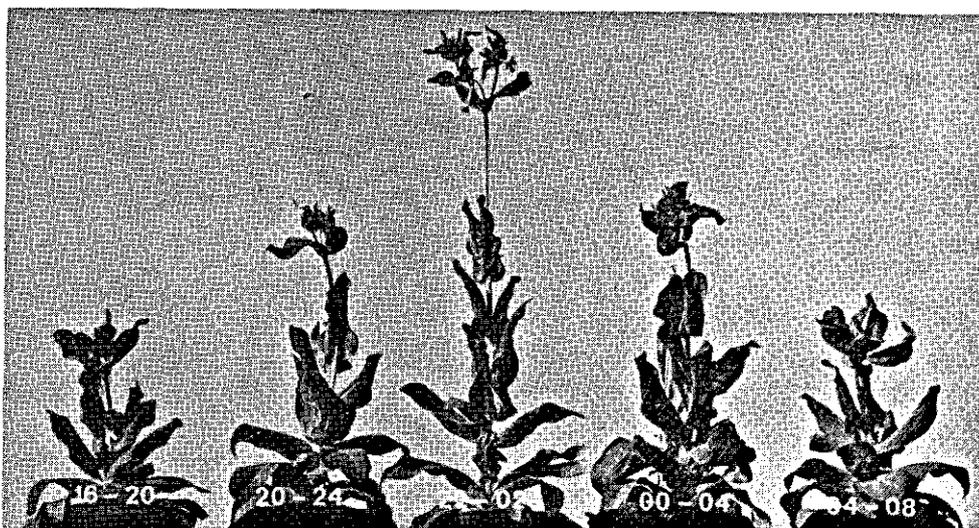


Fig. 1. Intermittent temperature treatment for E_1 during 7 cycles. The temperature during different sub-periods of a 16 h dark period (16–08 h) was 32° C and the temperature during the rest of the dark period and the 8 h light period (08–16 h) was 20° C. After-treatment SD_{20}° . Photograph 30 days after 32° C treatment.

I. The influence of high temperature on the flower inducing mechanism of *Silene armeria* L. I. Intermittent temperatures (VAN DE VOOREN 1969a). High temperature has its effect on flower induction in E_1 only during the middle of a 16 h dark period (figure 1). This high temperature treatment acts quantitatively and not qualitatively, as was concluded from treatments of different lengths during this particular sub-period of the dark period. About 15 cycles SD with 4 h at 32° C during the middle of the dark period were needed to get an optimal induction.

II. The influence of high temperature on the flower inducing mechanism of *Silene armeria* L. II. Comparison of an early and a late strain (VAN DE VOOREN 1969b). An early strain (E_1) flowers after an inductive treatment of about 10 cycles 32° SD, whereas a late one (L_1) flowers after 22 cycles 32° SD only for two third (figures 2 and 3). E_1 and L_1 have a quantitative juvenility for 32° SD, which may last for E_1 ap-

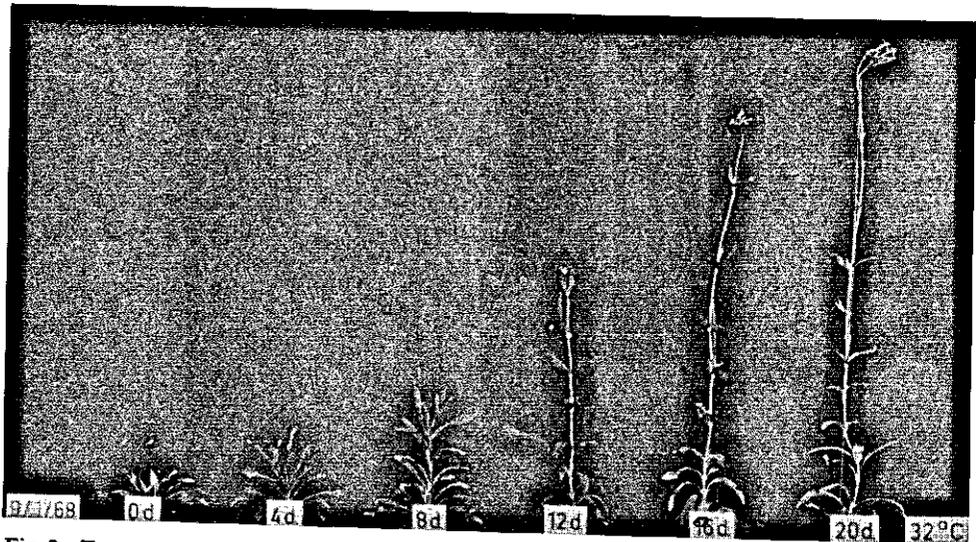


Fig. 2. Temperature treatment for E_1 : 0, 4, 8, 12, 16 or 20 cycles 32° SD. After-treatment SD_{20}° . Photograph 35 days after 32° SD treatment.

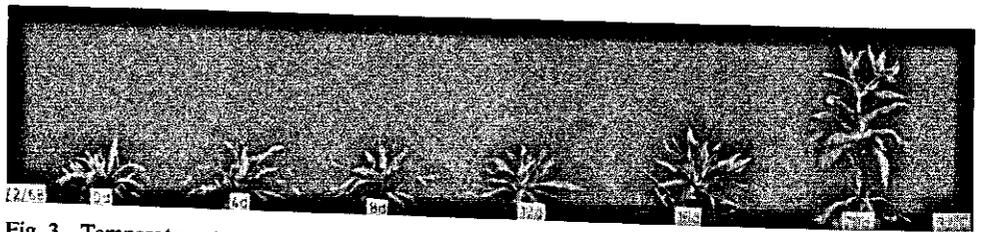


Fig. 3. Temperature treatment for L_1 : 0, 4, 8, 12, 16 or 20 cycles 32° SD. After-treatment SD_{20}° . Photograph 50 days after 32° SD treatment.

proximately 8 weeks and for L_1 1 week more. Interaction of 32° SD and LD_{20} exists in both strains. A partial induction by a suboptimal 32° SD treatment disappears after 12 and 21 days of SD_{20} for E_1 and L_1 respectively.

III. The influence of high temperature on the flower inducing mechanism in *Silene armeria* L. III. Relation to photoperiods (VAN DE VOOREN 1971a). A critical photoperiod was found only for 20° C in the photoperiodic response curves at 20° C and 32° C for E_1 . At 20–24 h photoperiods both curves are identical. The sensitivity of the flower induction for high temperatures was more precisely determined for photoperiods of 8 till 16 h. An increasing sensitivity was found some hours after the onset of darkness, reaching a maximum after 6–7 h and decreasing afterwards for about 5 h. For photoperiods of 20–24 h no sensitive period was found, as could be expected from the foregoing.

IV. The influence of high temperature on the flower inducing mechanism of *Silene armeria* L. IV. Temperature level (VAN DE VOOREN 1971b). In the temperature range 10° – 30° C no influence on flower induction was found for E_1 during the sensitive sub-period of the dark period of a SD cycle. An increasing response is apparent in the range 35° – 50° C, hence a critical temperature exists between 30° and 35° C.

From these studies a hypothesis on the mechanism of flower induction originated. In the present paper additional experimental results will be presented, followed by an elaboration of this hypothesis.

4 MATERIALS AND METHODS

4.1 Plant material

Strains E₁ and L₁ of *Silene armeria* have been used exclusively in the present research. They were selected by WELLENSIEK (1969b) from the original unselected material supplied by ANTON LANG and differ in their photoperiodic and temperature requirements for flower induction (WELLENSIEK 1969b, II).

The habits of E₁ and L₁ are quite different in SD_{20°}. E₁ has bright green, broad leaves with wavy margins. L₁ has greyish green, narrow leaves with straight margins. A small percentage generative plants in E₁ is found in continuous SD_{20°}. This percentage "day neutral" plants is highest in winter.

4.2 Equipment

Glasshouse for SD_{20°}, LD_{20°}, CL_{20°} and growth cabinets for the proper treatments have been described earlier (I, II, III, IV).

Light intensities have been measured with a cosine corrected Selenium photo cell for visible radiation (0.4–0.7 μ) at plant level.

Temperatures have been measured with copper/constantan thermocouples.

4.3 Measurement of flowering

4.3.1 General

Many criteria are used as a measurement of the response to a specific treatment, according to the peculiarities of the specific plant and the problem under discussion. A discussion on the measurement of flowering is given by HILLMAN (1964) and LANG (1965).

Some time after an optimal flower induction the first sign of differentiation of the flower bud becomes visible microscopically, followed by a macroscopical flower bud and open flower. The rate of differentiation depends on the preceding treatment and the prevailing environment.

It is obvious that realization or in other words the change of the apex from vegetative to generative is necessary to evaluate factors acting on induction. The stages of differentiation at a certain time and the time to attain a specific stage can be used as criteria.

Microscopical examination of the apex after a certain number of days presents stages of differentiation and sizes of buds, hence a quantitative measurement of the inductive effect of a treatment. Advantages of this scoring system are the rapid result and the minor dependence on realization, disadvantages are the subjective approach and the labour consuming microscopical dissection. Most of the research on flower induction in *Xanthium strumarium* is based on this criterion (SALISBURY 1963a).

Number of days till a specific stage of differentiation, being the macroscopical appearance of the flower bud and open flower, also presents a quantitative measurement, highly dependent on realization. This number of days turned out to be a good criterion for *Perilla* (ZEEVAART 1969). Number of days till shooting, mostly accompanying flowering in LD plants, is used for *Hyoscyamus niger* (DE LINT 1960) as criterion. However, stem elongation without flowering may occur in several plants, e.g. *Hyoscyamus niger* (LANG et al. 1943, SEIDLOVA et al. 1967) and *Rudbeckia bicolor* (MURNEEK 1940). Stem elongation was discussed by WELLENSIEK (1960) and DE LINT (1960).

Disadvantages of the scoring system and the number of days are their dependence on time, hence they also reflect differences in the rate of realization of the flower, caused by the environment during this realization. This is not the case with the next criteria.

The leaf index, number of leaves preceding the first flower, gives a quantitative measurement independent on time. This criterion was used in the classical work on vernalization of *Secale cereale* (PURVIS et al. 1937) and on photoperiodism of *Hyoscyamus niger* (LANG et al. 1943) and *Lolium temulentum* (EVANS 1969).

The flowering-percentage gives a black and white, hence qualitative effect independent on time. For *Lemna perpusilla* it is even the exclusive criterion used (HILLMAN 1959). Difficulties may be presented by transitional inflorescences, intermediate forms between the vegetative and generative state, occurring under marginal conditions in many plants (LANG 1965).

The flowering of a plant is accompanied by changes of its habit. Criteria based on the change of the habit are: stem length in *Impatiens balsamina* (NANDA et al. 1967), branching of the inflorescence in *Kalanchoë blossfeldiana* (HARDER et al. 1940), number of flowers or flowering nodes in Biloxy soybean (HAMNER 1969), *Kalanchoë blossfeldiana* (HARDER 1948) and *Pharbitis nil* (TAKIMOTO 1969). It is clear that these phenomena are secondary and influenced by the environment to a high degree.

4.3.2 *Silene armeria*

As the induction of flowering of *Silene armeria* is the topic of the present research, a criterion as independent from realization as possible has to be used.

The percentage generative plants is evaluated in SD_{20} following an inductive treatment. LD_{20} cannot be used since then always 100% flowering occurs. This criterion is suitable for light intensity and high temperature treatments, as it is independent on time. The duration of the treatments has to be marginal to get differences

in percentages generative plants. The differences over a range of treatments reflect the genetical impurity of the material and the intensity of the treatment. The average, being 50% flowering, gives a very useful value (III). No change from the vegetative to the generative state has been observed any more later than 2 months after a photo-periodic or high temperature treatment.

A suboptimal induction by a treatment is detected only by means of interaction. Since the response to $CL_{20^{\circ}}$ is highly predictable, it is useful as a second treatment. Suboptimal induction is also detected in $LD_{20^{\circ}}$ by the number of days till the appearance of the flower bud. A refinement of this method is a second treatment of an optimal number of cycles $CL_{20^{\circ}}$ and realization in $SD_{20^{\circ}}$. Good controls are in any case necessary.

Leaf indices were used by TAKIMOTO (1961). No reliable results were obtained by the present author.

Microscopical stages in differentiation were described by TAKIMOTO (1955) and WELLENSIEK et al. (1967). The earlier described disadvantages and the impossibility to detect late flowering make this method unsuited for the present research.

Stem elongation and number of days till open flower are influenced too much by time. No differences were found in the number of flowers in the inflorescence.

5 EXPERIMENTAL

5.1 Flowering habits

The flowering habits of E_1 and L_1 in SD_{20° after an inductive treatment were found to be different, as is demonstrated in the following experiment.

Groups of 15 plants of E_1 and L_1 received 0, 1, 2, 3, 4, 5 cycles CL_{20° at $8000 \text{ erg cm}^{-2} \text{ sec}^{-1}$, obtained from 40 W 55 fluorescent Philips lamps + 120 W Philinea lamps. The results in the subsequent SD_{20° are presented in table 1.

Table 1

Vegetative stem length in cm, percentage generative plants, days from start of the treatment till budding and stem length in cm, days till flowering and stem length in cm, for E_1 and L_1 after 0, 1, 2, 3, 4, 5 cycles CL_{20° .

			cycles CL_{20°					
			0	1	2	3	4	5
vegetative	cm	E_1	3.0	4.2	9.6	—	—	—
		L_1	2.8	2.7	2.8	2.5	—	—
generative	%	E_1	0	0	86	100	100	100
		L_1	0	0	0	93	100	100
budding	days	E_1	—	—	23.8	19.9	19.2	19.5
		L_1	—	—	—	25.9	22.3	20.4
	cm	E_1	—	—	9.5	11.5	16.1	17.6
		L_1	—	—	—	7.0	7.5	9.5
flowering	days	E_1	—	—	38.2	33.5	31.0	28.7
		L_1	—	—	—	43.9	41.2	38.8
	cm	E_1	—	—	21.3	27.4	32.7	32.1
		L_1	—	—	—	18.1	23.1	28.1

The stem of vegetative plants of E_1 , measured 100 days after the start of the treatment, is elongated, whereas the stem of L_1 does not elongate. The percentage generative plants after 2 cycles CL_{20° is much higher for E_1 than for L_1 . Days till visible flower bud ("budding") and open flower ("flowering"), counted from the start of the experiment, are less for E_1 than for L_1 . Stem length at the time of budding and flowering is greater for plants of E_1 than of L_1 .

The realization of flowers in SD_{20° for L_1 is influenced by the low light intensities of winter. After marginal inductive treatments the branching of the inflorescence is normal. However, in the inflorescence leaves and vegetative flowers develop. In spring

such plants develop normal flowers. At marginal conditions vegetative inflorescences are a general phenomenon in photoperiodically sensitive plants. In *Kalanchoë blossfeldiana* it is even a measurement for the inductive treatment (HARDER et al. 1940).

5.2 Sensitive period for 32° C

The most sensitive period for high temperature in E_1 was found to be the 5th–9th h after the onset of darkness. In the next experiment E_1 and L_1 will be compared for this topic.

Groups of 20 plants of E_1 and L_1 received 4 h of 32° C during different sub-periods of a 16 h dark period of a SD cycle. Temperature during the rest of the dark period, the 8 h light period and the SD control was 20° C. Light was obtained from 40 W 55 Philips fluorescent lamps + 60 W Philips incandescent bulbs, giving at plant level 5000 erg cm⁻² sec⁻¹. The plants received 7 cycles of this treatment. As a second treatment E_1 and L_1 received 1 and 2 cycles CL_{20° respectively. The percentages generative plants in the subsequent SD_{20° are presented in table 2.

Table 2
Percentages generative plants in E_1 and L_1 after 4 h 32° C treatment during different sub-periods of a 16 h dark period. Temperature during the rest of the dark period, the 8 h light period and the SD control was 20° C. Second treatment of 1 cycle (E_1) and 2 cycles (L_1) CL_{20° .
After-treatment in SD_{20° .

sub-period	E_1	L_1
control	15	33
0– 4	0	24
2– 6	0	40
4– 8	60	47
5– 9	60	56
6–10	20	39
8–12	0	33
10–14	11	26
12–16	0	22

Large differences in percentages are found for E_1 , whereas the differences for L_1 are much smaller. However, in both strains high temperature is most effective during the 4th–9th h after the onset of darkness.

5.3 Photoperiodic response curves

Photoperiodic response curves were constructed by Liverman for the unselected material at about 20° C (LANG 1965) and in III for E_1 at 20° and 32° C. The 20° curve agrees with response curves for most LD plants (BEST 1960). Curves for both E_1 and L_1 at 20° and 32° C were constructed in the same way as in III.

Groups of 15 plants of E_1 and L_1 received marginal numbers of cycles of different

photoperiods at 20° or 32° C. Light was obtained from 40 W 55 Philips fluorescent lamps + 60 W Philips incandescent bulbs, giving at plant level approximately 8000 erg cm⁻² sec⁻¹. From the percentages generative plants in the subsequent SD₂₀ the number of cycles necessary for 50% generative plants was calculated. These numbers are used to construct the photoperiodic response curves in figure 4.

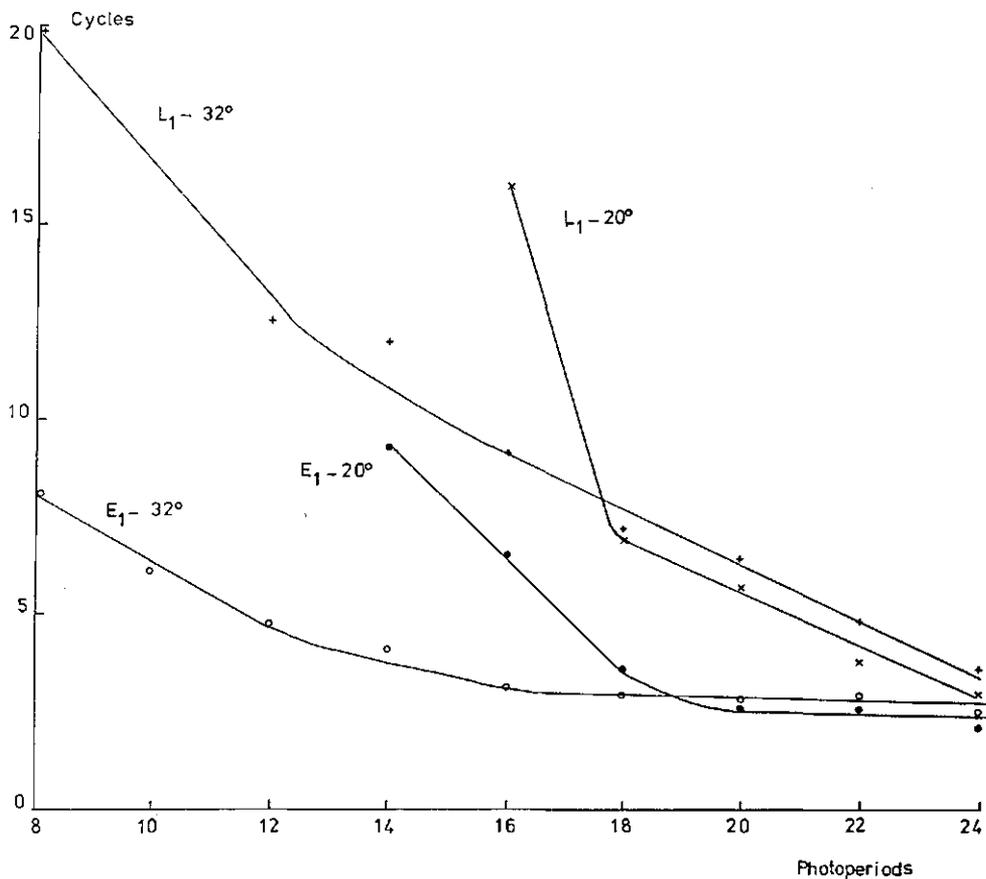


Fig. 4. Photoperiodic response curves for strain E₁ and L₁ at 20° and 32° C. The numbers of cycles required for 50% flowering (ordinate) for different photoperiods (abscissa).

The E₁-20° curve shows a strong decrease in number of cycles for 14–18 h photoperiods and an equal number for 20–24 h. The E₁-32° curve shows a slow decrease in number of cycles for 8–16 h photoperiods and an equal number for 16–24 h. The curves for E₁ cross each other at about 19 h and run parallel at longer photoperiods.

The L₁-20° curve shows a rapid decrease in number of cycles for 16–18 h photoperiods and a slow decrease for 18–24 h. The L₁-32° curve shows a decrease in number

of cycles for the whole range of photoperiods studied. The curves for L_1 cross each other at 18 h photoperiod and run parallel at longer photoperiods.

The decrease in number of cycles for increasing photoperiods in the range 8–18 h is larger at 20° than at 32° C in both strains. The number of cycles for photoperiods in the range 18–24 h is equal at 20° and 32° C in both strains, for E_1 at the same and for L_1 at a slowly decreasing level.

These results will be discussed in more detail in chapter 6.5.

5.4 Introduction to experiments on light

Light intensity and light quality are important factors in flower induction in LD plants (BONNER et al. 1953). Increasing light intensities during 7 cycles CL resulted in an increasing flowering percentage (LIVERMAN et al. 1956) and during a 16 h light period in a decreasing number of days till visible flower bud (WELLENSIEK 1969a) in unselected *Silene armeria*.

Continuous fluorescent light was ineffective for flower induction, but continuous incandescent light was very effective (TAKIMOTO 1957, WELLENSIEK 1964). The flower induction by incandescent light is most probably caused by the far red part of the light, since continuous far red irradiation caused optimal flower induction (TAKIMOTO 1961). Supplementary incandescent light is most effective during the last part of the light period (TAKIMOTO 1957).

For LD plants BONNER et al. (1953) discussed two light processes: a high intensity light process, which would provide photosynthates and a low intensity light process, at a low total energy need with an action spectrum similar to that for the inhibition of flowering in SD plants. TAKIMOTO (1957) concluded from his experiments with *Silene armeria* that the first process requires high intensity light for photosynthesis and that the second, depending on far red, is directly flower promoting. These processes are considered to be different from the low intensity light process, which destroys the dark inhibition.

5.5 Light intensity during SD_{20°

The influence of light intensity during the 8 h light period of a SD will be investigated at a 4 h high temperature induction.

Groups of 15 plants of E_1 and L_1 received 7 cycles of 8 h light at 4 light intensities and 16 h darkness at 20° C, except for the 5th–9th h of the dark period when the temperature was 40° C. Light was obtained from 40 W 55 Philips fluorescent lamps, giving at plant level approximately 1.5, 7.5, 15 or $22.5 \times 1000 \text{ erg cm}^{-2} \text{ sec}^{-1}$. A treatment of 1 cycle CL_{20° was added after the proper treatment. The percentages generative plants and days till budding in the subsequent SD_{20° are presented in table 3.

Table 3

Percentages generative plants and days till budding in E_1 and L_1 after 7 cycles of 8 h light at 4 light intensities and 16 h darkness at 20° C, except for the 5th–9th h of the dark period when the temperature was 40° C. Second treatment of 1 cycle CL_{20}° and after-treatment in SD_{20}° .

		light intensity in $\text{erg cm}^{-2} \text{sec}^{-1} \times 1000$			
		1.5	7.5	15	22.5
%	E_1	0	91	100	100
	L_1	0	17	29	43
days	E_1	–	25.2	24.3	22.4
	L_1	–	36.5	29.8	23.0

The percentage generative plants increases and the number of days decreases at increasing light intensities. This indicates that light intensity during the 8 h photoperiod is a major factor in flower induction, especially in L_1 .

5.6 Light intensity during CL_{20}° .

The influence of light intensity during CL_{20}° will be investigated for E_1 and L_1 .

Groups of 15 plants of E_1 and L_1 received 0, 1, 2, 3, 4, 5, 6 cycles CL_{20}° . Light was obtained from 40 W 55 Philips fluorescent lamps + 120 W Philips incandescent lamps, giving at plant level approximately 1.5, 9, 16.5 or $24 \times 1000 \text{ erg cm}^{-2} \text{sec}^{-1}$. The first light intensity was obtained from incandescent light only. The percentages generative plants in the subsequent SD_{20}° are presented in table 4. The numbers of cycles necessary for 50% flowering were calculated from these percentages.

Table 4

Percentages generative plants in E_1 and L_1 after 0, 1, 2, 3, 4, 5, 6 cycles CL_{20}° at 4 light intensities and the calculated average number of cycles for 50% generative plants. After-treatment in SD_{20}° .

		light intensity in $\text{erg cm}^{-2} \text{sec}^{-1} \times 1000$			
cycle number		1.5	9	16.5	24
0	E_1	0	0	0	0
1	E_1	0	0	0	0
2	E_1	31	47	87	86
3	E_1	73	100	100	100
4	E_1	93	100	100	100
5	E_1	100	100	100	100
6	E_1	100	100	100	100
0	L_1	0	0	0	0
1	L_1	0	0	0	0
2	L_1	0	0	30	60
3	L_1	15	93	100	100
4	L_1	13	100	100	100
5	L_1	42	100	100	100
6	L_1	100	100	100	100
50%	E_1	2.53	2.03	1.63	1.64
	L_1	4.80	2.57	2.20	1.90

The number of cycles necessary for 50% flowering decreases considerably for both E_1 and L_1 at increasing light intensities. E_1 needs a lower number of cycles than L_1 . Since only E_1 reaches a minimum of 1.6 cycles, this difference tends to diminish at increasing light intensities. An optimal light intensity for E_1 is indicated by this minimal number.

5.7 Different photoperiods followed by 32° SD

The influence of supplementary incandescent light in relation with 32° SD has been investigated.

Groups of 18 plants received photoperiods of 8, 9, 10, 11, 12, 13 or 14 h during 3 weeks. Light was obtained for the first 8 h from 40 W 55 Philips fluorescent lamps + 60 W Philips incandescent bulbs, giving at plant level approximately $6500 \text{ erg cm}^{-2} \text{ sec}^{-1}$. Supplementary light was obtained from 60 W Philips incandescent bulbs, giving at plant level approximately $500 \text{ erg cm}^{-2} \text{ sec}^{-1}$. This photoperiodic treatment was followed by a 32° SD treatment. The results are presented in table 5 for 0, 10 and 12 cycles 32° SD.

Table 5
Percentages generative plants and days till budding in E_1 after 3 weeks at different photoperiods, followed by 0, 10 or 12 cycles 32° SD. After-treatment in SD_{20}° . The number of days was determined from the start of the 32° SD treatment.

photoperiod	32° SD					
	0 c		10 c		12 c	
	%	days	%	days	%	days
8	9	-	44	35.0	82	34.4
9	0	-	33	37.0	91	37.3
10	10	-	50	37.9	82	34.4
11	0	-	42	38.4	82	34.7
12	9	-	45	35.4	80	37.3
13	10	-	73	35.5	100	31.1
14	91	16.8	90	25.1	100	20.3

Treatment 0 cycles 32° SD shows very low and almost equal percentages generative plants for 8, 9, 10, 11, 12 and 13 h photoperiod. A high percentage is apparent for 14 h photoperiod. This indicates that 3 weeks at 14 h photoperiod is inductive. Treatment 10 cycles 32° SD shows an almost equal percentage generative plants for 8, 9, 10, 11 and 12 h photoperiod. A slightly higher percentage is apparent for 13 h photoperiod. The high percentage for 14 h photoperiod confirms the results of treatment 0 cycles 32° SD. No clear difference in days till budding is apparent, except for 14 h photoperiod; the higher number of days for 10 cycles compared with 0 cycles is most probably caused by the retarding influence of the high temperature on realization.

The results of 32° SD indicates that no suboptimal induction is found in 8, 9, 10, 11 and 12 h photoperiod and a slight one in 13 h. Treatment 12 cycles 32° SD shows a slightly higher percentage generative plants for 13 and 14 h photoperiod, but a distinct decrease in number of days. This confirms that no suboptimal flower induction occurs in *Silene armeria* after 3 weeks 8, 9, 10, 11 or 12 h photoperiod, a suboptimal one in 13 h and an optimal in 14 h.

6 THE MECHANISM OF FLOWER INDUCTION

A preliminary hypothesis on the flower inducing mechanism of *Silene armeria* was arrived at in I, II, III, IV. In short, light has a deblocking action, while in darkness reblocking, formerly called blocking, occurs. At a balance of deblocking and reblocking the plant remains vegetative. When the balance is disturbed and more deblocking than reblocking occurs, the plant becomes generative.

The meaning of the terms deblocking and reblocking is not more than an approach to and a removal from the generative state respectively, or in other words a lowering and raising of the level of the non-flowering condition.

In the present chapter this hypothesis will be reconsidered with regard to the experimental results in chapter 5.

6.1 High temperature

Time of maximal action of high temperature is about 7 h after the onset of darkness, regardless the length of the dark period (I, III). A similar result was found in light break experiments e.g. in *Xanthium strumarium* with a maximal effect 8 h after the onset of darkness and nearly regardless the length of the dark period (SALISBURY et al. 1956). This could indicate that high temperature is acting on a timing mechanism, which however is unlikely, since timing is resistant to such temperatures (SALISBURY 1963b, 1965, BÜNNING 1967). Light break experiments have not been carried out in detail (cp. WELLENSIEK 1969a). The relation between high temperature and light break experiments was investigated by NAKAYAMA (1958) for *Pharbitis nil*. In this plant the maximal effect for light interruption and high temperature experiments is 8–10 h and 12–14 h respectively after the onset of darkness. So it is not likely that light and high temperature are acting on the same process.

SALISBURY (1963a) proposed a destruction of the flowering hormone by high temperatures. The flowering hormones of SD and LD plants may be identical, as can be concluded from grafting-experiments (LANG 1952). WELLENSIEK (1968b, 1970) demonstrated this identity from grafting experiments between *Silene armeria* and *Xanthium strumarium*. As the effect of high temperature during SD is promoting in LD plants and inhibiting in SD plants (SCHWEMMLE 1960, IV), it is unlikely that high temperature destroys the flowering hormone.

The results of experiments on critical temperature and temperature level (IV) could

make it possible to find a process in the plant with the same properties, but since such a process has not been established, only a suggestion can be made. This is that high temperature prevents a process, which is supposed to be the building up of an inhibition. This inhibition was tentatively suggested to consist of a blocking of DNA by histones, preventing it to produce the flowering hormone (WELLENSIEK 1966c). This building up of an inhibition is called reblocking. Since it is possible to obtain flower induction by preventing the reblocking by high temperature in the dark, a deblocking process must be present in the light period. This agrees well with the results of TAKIMOTO (1955) with light/dark cycles of different lengths: the longer the light period or the shorter the dark period, the more flower bud formation.

6.2 Light

Light intensity is an important factor in flower induction during an 8 h photoperiod (5.5) and a decisive factor during CL (5.6). A rather low optimal light intensity during CL was found for E_1 (5.6). The conclusion can be drawn that deblocking depends on light intensity to a certain extent.

Low intensity incandescent light at photoperiods shorter than 12 h has no effect on flower induction in E_1 (5.7). Since reblocking is only decreased at dark periods shorter than 12 h (III), low intensity incandescent light has no effect on reblocking. Consequently, the induction in 13 and 14 h photoperiod has to be ascribed to prevention of reblocking due to the postponement of the onset of darkness.

6.3 Hypothesis

The mechanism of flower induction in *Silene armeria* can be indicated as:

1. A deblocking process in high intensity light; increasing light intensities accelerate it with an optimal action; temperature insensitive.
2. A reblocking process in darkness, starting about 4 h after the onset of darkness, reaching its maximum after 6–7 h and decreasing for about 5 h; temperature sensitive, temperatures higher than 30°–35° C slowing it down increasingly. Incandescent light postpones the onset of darkness and hence the reblocking.

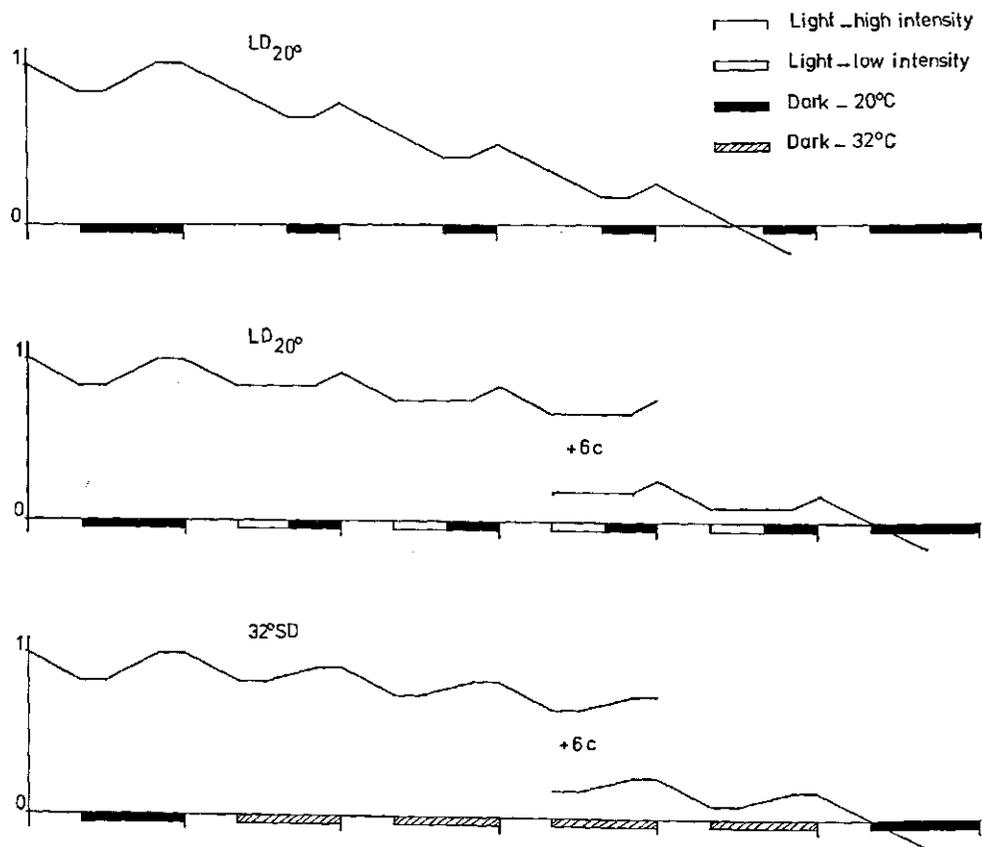
In a vegetative plant these two processes are in balance on a certain level of the block. A critical level or complete disappearance, causing optimal flower induction, is achieved when the balance is disturbed and more deblocking than reblocking occurs.

6.4 Models

Three cases of flower induction in E_1 will be discussed with the hypothesis described

in 6.3 and constructed graphically by way of illustration. In these models the level of the block is represented by the ordinate, the time by the abscissa. Flower induction is achieved when the critical level is reached, represented by the zero value of the ordinate.

For all the three cases in figures 5-7 the first cycle is a SD_{20° . Deblocking starts at the onset of light and lasts for 8 h till the onset of darkness. Reblocking starts 4 h after the onset of darkness and lasts about 8 h. During the last 4 h of darkness no reblocking occurs, so that the original level of the block is reached.



Figs. 5, 6 and 7. Flower induction, preceded by one cycle SD_{20° , by LD_{20° - high intensity light, LD_{20° - high intensity + low intensity incandescent light or $32^\circ SD$. The ordinate represents the block, the abscissa the time. For details see the text.

6.4.1 LD_{20° - high intensity light (figure 5)

The LD_{20° starts with deblocking, which lasts for 16 h till the onset of darkness. Reblocking starts 4 h later and lasts 4 h till the onset of light. A lower level of the

block is reached. This is repeated and the critical level, so flower induction, is reached after 4 cycles LD_{20° and flower formation can start.

6.4.2 LD_{20° – high intensity + supplementary light (figure 6)

The LD_{20° starts with deblocking, which lasts for 8 h till the onset of the low intensity supplementary light. No deblocking occurs for 8 h till the onset of darkness. Reblocking starts 4 h after the onset of darkness and lasts 4 h till the onset of light. A lower level is reached now. This is repeated and the critical level is reached after 10 cycles LD_{20° .

6.4.3 $32^\circ SD$ (figure 7)

The $32^\circ SD$ starts with deblocking, which lasts for 8 h till the onset of darkness. Reblocking starts 4 h after the onset of darkness and lasts 8 h. No reblocking occurs during the last 4 h of darkness. The reblocking is retarded by the high temperature, so that a lower level is reached. This is repeated and the critical level is reached after 10 cycles $32^\circ SD$.

6.5 Photoperiodic response curves

An attempt can be made now to explain the photoperiodic response curves in figure 4. The next general considerations are based on the above hypothesis (6.3). A decrease in number of cycles for photoperiods from 8 to 12 h indicates an increasing deblocking, since reblocking remains the same at nyctoperiods longer than 12 h. A decrease in number of cycles for photoperiods from about 20 to 24 h indicates an increase in deblocking, since no reblocking occurs in nyctoperiods shorter than about 4 h. An equal number of cycles for the 20° and 32° curves indicates that no reblocking occurs. An equal number of cycles at increasing photoperiods indicates that no increase in deblocking and no decrease in reblocking occurs.

The decrease for the E_1-20° curve from 14 to 18 h is possibly caused by increased deblocking and certainly by decreased reblocking. Since the 20° and 32° curve are identical from 19 to 24 h, no reblocking occurs. The equal number from 20 to 24 h indicates no additional deblocking or reblocking. The decrease for the E_1-32° curve from 8 to 12 h is caused by increased deblocking. The slow decrease from 12 to 16 h is caused by the increased deblocking and the retarded reblocking. The almost equal number from 16 to 24 h indicates no additional deblocking or reblocking. The conclusion for E_1 is that deblocking takes place during the first 16 h of the photoperiod and that reblocking does not take place during the first 5 h of darkness.

The decrease for the L_1-20° curve from 16 to 18 h is caused by increased deblocking and decreased reblocking. Since the 20° and 32° curve are identical from 18 to 24 h, no reblocking occurs. The decrease from 18 to 24 h indicates an increased deblocking. The decrease for the L_1-32° curve from 8 to 12 h is caused by increased deblocking

and decreased reblocking, from 18 to 24 h by increased deblocking. The conclusion for L_1 is that deblocking takes place in light and that reblocking does not take place during the first 6 h of darkness.

6.6 Discussion

The described models fit very well and a programme for computer simulation of the induction of flowering seems possible, when more data are available on light intensity and duration of the action of light. Variables in this programme will be the light intensity, ratio of light and dark in a cycle and night temperature. It will then be possible to predict for a combination of these factors when induction will take place. It seems possible to use the described models for other LD plants. Since most plants need a special treatment for realization, the consideration that once a critical value of the block is reached, flowering occurs, does not hold for these plants. Flower induction took place, but the plant reverses to the vegetative state.

Remaining problems are:

1. Induction by 5° SD (WELLENSIEK 1966a). Research has to be carried out in the same way as was done for high temperature (SCHWEMMLE 1960).
2. Induction by high root temperature (WELLENSIEK 1968a, 1969b). An interesting problem would be the period of action during a photoperiodic cycle. In preliminary experiments no such period was found by the present author, but the responses were highly influenced by damage to the plant.
3. Partial induction and desinduction in SD_{20° (WELLENSIEK 1966b, II).
4. Induction by low light intensities in SD_{20° for some segregates, the so called "day neutral" plants. Strains selected from these plants flower very irregularly over a long period of time in SD_{20° .
5. Induction by GA_3 during SD_{20° in some strains (WELLENSIEK 1969b).
6. Differences between E_1 and L_1 . The differences regarding high temperature action between E_1 and L_1 were studied in II. Three possibilities were discussed:
 - a. deblocking is faster in E_1 than in L_1 , b. reblocking is faster in L_1 than in E_1 ,
 - c. the block is greater in L_1 than in E_1 . In this paper the sensitive period for E_1 and L_1 was found to be the same (5.2). The responses to light intensity (5.5,6) and duration of light action (5.3) present great differences for E_1 and L_1 . However, with the available data no choice between these three possibilities can be made.

7 LITERATURE

- BEST, R.: Proc. Kon. Ned. Akad. Wetensch. Amsterdam *C63*, 676-691 (1960).
- BONNER, J. and LIVERMAN, J. L.: in "Growth and differentiation in plants", ed. W. E. Loomis. Iowa State College Press, Ames, 283-304 (1953).
- BÜNNING, E.: The physiological clock. Springer Verlag, New York, 167 p. (1967).
- EVANS, L. T.: *Lolium temulentum*, in "The induction of flowering", ed. L. T. Evans. MacMillan, Melbourne, 328-349 (1969).
- HAMNER, K. C.: *Glycine max*, in "The induction of flowering", ed. L. T. Evans. MacMillan, Melbourne, 62-89 (1969).
- HARDER, R.: Symp. Soc. Exp. Biol. *II* Growth, 117 (1948).
- HARDER, R. and WITSCH, V. H. VON: *Planta* *31*, 523 (1940).
- HILLMAN, W. S.: Amer. J. Bot. *46*, 466 (1959).
- : The physiology of flowering. Holt, Rinehart and Winston, New York, 164 p. (1964).
- LANG, A.: Ann. Rev. Plantphysiol. *3*, 265-306 (1952).
- : Encycl. Plant Physiol. *XV* (1), 1380-1536 (1965).
- and MELCHERS, G.: *Planta* *33*, 653-702 (1943).
- LINT, P. J. A. L. DE: Meded. Landbouwhogeschool, Wageningen *60* (14), 59 p. (1960).
- LIVERMAN, J. L.: Doctor's Thesis Caltech. 203 p. (1952).
- and LANG, A.: *Plant Physiol.* *31* (2), 147-150 (1956).
- MURNEEK, A. E.: Bot. Gaz. *102*, 269 (1940).
- NAKAYAMA, S.: Sci. Rep. Tôhoku Univ. 4 Ser. *24* (4), 137-183 (1958).
- NANDA, K. K. and KRISHNAMOORTHY, H. N.: *Planta* *72*, 338-343 (1967).
- PURVIS, O. N. and GREGORY, F. G.: Ann. Bot. Lond. *1*, 569-591 (1937).
- SALISBURY, F. B.: The flowering process. MacMillan, New York, 234 p. (1963a).
- : *Planta* *59*, 518-534 (1963b).
- : *Planta* *66*, 1-26 (1965).
- and BONNER, J.: *Plant Physiol.* *31* (2), 141-147 (1956).
- SCHWEMMLE, B.: Naturwiss. *47* (3), 68-69 (1960).
- SEIDLOVA, F. et al.: Nature *214*, 1146-1147 (1967).
- TAKIMOTO, A.: Bot. Mag. Tokyo *68* (809), 308-314 (1955).
- : Bot. Mag. Tokyo *70* (832), 312-326 (1957).
- : Plant & Cell Physiol. *2*, 71-75 (1961).
- : *Pharbitis nil*, in "The induction of flowering", ed. L. T. Evans. MacMillan, Melbourne, 90-115 (1969).
- VOOREN, J. VAN DE: Z. Pflanzenphysiol. *61* (2), 135-139 (1969a).
- : Z. Pflanzenphysiol. *61* (4), 332-337 (1969b).
- : Z. Pflanzenphysiol. *64* (1), 52-59 (1971a).
- : Z. Pflanzenphysiol. in press (1971b).

- WELLENSIEK, S. J.: Proc. Kon. Ned. Akad. Wetensch. Amsterdam C63, 159-166 (1960).
- : in "Differentiation of apical meristems and some problems of ecological regulation of development of plants. Proc. Symp. Praha-Nitra, 115-118 (1964).
 - : Z. Pflanzenphysiol. 54 (5), 377-385 (1966a).
 - : Z. Pflanzenphysiol. 55 (4), 363-373 (1966b).
 - : Naturwiss. 53 (16), 411 (1966c).
 - : Acta Bot. Neerl. 17 (1), 5-8 (1968a).
 - : Acta Bot. Neerl. 17 (3), 197-198 (1968b).
 - : *Silene armeria*, in "The induction of flowering", ed. L. T. Evans. MacMillan, Melbourne, 350-364 (1969a).
 - : Z. Pflanzenphysiol. 61 (5), 462-471 (1969b).
 - : Z. Pflanzenphysiol. 63 (1), 25-30 (1970).
 - and ELINGS, C. G.: Proc. Kon. Ned. Akad. Wetensch. Amsterdam C70, 187-191 (1967).
- ZEEVAART, J. A. D.: *Perilla*, in „The induction of flowering”, ed. L. T. Evans. MacMillan, Melbourne, 116-155 (1969).

Nicht einzeln im Buchhandel!

Überreicht vom Verfasser

Sonderdruck aus
Zeitschrift für Pflanzenphysiologie
Band 61, Heft 2, Seite 135—139 (1969)
Gustav Fischer Verlag Stuttgart

From: *Laboratorium voor Tuinbouwplantenteelt, Landbouwhogeschool,
Wageningen, the Netherlands. Publication 316*

The Influence of High Temperature on the Flower Inducing Mechanism of *Silene armeria* L.

I. Intermittent Temperatures

JAN VAN DE VOOREN

Received March 12, 1969

Summary

Silene armeria, a qualitative long day («LD») plant at 20° C, is induced to flower formation in short day at 32° C («32° SD»).

From experiments with intermittent temperatures follows, that the high temperature is exclusively active on the flower inducing mechanism during the middle of a long night. By applying 1, 2 or 4 hours of 32° C during the middle of the dark period, the conclusion is drawn, that not a temperature shock, but a quantitative action of the high temperature is the cause of the effect on the flower inducing mechanism. An optimal induction in SD by 4 hours 32° C in the middle of the dark period requires at least 15 cycles.

The action of 32° C is explained by assuming, that the blocking is slowed down by the high temperature, so that the critical value can be reached in SD.

Zusammenfassung

Silene armeria, bei 20° C eine qualitative Langtagpflanze («LD»), wird im Kurztag bei 32° C («32° SD») zur Blütenbildung induziert.

Versuche mit Wechseltemperaturen (20° und 32° C) wurden mit zwei Monate alten Pflanzen durchgeführt. Hohe Temperaturen wirken nur in der Mitte der Dunkelphase des SD auf die Blüteninduktion. Die Anwendung von 1, 2 oder 4 Stunden 32° C in der Mitte der Dunkelphase hat gezeigt, daß nicht ein Temperaturstoß, sondern eine quantitative Wirkung der hohen Temperaturen die Ursache der Blüteninduktion ist. Es sind 15 Zyklen notwendig, um eine optimale Induktion bei 4 Stunden 32° C in der Mitte der Dunkelphase eines SD zu erhalten.

Die Wirkung von 32° C wird als eine Hemmung der Blockierung erklärt, so daß der kritische Wert im SD erreicht werden kann.

Introduction

Since LIVERMAN (1952) and WELLENSIEK (1966 a) it is known, that the qualitative long day («LD») plant *Silene armeria* is induced to flower bud formation in short days by 32° C («32° SD»). This high temperature is exclusively active on flower induction during the dark period (WELLENSIEK 1966 a). SCHWEMMLE (1960) reports a similar response for the LD plant *Hyoscyamus niger*. Giving periods of 3 hours of 35° C, he found that this temperature was optimally active during the 6–9th hour of a 12 hours dark period. Other LD plants, induced by 32° SD, are *Scrofularia alata* and *Stenactis annua* (CHOUARD et al. 1965). On the other hand, in SD plants a period

of high temperature during a long dark period inhibits the flower induction, as was found for *Xanthium strumarium* (HAMNER et al. 1938), *Chenopodium amaranticolor*, *Kalanchoë blossfeldiana* (SCHWEMMLE 1957, 1960), *Pharbitis nil* (NAKAYAMA 1958).

The scope of the present research is to study whether a changing sensitivity of the flower inducing mechanism for high temperatures exists in *Silene armeria*. The most sensitive period of a SD cycle for high temperature, the duration of the high temperature in a SD cycle and the optimal number of SD cycles will be dealt with.

Materials and Methods

In a later publication will be demonstrated that the strain Early₁ («E₁»), as selected by WELLENSIEK (1969), reacts far better towards 32° SD than the strain Late₁ («L₁»). This is why the present work was done with this strain E₁. It is rather uniform, but sometimes a very small percentage of still earlier plants segregates. Multiplication by seed does not present any difficulty.

Plants were grown from seeds in 8 hours light and 16 hours darkness in the greenhouse at about 20° C («SD 20°»). A growth cabinet was used for the temperature treatments. This cabinet contained 15 plastic boxes, each with 15 potted plants on sand. The temperature was controlled at 32° C, while a high humidity was maintained by sprinkling. Watering was done by wetting the sand. This procedure was chosen to avoid damage of the plants and to assure a regular water supply. The intermittent temperature treatments were realized by transferring the plants by hand, which partly had to be done in complete darkness.

As a measurement of the response to the treatment two criteria were used:

1. Percentage of generative plants, obtained by an aftertreatment in SD 20° following the proper treatment. A disadvantage is, that a suboptimal induction is not detected. By adding a limited number of cycles of LD 20° (16 hours light and 8 hours darkness at 20° C), this partial induction can be revealed by interaction and a complete picture of the degree of induction is obtained without any disturbance by realization.
2. Number of days till the appearance of the first visible flower bud. This criterium can be determined by giving LD 20° as an aftertreatment, or a limited number of cycles of LD 20°, followed by realization in SD 20°. The second way will yield greater differences than the first one. The disadvantage of both procedures is, that the environment – light intensity – can obscure the effects of the treatments.

Differences between averages will be indicated as mathematically significant, when they are reliable for at least 95 %.

Special procedures will be indicated, when dealing with special experiments.

Experimental results

The most sensitive period of a 24 h SD cycle

As mentioned before, WELLENSIEK (1966 a) found that 32° C is exclusively active during the dark period of a SD cycle. To extend this work, my intention was to find out which part of the dark period is sensitive.

First of all, a SD cycle was divided into 3 periods of 8 hours. During 5 weeks groups of 15 plants were exposed to 8 hours 32° C at different periods of a 24 hours SD cycle. The flowering percentages in SD 20° for the 8 h light, the first 8 h darkness and the second 8 h darkness were 0, 93, 100 respectively. These results confirm those of Wellensiek and indicate, that the first and second part of the dark period both

are sensitive for 32° C. It is remarkable that 32° C, given during the light period, did elongate the stem, but without any subsequent flowering. This demonstrates that, although flowering and elongation are closely related, both phenomena can be disconnected.

Table 1

Flowering percentages and days till budding for groups of 8 plants after 4 hours of 32° C in different periods of a SD cycle (8 hours light and 16 hours darkness during 08–16h and 16–08h respectively) and a second treatment of 2, 3 or ~ LD 20°.

period	2 LD %	3 LD %	~ LD days
08h–12h	12.5	12.5	45.1
12h–16h	0.0	0.0	44.5
16h–20h	12.5	12.5	44.3
20h–24h	50.0	100.0	42.4
00h–04h	87.5	100.0	41.1
04h–08h	12.5	0.0	43.6

The next logical step was to use shorter periods of high temperature. Groups of 8 plants were treated for 7 SD cycles of 4 hours 32° C during different parts of that cycle. In order to get a good picture of the flowering percentages of the treatments, 0, 1, 2 or 3 LD 20° cycles were added before the aftertreatment at SD 20°. Since 0 and 1 LD 20° did not give any response, they are omitted in table 1. To evaluate the criterium «days till budding», part of the plants got a second treatment in LD 20°.

It appears from the results, summarized in table 1, that the most sensitive period is around the middle of the 16 h dark period, treatment 00–04 being the most active, directly followed by 20–24. The difference between these two treatments is not significant. The few plants, flowering after periods 08–12 and 16–20 are considered as genetical segregates.

Table 2

Number of days till budding in SD 20° for groups of 10 plants after 10 cycles of high temperature treatment within the period 20–24h for 1, 2 or 3 hours. A second treatment of 4 LD 20° and an aftertreatment in SD 20° were given.

period	hours	days
control	0	29.9
20h–21h	1	30.2
22h–23h	1	30.3
20h–22h	2	29.7
22h–24h	2	28.3
20h–24h	4	25.7

Duration of the high temperature in a SD cycle

The question arises, whether it is necessary to give such a long period as 4 hours of high temperature. It could be, that a temperature shock of one hour or even less could be sufficient.

Within the period 20–24 h, treatments of 1, 2 or 4 hours 32° C were given for 10 cycles of SD to groups of 10 plants. As a second treatment 4 cycles of LD 20° appeared to give an optimal induction, so that the criterium «days till budding» could be used.

In table 2 the treatments 1 and 2 hours do not differ significantly from the control, whereas 4 hours does so. Hence it is clear that a temperature shock is not the cause of flowering in 32° SD. A quantitative action of the high temperature on the flower inducing mechanism is a more reasonable explanation. Also, it is not justified to use shorter treatments than 4 hours in future experiments.

Optimal number of SD cycles with 4 h 32° C

Now the question arises how many cycles of 4 hours 32° C are optimal for flower induction. Four hours 32° C in the period 20–24 h were given for 0, 5, 10, 15 cycles of SD 20° to groups of 15 plants. To get a complete picture, 0, 1 or 2 days of continuous light («CL») were given as a second treatment. The flowering percentages in the subsequent SD 20° are listed in table 3. It appears, that 15 cycles were required for an optimal induction by 4 hours 32° C. This is about twice the number of cycles for an all day 32° C treatment. If the plant is only sensitive for induction by 32° C during the 8 hours in the middle of the dark period, this is understandable and confirms the opinion, that 32° C does not involve a qualitative temperature shock, but a quantitative action.

Table 3

Flowering percentages for groups of 15 plants after 0, 5, 10 or 15 cycles of SD 20° with 4 hours 32° C, in the period 20–24h, and a second treatment of 0, 1 or 2 cycles of CL.

cycles	Second treatment in CL		
	0	1	2
0	0.0	0.0	100
5	13.3	73.4	100
10	13.3	100	100
15	86.7	100	100

This experiment shows nicely that by applying some days of CL, a complete picture of a partial induction is obtained, by means of the criterium percentage flowering. An even more detailed picture arises from giving cycles of LD consisting of for instance 16 hours light. It is then possible to give more cycles of LD before an optimal induction is reached. A disadvantage is the greater number of plants required.

Discussion

The middle of the dark period being the most sensitive for flower induction by 32° C, makes it possible to extend WELLENSIEK's hypothesis on the flower inducing mechanism (1966 b), that flower induction in *S. armeria* can be considered as a deblocking of a specific DNA particle.

We can now consider that this deblocking takes place in the photophase. In the nyctophase the blocking is rebuilt. Those two mechanisms are in balance, when the photoperiod is shorter than the critical. By shortening the nyctophase, more deblocking takes place than blocking of the DNA, so that after several cycles the blocking of DNA is removed and a specific RNA is formed. This RNA can be, or can give rise to a flowering hormone, which must be a protein.

The high temperature action on flower induction, being strongest around the middle of the dark period, resembles the action of intermittent light periods, and reflects the timing mechanism as described by BÜNNING (1967). It is tempting to suppose that the blocking of the DNA is influenced by this timing mechanism.

An explanation of the high temperature action is a slowing down of the blocking of DNA. It could be possible that this slowing down is caused by a shortage of carbohydrates for the building of the block, brought about by a reduction of the translocation of carbohydrates by the high temperature (WENT and HULL 1948). Again blocking and deblocking will be out of balance, so that the total deblocking of DNA is achieved.

From the foregoing follows, that LD 20° and 32° SD are acting on different parts of the same mechanism: LD 20° results in a longer period of deblocking and 32° SD in a slowing down of the blocking of DNA. The final result is the same, viz. formation of a flowering hormone.

Experiments with a second treatment, viz. some days LD 20° after the proper treatment, give a good picture in flowering percentages of a suboptimal induction, as is shown in tables 1 and 3. This criterium is independent of the environment and is therefore very reliable. Very small differences, which cannot be detected by the criterium «days till budding» in LD 20°, can be revealed. Another advantage is the possibility to give short, in itself not inductive, treatments, which can be important for labour intensive or destructive treatments.

Literature

- BÜNNING, E.: The physiological clock. Springer Verlag, New York, 167 p. (1967).
 CHOUARD, P. et al.: Bull. Soc. franç. Physiol. Végétale 11 (1), 85-94 (1965).
 HAMNER, K. C., and BONNER, J.: Bot. Gaz. 100, 388-431 (1938).
 LIVERMAN, J. L.: Doctor's Thesis Caltech. 203 p. (1952).
 NAKAYAMA, S.: Sci. Rep. Tôhoku Univ. 4 ser. 24 (4), 137-183 (1958).
 SCHWEMMLE, B.: Naturwiss. 44 (12), 356 (1957).
 - Naturwiss. 47 (3), 68-69 (1960).
 WELLENSIEK, S. J.: Z. Pflanzenphysiol. 54, 377-385 (1966 a).
 - Naturwiss. 53 (16), 411 (1966 b).
 - Z. Pflanzenphysiol. in preparation (1969).
 WENT, F. W., and HULL, H. M.: Plant Physiol. 24, 505-526 (1949).

Ir. JAN VAN DE VOOREN, Landbouwhogeschool, Laboratorium voor Tuinbouwplantenteelt,
 Haagsteeg 3 (Postbus 30), Wageningen, the Netherlands.

Nicht einzeln im Buchhandel

Überreicht vom Verfasser

*Sonderdruck aus
Zeitschrift für Pflanzenphysiologie
Band 61, Heft 4, Seite 332-337 (1969)
Gustav Fischer Verlag Stuttgart*

*From: Laboratorium voor Tuinbouwplantenteelt, Landbouwhogeschool, Wageningen,
The Netherlands. Publication 317*

NG

**The Influence of High Temperature on the Flower Inducing
Mechanism of *Silene armeria* L.
II. Comparison of an Early and a Late Strain**

JAN VAN DE VOOREN

Received May 27, 1969

Summary

Silene armeria, a qualitative long day plant at 20° C («LD₂₀»), is induced to flower formation in short day at 32° C («32° SD»).

An Early («E₁») and a Late («L₁») strain with respect to their photoperiodic behaviour, were compared in their sensitivity for 32° SD. E₁ is the most sensitive for 32° SD, 10–12 cycles being necessary for optimal induction, whereas L₁ flowered after more than 22 cycles only for two third of the plants. E₁ and L₁ both have a quantitative juvenility for 32° SD, with E₁ reaching approximately one week sooner the highest sensitivity than L₁. Interaction of 32° SD and LD₂₀ exists in both strains. After a suboptimal 32° SD, partial induction does exist in E₁ and L₁. This partial induction can be completed with LD₂₀, is in itself persistent and disappears completely after approximately 12 and 21 days of a non-inductive treatment respectively.

The difference between E₁ and L₁ is explained in three ways: 1. the deblocking of a specific DNA particle in the photophase is faster for E₁ than for L₁; 2. the blocking in the nyctophase is faster for L₁ than for E₁; 3. the block in L₁ is greater. In all three cases the photoperiod can be shorter for E₁ to reach the critical value, a total deblocking, so that the DNA can give rise to the formation of a flowering-hormone.

The difficulty for L₁ to reach 100 % flowering is explained by supposing a multi-layered block.

Zusammenfassung

Silene armeria, bei 20° C eine qualitative Langtagpflanze («LD₂₀»), wird im Kurztag bei 32° C («32° SD») zur Blütenbildung induziert.

Eine frühe («E₁») und eine späte («L₁») Sippe in Beziehung auf photoperiodisches Verhalten, sind hinsichtlich ihrer Empfindlichkeit für 32° SD verglichen worden. E₁ ist die meist Empfindliche, 10–12 Zyklen sind für eine optimale Induktion nötig; dagegen blühen bei L₁ nach mehr als 22 Zyklen nur zwei Drittel der Pflanzen. E₁ und L₁ haben eine quantitative Jugendphase, welche für E₁ ungefähr eine Woche kürzer ist als für L₁. LD₂₀ und 32° SD wirken in E₁ und L₁ zusammen.

Suboptimale Induktion erscheint in E₁ und L₁ nach einer suboptimalen 32° SD und wird ganz beseitigt nach ungefähr 12 und 21 Tagen unter nicht-induzierenden Bedingungen.

Die Unterschiede zwischen E₁ und L₁ werden wie folgt erklärt: 1. die Enthemmung einer spezifischen DNA in der Photophase ist schneller für E₁ als für L₁; 2. die Blockierung in der Nyctophase ist schneller für L₁ als für E₁; 3. die Blockierung in L₁ ist größer als in E₁. Um dem kritischen Wert, eine vollständige Enthemmung zu erreichen, kann die Photophase in

diesen drei Fällen für E_1 kürzer sein als für L_1 . Enthemmung von DNA führt zur Blütenhormonbildung.

Die Schwierigkeit mit L_1 , 100 % generative Pflanzen zu erhalten, wird durch einen Block an mehreren Stellen erklärt.

Introduction

As is known from the literature, many species have strains with different photoperiodic responses (WHALEY 1965), which function as a major factor in the ability of species to spread over an extensive area, just as the response to temperature. Many cases of different responses to cold requirement for different strains of one species are known (NAPP-ZINN 1961). Different response types towards photoperiod were already found by LIVERMAN (1952) in his original material of *Silene armeria*.

After having dealt with the necessary number of inductive cycles 32° SD, the interactions between 32° SD and LD_{20}° and the suboptimal or partial induction for the original material (WELLENSIEK 1966 a, b, 1967), an Early (« E_1 ») and a Late (« L_1 ») flower inducing strain were selected for their photoperiodic and temperature requirements (WELLENSIEK 1969).

The scope of the present research is to study the difference towards 32° SD inductions between E_1 and L_1 and its implications on the flower inducing mechanism in *Silene armeria* (WELLENSIEK 1966 c, v. D. VOOREN 1969).

Materials and Methods

Two strains of *Silene armeria* selected by WELLENSIEK (1969) from an original sample of LIVERMAN (1952), kindly supplied by Anton Lang, were used. Plants were grown from seeds in the greenhouse in non-inductive SD_{20}° . A growth cabinet at 32° C was used for the temperature treatments, while a high humidity was maintained by sprinkling.

As a measurement of the response to the treatment two criteria were used:

1. Percentage of generative plants, obtained at an aftertreatment in SD_{20}° following the proper treatment.

2. Number of days till the appearance of the first visible flower bud.

This criterium can be used by giving LD_{20}° as an aftertreatment, but also by giving a limited, but optimal number of cycles of LD_{20}° followed by realization in SD_{20}° . Both methods result in 100 % flowering. The second way will yield greater differences than the first one.

Special procedures will be indicated, when dealing with special experiments.

Experimental results

Number of 32° SD cycles

Plants received 0, 2, ... 10 (E_1) or 0, 16, 18 ... 26 (L_1) cycles 32° SD. More than 26 cycles gave too high a mortality for conclusive results. Aftertreatment was given in SD_{20}° . The results are listed in table 1 and clearly indicate the enormous differences between E_1 and L_1 . For E_1 an optimal induction is reached after 12 cycles 32° SD, with 6–10 cycles as a transitory region. A quite different picture is obtained for L_1 . After 0–16 cycles 32° SD no optimal induction at all is reached. After 18–20 cycles some plants are optimally induced and resemble a transitory region. A maximum number of optimally induced plants is obtained after 22 and more cycles, but never reaches 100 %.

Table 1
Flowering-percentages in SD_{20}° for groups of 15 plants of E_1 and L_1 after 32° SD treatments.

E_1		L_1	
cycles 32° SD	%	cycles 32° SD	%
0	0	0	0
2	0	16	0
4	0	18	20
6	27	20	33
8	40	22	60
10	80	24	60
12	100	26	67

Effect of plant age

HIGAZY (1962) found that plants, younger than 11 weeks, have a lower sensitivity for LD_{20}° than older ones. However, from his experiments the conclusion, that *Silene armeria* has a quantitative juvenility for flower induction cannot be drawn, as he observed his plants in LD_{20}° , which confuses flower induction and -realisation. These can be separated by applying a marginal treatment with LD_{20}° and observing its effect in SD_{20}° . An increase in percentage of flowering for increasing plant age then indicates an increase in sensitivity for the photoperiod. To study whether such a juvenility exists for a 32° SD induction, an age series of 6, 7, 8, 9 weeks old plants was grown. A 32° SD treatment of 7 cycles (E_1) or 21 cycles (L_1) was given. The results obtained, are listed in table 2 and indicate that E_1 reaches the highest sensitivity approximately one week sooner than L_1 .

Table 2
Flowering-percentages in SD_{20}° for groups of 15 plants of 4 ages (in weeks) of E_1 and L_1 after 7 and 21 cycles of 32° SD respectively.

age	E_1	L_1
6	20	13
7	87	53
8	73	93
9	87	80

Interaction between 32° SD and LD_{20}

Now it becomes interesting to know whether in both strains the different flower inducing factors are working in the same way, viz. can they complete and supplement each other. This can be revealed by interaction studies. By so doing it is also possible to reveal whether partial induction of 32° SD takes place in both strains.

Plants of E_1 and L_1 obtained the treatments: 0, 4, 8, 12 cycles 32° SD + 0, 2, 4, 6 LD_{20}° cycles (E_1) or 0, 4, 8, 12 cycles 32° SD + 0, 4, 8, 12 LD_{20}° cycles (L_1). The results in tables 3 and 4 enable to draw the conclusion, that in both E_1 and L_1 interaction exists; 32° SD and LD_{20}° can complete and supplement each other. As was

shown in table 1, L_1 never reaches 100 % flowering. In table 4 an additional $LD_{20^{\circ}}$ results into a percentage of 100 %, in cases where $LD_{20^{\circ}}$ in itself gives no flowering at all. Hence in all the L_1 plants a 32° SD action is at hand.

Table 3

Flowering-percentages in $SD_{20^{\circ}}$ for groups of 8 plants of E_1 after treatments with different cycles of 32° SD followed by different cycles of $LD_{20^{\circ}}$.

cycles 32° SD	cycles		$LD_{20^{\circ}}$	
	0	2	4	6
0	0	13	75	100
4	0	75	100	100
8	38	100	100	100
12	100	100	100	100

VG

Table 4

Flowering-percentages in $SD_{20^{\circ}}$ for groups of 8 plants of L_1 after treatments with different cycles of 32° SD followed by different cycles of $LD_{20^{\circ}}$.

cycles 32° SD	cycles		$LD_{20^{\circ}}$	
	0	4	8	12
0	0	0	38	100
4	0	63	100	100
8	0	88	100	100
12	0	100	100	100

Partial induction

According to the foregoing results, a partial induction for 32° SD does exist. This raises the question, whether this partial induction persists or fades away in both strains. To elucidate this, plants of E_1 and L_1 were first exposed to a partial induction of 0, 1, 2, 3, 4 (E_1) or 0, 3, 6, 9, 12 (L_1) cycles 32° SD, next to a non-inductive period of 0, 4, 8, 12, 16 (E_1) or 0, 7, 14, 21, 28 (L_1) cycles $SD_{20^{\circ}}$. The existing partial induction was made visible by 3 (E_1) or 4 (L_1) cycles of continuous light («CL»). The results, listed in tables 5 and 6, enable the following conclusions. In E_1 the partial induction does persist during the first 4 days in $SD_{20^{\circ}}$. During the next 8 days this induction fades

Table 5

Accelerations of flowering in days in $SD_{20^{\circ}}$ for groups of 15 plants of E_1 after partial induction by 32° SD and an optimal induction of 3 cycles CL, separated by non-inductive $SD_{20^{\circ}}$.

cycles 32° SD	cycles		$SD_{20^{\circ}}$		
	0	4	8	12	16
1	0.8	1.6	0.2	0.3	0.0
2	2.3	2.1	1.8	0.4	0.5
3	4.1	4.0	1.2	0.6	0.1
4	5.2	4.6	1.8	0.7	0.5

away, the soonest for 1 cycle 32° SD. After 12 days the partial induction has gone completely. In L₁ a similar picture is obtained. After 7 days the partial induction is partly gone. This goes on the next 14 days to reach its completion. Hence it is concluded that in both E₁ and L₁ partial induction exists and that this induction fades away in a non-inductive environment in 12 days for E₁ and in 21 days for L₁.

Table 6

Accelerations of flowering in days in SD₂₀^o for groups of 15 plants of L₁ after partial induction by 32° SD and an optimal induction of 4 cycles CL, separated by non-inductive SD₂₀^o.

cycles 32° SD	0	cycles 7	SD ₂₀ ^o 14	21	28
3	2.9	1.1	0.8	0.4	0.7
6	5.5	2.6	1.1	0.5	0.3
9	7.5	6.4	2.2	1.1	0.9
12	10.0	6.4	1.8	-0.3	0.7

Discussion

The great differences between E₁ and L₁ for a 32° SD induction, for a LD₂₀^o induction, the interaction 32° SD and LD₂₀^o and the partial induction make it possible to extend our hypothesis on the flower inducing mechanism for *Silene armeria* (WELLENSIEK 1966 c, v. D. VOOREN 1969). It was postulated there, that the DNA, giving rise to a flowering-hormone, is blocked in the vegetative state. In the light period deblocking takes place, in the dark period blocking. Those two mechanisms are in balance, when the light period lasts shorter than the critical one. The action of 32° SD is a slowing down of the blocking, so that after several cycles the critical value, an irreversible totally deblocked DNA, is reached.

From the foregoing, the differences between E₁ and L₁ could be explained by three possibilities:

1. Deblocking in E₁ is faster than in L₁ and the photoperiod can be shorter to attain the critical value.
2. Blocking in L₁ is faster than in E₁ and the nyctoperiod can be shorter to attain the critical value.
3. The block of L₁ is greater, from which a longer necessary period of time for deblocking follows.

The material at hand does not allow to choose between these possibilities.

The difficulties for L₁ to reach 100 % flowering after 32° SD can be explained from the interaction and partial induction experiments, by assuming a multi-layered block. The outer layers are removed more easily by inductive treatments than the inner ones. The last part of the block is bound stronger for L₁ than for E₁ and cannot be removed by 32° SD alone. But in combination with LD₂₀^o or perhaps with longer photoperiods, shorter than the critical, this last part can be removed. This opens up a new interesting field of research: the action of suboptimal photoperiods.

Literature

- HIGAZY, M. K. M. T.: Meded. Landbouwhogeschool, Wageningen 62 (8), 53 p. (1962).
 LIVERMAN, J. L.: Doctor's Thesis Caltech. 203 p (1952).
 NAPP-ZINN, K.: Encycl. Plant Physiol. XVI, 46-49 (1961).
 VOOREN, J. v. D.: Z. Pflanzenphysiol., in press (1969).
 WELLENSIEK, S. J.: Z. Pflanzenphysiol. 54 (5), 377-385 (1966 a).
 - Z. Pflanzenphysiol. 55 (4), 363-373 (1966 b).
 - Naturwiss. 53 (16), 411 (1966 c).
 - Z. Pflanzenphysiol. 56 (1), 33-39 (1967).
 - Z. Pflanzenphysiol., in preparation (1969).
 WHALEY, W. G.: Encycl. Plant Physiol.. XV (1), 86-90 (1965).
- Ir. JAN VAN DE VOOREN, Landbouwhogeschool, Laboratorium voor Tuinbouwplantenteelt,
 Haagsteeg 3 (Postbus 30), Wageningen, the Netherlands.

NG

From: Laboratorium voor Tuinbouwplantenteelt, Landbouwhogeschool,
Wageningen, The Netherlands. Publication 342.

THE INFLUENCE OF HIGH TEMPERATURE ON THE FLOWER INDUCING
MECHANISM OF *SILENE ARMERIA* L.
III. RELATION TO PHOTOPERIODS.

with 10 figures

JAN VAN DE VOOREN

Received

Summary

Silene armeria, a qualitative long day plant at 20°C, is induced to flower formation in short day at 32°C.

Photoperiodic response curves for 20° and 32°C were constructed. A critical value at photoperiods from 8h was only found at 20°C. At 20-24h photoperiods there was no difference in the number of cycles necessary for 50% flowering and between the 20°- and 32° curve.

Experiments with intermittent temperatures demonstrated that for 8-16h photoperiods the most sensitive period to high temperature was 6-7 hours after the onset of darkness. There was at longer photoperiods no particular sensitive period.

The above led to the following conclusions:

Flower induction depends on a balance between a deblocking process during the light period - temperature insensitive - and a blocking process during the dark period - very temperature sensitive, with high temperature slowing down the blocking. At the onset of darkness the rate of blocking increases gradually, reaches a maximum after 6-7 hours and then decreases for another 5 hours.

Zusammenfassung

Silene armeria, bei 20°C eine qualitative Langtagpflanze, wird im Kurztag bei 32°C zur Blütenbildung induziert.

Photoperiodische Reaktionskurven bei 20° und 32°C wurden zusammengestellt. Eine kritische Photoperiode gibt es nur bei 20°C für Photoperioden über 8 Stunden. Die Photoperioden 20-24 Stunden zeigen keinen Unterschied a. in Zyklenzahl und b. zwischen die 20°- und 32° Kurve.

Versuche mit Wechseltemperaturen wurden für verschiedene Photoperioden durchgeführt. Es zeigte sich, dass hohe Temperaturen 6-7 Stunden nach dem Anfang des Dunkels maximal wirken für Photoperioden von 8 bis 16 Stunden, aber wenig oder nicht während Photoperioden von 16 bis 24 Stunden.

Beide Phänomene haben Beziehungen mit einander und führten zum Bild, dass Blüteninduktion abhängt von der Waage zwischen ein Enthemmung in der Photophase - temperaturempfindlich - und ein Blockierung in der Nyctophase - sehr temperaturempfindlich, mit Hemmung von hohe Temperaturen. Nach dem Anfang des Dunkels nimmt die Reaktion der Blockierung allmählich zu, hat nach 6-7 Stunden ein Maximum und nimmt während 5 Stunden ab.

Introduction

Hyoscyamus niger L. has an increasing critical photoperiod with increasing temperature in the range 15.5 - 28.5°C (LANG et al. 1943). LIVERMAN (1952) tentatively reports the opposite response for Silene armeria L. Vegetative plants of Silene armeria are also induced to flower formation by high temperature of 32°C in short day ("32°SD") according to LIVERMAN (1952), effective only during a dark period of 16 hours (WELLENSIEK 1966a) and exclusively during a 4 hours period in the middle of this dark period (VAN DE VOOREN 1969a).

The scope of the present paper is to study the interactions of these responses by comparing the photoperiodic response curves at 20° and 32°C and to define the period sensitive to high temperature during light/dark cycles of different photoperiods.

Materials and General Methods

The strain E₁ of Silene armeria L. was used exclusively and was selected by WELLENSIEK (1969) from the original seed sample of LIVERMAN (1952), reacts well to 32°SD, is rather uniform, but may segregate a small percentage of "day neutral" plants.

Plants were grown from seeds in short day (8h) in the greenhouse at approximately 20°C ("SD₂₀°"). In winter natural daylight was supplemented by HPLR 400W Philips lamps. In order to avoid juvenility complications, plants of older than 2 months were used (VAN DE VOOREN 1969b).

For the photoperiod-temperature treatments the following equipment was used:

1. A greenhouse at about 20°C with continuous light ("CL_{20°}"), i.e. natural daylight supplemented by MLL 160W Philips lamps, giving at plant level at least 1750 erg.cm⁻².sec⁻¹.
2. Two growth cabinets at 20°C and a relative humidity of 80% for the different photoperiods. Light was obtained from 40W 55 Philips fluorescent lamps + 60W Philips incandescent bulbs.
3. Two growth cabinets at 32°C and a relative humidity of 40% at the same light conditions as 2.

The photoperiod consisted of incandescent + fluorescent light. The photoperiod for all the treatments commenced at 8 AM. Light intensity in the growth cabinets was measured with a Selenium photocell for visible radiation (0.4 - 0.7 μ). Realization of the induced flowering was obtained in SD_{20°}.

The response to the treatments was measured as:

1. Percentage of generative plants.
2. Number of days till visible flower bud.

Experimental Results

Photoperiodic response curves

In order to construct photoperiodic response curves for 20° and 32°C, groups of 15 plants received 1, 2, 3, 4, 5, 6, 8, 10, 12 cycles of 8, 10, 12, 14, 16, 20 or 24h photoperiods at 20° or 32°C. Light intensity was 8000 erg/cm²/sec.

From the percentages of flowering plants the numbers of cycles necessary for 50% flowering were calculated (table 1) and these values were plotted against the photoperiod in figure 1. The 50% flowering data represents the reaction of a sample of a group of plants for a given treatment and greatly eliminates differently reacting plants.

Table 1

Fig. 1

The response curve for 20°C is similar to that constructed by LIVERMAN for about 22°C (LANG 1965) and agrees with response curves for most LD plants (BEST 1960). The curve is very steep for photoperiods shorter than 18-20h and levels off at longer photoperiods.

The shape of the 32° curve is quite different from the 20° curve. A critical photoperiod is not attained within the range of studied photoperiods. The curve is rather steep for photoperiods shorter than 16h and levels off at longer photoperiods. At 20-24h the 32° curve is quite the same as the 20° curve, indicating no influence of the high temperature.

Intermittent temperatures

To determine the most sensitive period for high temperatures of a light/dark cycle at a large range of photoperiods, groups of 60 plants received 4 hours 32°C at different periods of this cycle. For the remaining 20 hours of this cycle the plants were kept at 20°C . These treatments were realised by transferring the plants from one cabinet to the other. The controls remained at 20°C permanently. The lengths of the photoperiods were 8, 10, 12, 14, 16, 20 or 24h. Light intensity was $5000 \text{ erg.cm}^{-2}.\text{sec}^{-1}$. The treatment was given for 5 cycles. To reveal a suboptimal induction, 0, 1, 2 or 3 cycles $\text{CL}_{20^{\circ}}$ were added.

The most essential results are summarized in figures 2-8. Percentages of flowering plants (left ordinate, broken lines) and days till visible flower bud (right ordinate, solid lines), are plotted against the 32° treated period of the light/dark cycle (abscissa). Every 4 hours treatment is in this way indicated in the abscissa of the figures by the middle of the 4 hours, e.g. the treatment 18-22h is "20" in the figure. In the right top corners of each figure the number of cycles $\text{CL}_{20^{\circ}}$, as a second treatment, is mentioned.

Fig. 2-8

According to figures 2-6, for the photoperiods 8, 10, 12, 14 and 16h, the percentages are the highest, the number of days the lowest for periods 23(21-01), 01(23-03), 03(01-05), 04(02-06) and 06(04-08) respectively, so that these periods are the most sensitive.

Only a weak response was obtained with 20h photoperiod, 06(04-08) and 08(06-10) being the most sensitive (fig. 7). With 24h photoperiod there was no response (fig. 8).

In figure 9 the most sensitive periods are plotted against the photoperiod. Change from light to darkness is indicated.

Figure 9

The line, connecting the most sensitive periods, remains at a rather fixed distance from the light/dark change at 8-16h photoperiods and becomes uncertain at 16-24h photoperiods. This indicates the onset of darkness determines the most sensitive period, which is 6-7 hours after this onset.

Figure 10 gives the results of an intermittent temperature treatment at a photoperiod of 8h, followed by 3 cycles $CL_{20^{\circ}}$. As a result of this second treatment all plants flowered, but there is a considerable difference in number of days till visible flower bud.

Fig. 10

The number of days decreases gradually with increasing darkness for 7 hours after the onset of this darkness and then increases for the next 5 hours, a constant level is reached for the last 4 hours of darkness and the 8 hours of the light period. The control differs not significantly with this constant level. These responses indicate that during the dark period the sensitivity of the plant to high temperature gradually increases, reaches a maximum and then decreases. The last 4 hours of a 16 hour dark period and the light period are not sensitive.

Discussion

Flower induction in Silene armeria is regarded by WELLENSIEK (1966b) as a deblocking of a specific DNA particle. VAN DE VOOREN (1969a) extended this hypothesis by suggesting, that deblocking takes place in the photophase and is temperature insensitive. Blocking, taking place in the nyctophase, is very temperature sensitive, high temperature slowing it down. The two mechanisms of blocking and deblocking are in balance in SD₂₀. Shortening the nyctophase or raising the temperature during the nyctophase disturbs the balance and the blocking can be entirely removed.

In the light of this hypothesis the increase of the flowering percentage and the acceleration of budding can be considered as a reflection of the retarded rate of blocking.

From the intermittent temperature treatments it follows that at the onset of darkness the rate of blocking is slow, increases to a maximum after 6-7 hours, then slows down and finally stops after about 12 hours.

The response curves can be explained as follows. At photoperiods longer than 18-20h little or no difference was found between the 20°- and 32° curve, as little or no blocking occurs during the first hours of darkness. In this way the absence of a sensitive period and the slight difference in the required numbers of cycles for 50% flowering at the photoperiods 20 and 24h can also be explained. Every shortening of the photoperiod increases the blocking considerably, resulting in a higher number of cycles for 50% flowering. At dark periods of about 12h only a very limited increase in blocking occurs, hence the critical photoperiod has to be about 12h, which agrees with the critical photoperiod for E_1 given by WELLENSIEK (1969). At photoperiods, shorter than the critical, high temperature has a strong effect on flowering, as the balance of blocking and deblocking processes are disturbed by a slowing down of blocking and an extending of deblocking.

The hypothesis on the flower inducing mechanism of Silene armeria becomes in brief, that flower induction depends on the balance of two reactions:

1. A deblocking process in the light; temperature insensitive.
2. A blocking process in the darkness, starting shortly after the onset of darkness, reaching its maximum after 6-7 hours and decreasing afterwards; very temperature sensitive, high temperature slowing it down.

Literature

- BEST, R.: Proc.Kon.Ned.Akad.Wet. C63, 676-691 (1960).
- LANG, A.: Encycl. Plant PHysiol. XV (1), 1380-1536 (1965).
- LANG, A. and MELCHERS, G.: Planta 33(5), 653-703 (1943).
- LIVERMAN, J.L.: Doctor's Thesis Caltech. 203 p. (1952).
- VOOREN, J. VAN DE: Z. Pflanzenphysiol. 61(2), 135-139 (1969a).
- " " " " : Z. Pflanzenphysiol. 61(4), 332-337 (1969b).
- WELLENSIEK, S.J.: Z. Pflanzenphysiol. 54(5), 377-385 (1966a).
- " " " : Naturwiss. 53(16), 411 (1966b).
- " " " : Z. Pflanzenphysiol. 61(5), 462-471 (1969).

Ir. JAN VAN DE VOOREN, Landbouwhogeschool, Laboratorium voor
Tuinbouwplantenteelt, Haagsteeg 3 (Postbus 30), Wageningen,
The Netherlands.

Table 1

Percentages of flowering plants in SD_{20°} after varying numbers of cycles of different photoperiods, and the numbers of cycles necessary for 50% flowering plants.

	Length of photoperiod (h)							
	8	10	12	14	16	18	20	24
	at 20°C							
cycles								
1	-	-	-	-	-	0	5	10
2	-	-	-	0	0	0	20	35
3	-	-	-	-	-	40	70	100
4	-	-	-	0	7	67	100	100
5	-	-	-	-	-	87	100	100
6	-	-	0	7	20	100	100	100
8	0	0	7	27	100	100	-	-
10	0	7	7	53	100	100	-	-
12	0	0	20	100	-	-	-	-
50% flowering	5	5	5	9.3	6.5	3.6	2.6	2.1
	at 32°C							
cycles								
1	-	-	-	-	-	7	5	5
2	0	0	0	13	33	20	10	10
3	-	-	-	-	-	40	60	85
4	7	7	13	33	60	93	100	100
5	-	-	-	-	-	100	100	100
6	27	47	100	100	100	100	-	-
8	40	93	100	100	100	-	-	-
10	73	100	-	-	-	-	-	-
12	100	100	-	-	-	-	-	-
50% flowering	8.1	6.1	4.7	4.1	3.1	2.9	2.8	2.5

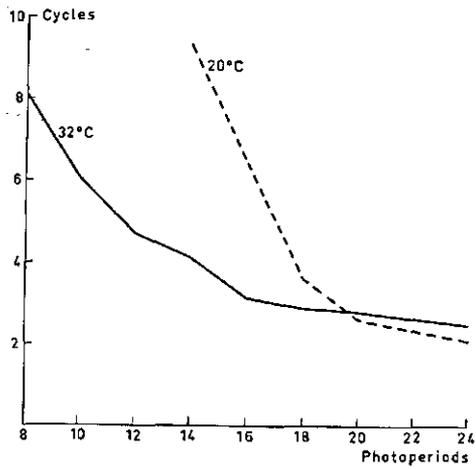


Fig. 1 Photoperiodic response curves for 20° and 32 °C. The numbers of cycles required for 50% flowering (ordinate) for different photoperiods (abscissa) at 20° and 32 °C.

Fig. 2-8 Percentages of flowering (broken lines, left ordinate) and number of days till flower bud formation (solid lines, right ordinate) after different 4 hours high temperature treatments (abscissa) for photoperiods 8, 10, 12, 14, 16, 20 and 24 h respectively. A second treatment of CL_{20}° is indicated in the right corner of each figure. (See also text).

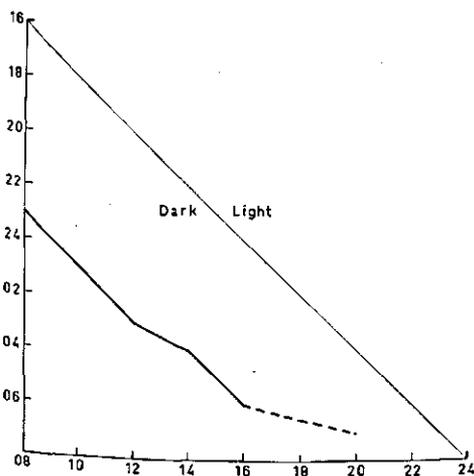
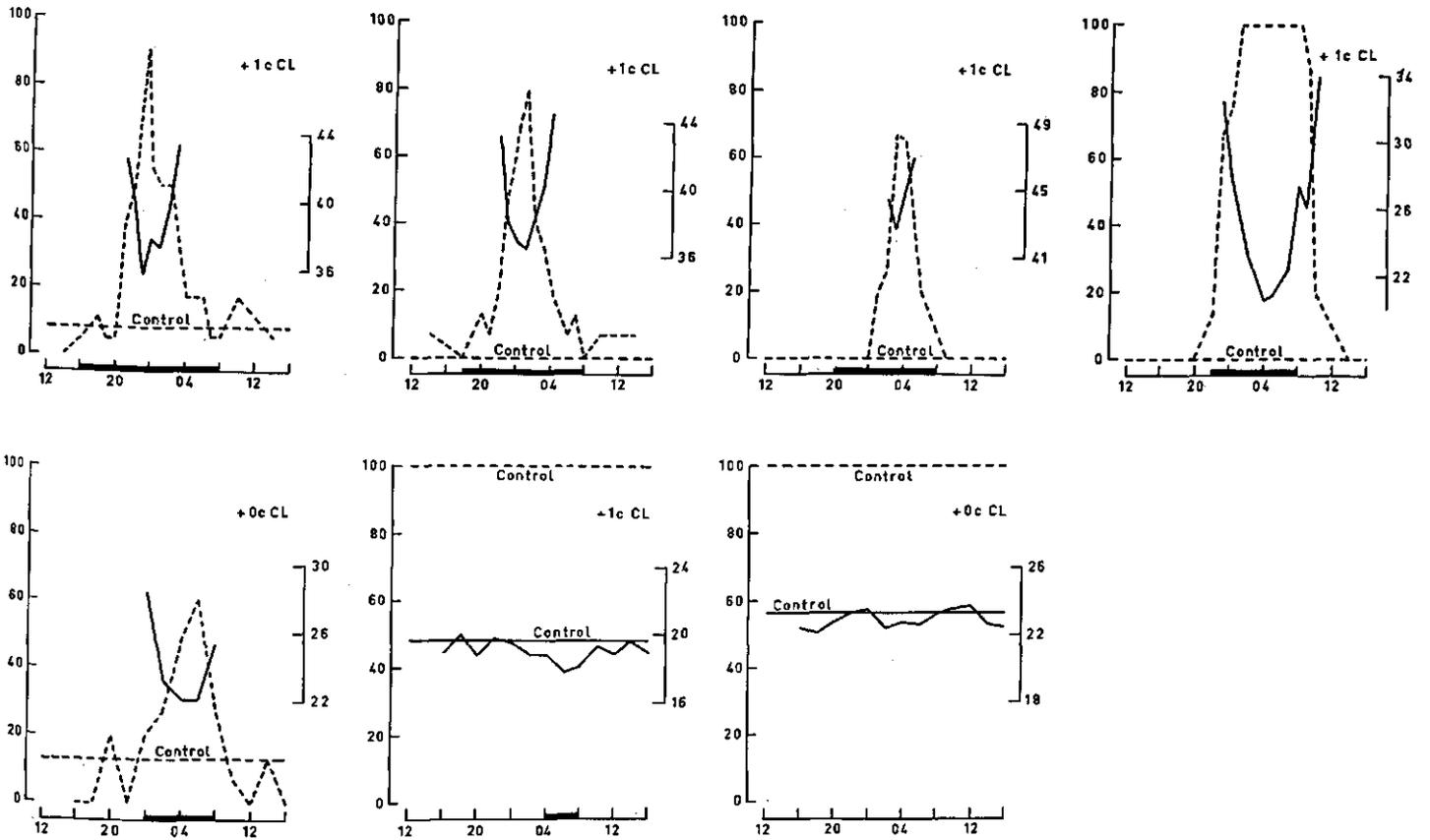
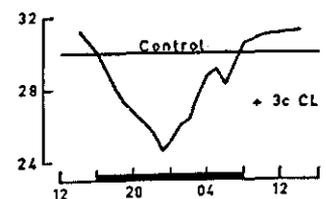


Fig. 9 The most sensitive period of a light/dark cycle (ordinate) at different photoperiods (abscissa). The numbers on the ordinate represents the time of the day. Change of light and darkness is indicated in the figure by the straight line from 16 (ordinate) to 24 (abscissa).

Fig. 10 Number of days till flower bud formation (ordinate) after different 4 hours high temperature treatments (abscissa) and a second treatment of 3 cycles CL_{20}° . (See also text).



Laboratorium voor Tuinbouwplantenteelt, Landbouwhogeschool,
Wageningen, the Netherlands. Publication 345.

The Influence of High Temperature on the Flower Inducing
Mechanism of Silene armeria L.

IV Temperature Level

JAN VAN DE VOOREN

Received , 1970

Summary

Silene armeria, a qualitative long day plant at 20°C is induced to flower formation by a short period of high temperature during the middle of a 16h dark period of a short day cycle.

The influence of the temperature level during this period was investigated.

No influence of temperatures in the range 10° - 30°C and an increasing response of temperatures in the range 35° - 50°C was found.

Increasing temperature from 30° - 35°C is supposed to slow down increasingly the rate of blocking during the middle of the dark period, so that the balance of blocking and deblocking becomes disturbed, which can result in the formation of a flowering hormone.

Zusammenfassung

Silene armeria, bei 20°C eine qualitative Langtagpflanze, wird zur Blütenbildung induziert durch kurze Zeitabschnitte von hohen Temperaturen während der Mitte der Dunkelphase einer Kurztagzyklus.

Der Einfluss der Temperaturhöhe während dieser Periode wurde festgestellt.

Es zeigte sich keine Wirkung bei Temperaturen von 10 bis 30°C und eine zunehmende Wirkung ab 35° bis 50°C.

Die Wirkung zunehmender Temperaturen ab 30° - 35°C wird als eine zunehmende Hemmung der Blockierung erklärt. Diese Erstörung der Waage zwischen Blockierung und Deblockierung führt zur Blütenhormonbildung.

Introduction

High temperature has a long day ("LD") effect on flower formation during short day ("SD") conditions. This involves that SD plants are inhibited to flower at high temperatures under SD conditions, as was shown for Xanthium strumarium (HAMNER et al. 1938), Biloxi soybean (PARKER et al. 1943), Kalanchoë blossfeldiana (SCHWEMMLE 1957), Pharbitis nil (NAKAYAMA 1958), Lemna perpusilla 6746 (HILLMAN 1959), Chenopodium amaranticolor (SCHWEMMLE 1960). On the other hand, LD plants are induced to flower at high temperatures under SD conditions, as was shown for Silene armeria (LIVERMAN 1952). Hyoscyamus niger (SCHWEMMLE 1960), Scrofularia alata, Stenactis annua (CHOUARD et al 1965).

The temperature has to be more than about 30°C to give this effect on flower induction. For Pharbitis nil (NAKAYAMA 1958) and Lemna perpusilla (HILLMAN 1959) the critical temperature is 31.5°C. WELLENSIEK (1965) found an increasing response from 30° to 34°C for Silene armeria.

The high temperature acts exclusively during a special part of the dark period, as was found for Pharbitis nil (NAKAYAMA 1958), Lemna perpusilla 6746 (HILLMAN 1959), Chenopodium amaranticolor, Hyoscyamus niger (SCHWEMMLE 1960), Xanthium strumarium (SALISBURY 1963) Silene armeria (VAN DE VOOREN 1969a). For Silene armeria VAN DE VOOREN (1970) found a maximal effect of the high temperature, when applied during a period of 4 hours and determined this sensitive period more precisely at about 7 hours after the onset of darkness for photoperiods of 8 till 16 hours.

The scope of the present paper is to study a wide range of temperatures for Silene armeria by means of a 4h temperature treatment during the 5th till 9th h dark period.

Materials and Methods

Strain E₁ of Silene armeria L. was used exclusively. For details see WELLENSIEK (1969) and VAN DE VOOREN (1969b).

The following equipment was used:

1. A greenhouse at about 20°C with SD ("SD_{20°}") for growing vegetative plants and realization of induced flowering. In winter natural day light was supplemented by HPLR 400W Philips lamps.
2. A growth cabinet with alternating temperatures. The 8h photoperiod was obtained from 40W 55 Philips fluorescent lamps + 120W Philips incandescent lamps, giving at plant level 23.000 erg cm⁻²sec⁻¹.
3. A greenhouse at about 20°C with continuous light ("CL_{20°}"), i.e. natural day light supplemented by MLL 160W Philips lamps, giving at plant level at least 1750 erg cm⁻²sec⁻¹.

Change of air and leaf temperature was achieved in some minutes, soil temperature lagging about half an hour behind.

Realization of the induced flowering was obtained in SD_{20°}.

The response to the treatments was measured as:

1. Percentage generative plants.
2. Number of days till visible flower bud.

Experimental results

To investigate the influence of the temperature level groups of 60 plants received 4, 8 or 12 cycles of 10°, 15°,.....50°C during the 5th till 9th h of a 16 h dark period of a SD cycle. Temperature of the rest of the cycle was 20°C. The temperature of the control was continuously 20°C. To reveal a suboptimal induction 0, 1, 2 or 3 cycles CL₂₀° were added. 15 plants were used per treatment.

The essential results are listed in table 1.

Table 1

For the range 10° - 30°C no significant difference is found, however for range 35° - 50°C a significant increase in percentages generative plants and acceleration in days, compared with the control, is apparent. A second treatment of 3 cycles CL₂₀° gave always 100% generative plants. These results indicate, that the influence of high temperature on flower induction starts at 30° - 35°C and increases with higher temperature. The acceleration in number of days for treatment 50°C is, at least partly, influenced by high temperature damage of the plants.

Discussion

As hypothesis on the flower inducing mechanism of Silene armeria VAN DE VOOREN (1970) suggested the balance of two reactions:

1. A deblocking process in light; temperature insensitive.
2. A blocking process in darkness, starting shortly after the onset of darkness, reaching its maximum after 6-7 hours and decreasing

afterwards; temperature sensitive, high temperature slowing it down.

The increase of the flowering percentages and the acceleration of days till budding in high temperature treatments can be considered as a reflection of the slowed down rate of blocking.

The obtained results with regard to the temperature level can be explained as follows. Temperatures of 10 to 30°C have no effect on blocking, whereas temperatures from 30°C have an increasing retarding effect on blocking. The critical value agrees well with the results for the SD plants Pharbitis nil (NAKAYAMA 1958) and Lemna perpusilla 6746 (HILLMAN 1959).

It is not possible to decide, whether 45°C represents an optimum, because 50°C causes damage to the plant.

Literature

- CHOUARD, P. et al.: Bull.Soc.frang.Physiol. Végétale 11 (1), 85-94 (1965).
- HAMNER, K.C. and BONNER, J.: Bot.Gaz. 100, 388-431 (1938).
- HILLMAN, W.S.: Amer.J. Bot. 46, 489 (1959).
- LIVERMAN, J.L.: Doctor's Thesis Caltech. 203 p. (1952).
- NAKAYAMA, S.: Sci.Rep. Tôhoku Univ. 4 Ser. 24 (4), 137-183 (1958).
- PARKER, M.W. and BORTHWICK, H.A.: Bot.Gaz. 104, 612-619 (1943).
- SALISBURY, F.B.: Planta 59, 518-534 (1963).
- SCHWEMMLE, B.: Naturwiss. 44 (12), 356 (1957).
- " : Naturwiss. 47 (3), 68-69 (1960).
- VOOREN, J. VAN DE: Z. Pflanzenphysiol. 61 (2), 135-139 (1969a).
- " : Z. Pflanzenphysiol. 61 (4), 332-337 (1969b).
- " : Z. Pflanzenphysiol. in press (1970).

WELLENSIEK, S.J.: Z. Pflanzenphysiol. 54 (5), 377-385 (1965).

" : Z. Pflanzenphysiol. 61 (5), 462-471 (1969).

Ir. JAN VAN DE VOOREN, Landbouwhogeschool, Laboratorium voor
Tuinbouwplantenteelt, Haagsteeg 3 (Postbus 30), Wageningen,
the Netherlands.

Table 1

Percentages generative plants and acceleration of days till budding after 12 cycles of 4 hours of different temperatures during the 5th-9th h of a 16h dark period and second treatments of 0, 1 or 3 cycles CL_{20°}.

Temperature	cycles		CL _{20°}
	0	1	3
	%	%	days
10°	0	0	0.7
15°	0	0	-0.2
20°	0	0	0.0
25°	0	0	0.4
30°	13	7	0.7
35°	20	93	1.6
40°	57	100	5.0
45°	100	100	7.2
50°	100	100	5.0

STELLINGEN

I.

De uitspraak, dat het bloeihormoon door hoge temperaturen vernietigd wordt, is onjuist.

F. B. SALISBURY, *The flowering process*, p. 165 (1963).

II.

Hoge temperaturen gedurende een bepaald gedeelte van de nacht kunnen een vervanging zijn van dagverlenging met gloeilampen.

III.

Het kwantitatieve aspect van niet-bloei is meetbaar.

A. LANG, *Enc. Plantphysiol.* XV (1), 1390 (1965).
M. K. JOUSTRA, *Meded. Landbouwhogeschool, Wageningen* 70 (19), 78 p., (1970).

IV.

Bloefysiologie kan van grote waarde zijn voor de plantensystematiek.

V.

Stengelstrekking en bloei worden grotendeels door dezelfde omstandigheden geïnduceerd.

M. KH. CHALAKHYAN, *Flowering hormones of plants*, in: *Biochemistry and plant growth substances* ed. Wightman and Setterfield, 1317-1340 (1968).

VI.

Het scheppen van uitgebreide recreatiemogelijkheden aan de rand van natuurgebieden komt de rust in deze gebieden zeer ten goede.

VII.

De doeltreffendheid van het persoonlijk literatuursysteem zou sterk bevorderd worden door verwerking op ponskaarten, gekoppeld aan een computerprogramma.

K. W. BRIDGES, *BioScience* 20 (2), 94-97 (1970).