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FLORAL INDUCTION, FLORAL HORMONES AND FLOWERING

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Abbreviations, Symbols and Terms.

LD	= long day, 16 h light and 8 h darkness.
SD	= short day, 8 h light and 16 h darkness.
LDP	= long-day plant.
SDP	= short-day plant.
LSDP	= long-short-day plant.
DNP	= day-neutral plant.
CL	= continuous light.
r	= receptor, being the non induced graft partner.
r–	= defoliated receptor.
d	= donor, being the induced graft partner.
r/r	= receptor grafted on receptor (control).
r/d	= receptor grafted on donor.
d/r	= donor grafted on receptor.
$d/r_1 \rightarrow r_1/r_2$	= shoot of a grafted receptor r_1, regrafted on a new receptor r_2.
nr	= treatment number.
q	= quotient of the number of flowering receptors (in a grafting-experiment) or flowering plants (in an induction experiment) on the total number of survived receptors or plants.
%	= percentage of flowering plants or shoots.
days	= average number of days from the beginning of a treatment until macroscopically visible flower buds.
GA_3	= gibberellic acid, gibberellin number 3.
°l	= temperature in °C during the light.
°d	= temperature in °C during the dark.
h	= hour.
sum	= sum of the products of temperature times hours per 24 hours.
induction	= deblocking of the synthesis of floral hormone.
floral hormone	= one or more substances, usually synthesized in induced leaves and initiating the formation of floral primordia in the receptive meristems.

1. GENERAL INTRODUCTION

1.1. HISTORICAL REVIEW

Since the invention of an incandescent lamp by EDISON in 1879 many investigators showed that flowering of several horticultural plants could be accelerated by extending natural daylengths with the aid of these lamps (EVANS, 1969). The general explanation was that the growth and development were promoted by additional light. However, TOURNOIS (1912) was the first to demonstrate the promotive influence of short daylength on the flowering of hops and hemp. It was KLEBS (1913), investigating the influence of continuous light on the flowering of *Sempervivum funkii*, who concluded that the additional light was acting 'catalytically and not as a nutritional factor'.

The recognition of daylength as a major flowering controlling factor dates from GARNER and ALLARD (1920), who found the SD response of Mammoth tobacco and Peking soybeans and introduced the terms photoperiod for daylength, photoperiodism for the response of organisms to the relative lengths of light and darkness.

Experiments by GARNER and ALLARD (1925) with COSMOS and by RAZUMOV (1931) on photoperiodic control of tuberization in potatoes suggested that it were the leaves which initially respond to daylength.

The term photoperiodic induction was introduced into flowering-physiology by LUBIMENKO and SZEGLOVA (1932). KNOTT (1934) gave different daylengths separately to the shoot apex and to the leaves of spinach, demonstrated that the leaves are the organs reacting to daylength, and concluded that the response to a photoperiod, favourable for reproductive growth, may be the production of some substance or stimulus, transported from the leaves to the growing-point. In 1865 SACHS already suggested the existence of 'flower forming substances' which could move within a plant (in EVANS, 1969). KNOTT's technique was used by other investigators, who found similar mechanisms in several plants.

The definite proof of the concept of a floral hormone followed soon from grafting-experiments, in which was shown that receptor plants under non inductive conditions could be brought to flowering by grafting them to donor plants of the same strain, which had previously been kept under inductive daylength conditions. The first experiments of this kind were carried out by KUIJPER and WIERSUM (1936) with *Glycine max* and almost simultaneously by CHAJLAKHYAN (1936b) with *Perilla* and *Helianthus* and MOSHKOV (1937) with tobacco. CHAJLAKHYAN (1936a) postulated that a flower hormone regulates the processes of development. He christened the hypothetical substance *florigen*. Afterwards CHOLODNY (1939) proposed the term *anthesin* and VAN DE SANDE BAKHUYZEN (1947) following WENT's nomenclature (1938) of organ-forming substances introduced the term *anthocaline*. Many cases of successful transmission of flowering by grafting followed. LANG (1965) summarized about 35 cases.

The nature of floral hormone is often discussed. Evidence for a more general character came from experiments in which donors of one daylength response type could induce flowering in receptors of a different response type. Extracts of flowering plants have never induced reproduceable flower formation in vegetative plants. Moreover none of the efforts of chemical identification of the floral hormone met with success. So the character of floral hormone can until now only be studied in vivo, by inducing parts of plants and by grafting experiments.

Besides floral hormones, in some plants floral inhibitors could be demonstrated. BHARGAVA (1964, 1965) even proved transmissable floral inhibitors in *Salvia occidentalis* and *Perilla crispa*. In the latter case a hormone-like floral inhibitor is formed, which is directly translocated to the growing-tip, where it interferes with the functioning of the floral stimulus. This inhibitor does not interfere with the production or the translocation of the floral stimulus.

Another factor which may determine flowering is temperature, with a mechanism, sometimes differing from photoperiodism. The action of low temperature has been called vernalization by WHYTE and HUDSON (1933). In most cases the site of perception of low temperature is not separated from the place where the effect manifests itself: both are localized in a growing apex (WHYTE, 1948), or perhaps in a meristem. For biennial *Hyoscyamus niger*, MELCHERS (1936) showed that the low temperature requirement can be fulfilled by grafting a vernalized growing-point near to a non-vernalized one. After grafting with the short-day plant 'Maryland Mammoth' tobacco, flower formation in cold requiring *H. niger* occurred irrespective whether the donors were kept in short day or in long day. So, even when this tobacco variety was not capable of flowering itself, it could still supply a stimulus which caused flowering in the unvernalized *H. niger* stocks. Therefore, MELCHERS (1939) concluded that the transmissable stimulus in biennials (produced after a low-temperature treatment) is different from floral hormone. To stress their specific natures he called the former vernalin.

A second case of temperature determining flowering is induction by high temperature. VAN DE VOOREN (1969) intensively worked out this phenomenon for the LDP *Silene armeria*. He concluded that high temperatures above 30 °C. prevent a process, which is supposed to be the rebuilding of an inhibition ('reblocking') during the dark period.

Besides photoperiodism and temperature, some chemicals, especially some gibberellins, promote flowering. Studies about differences in behaviour of floral hormones in different plants gave more information about the character of the floral hormones. Until now only 3 plants have been reported with the particular property that receptors, brought into flowering by grafting, can act as a donor in a next grafting. These plants are *Xanthium strumarium* (LONA, 1946), *Bryophyllum daigremontianum* (ZEEVAART and LANG, 1962) and *Silene armeria* (WELLENSIEK, 1966). In literature this phenomenon is called: 'indirect induction by the floral hormone' (ZEEVAART and LANG, 1962, p. 537); 'auto-

catalytical multiplication of the floral hormone' (WELLENSIEK, 1966, p. 9) or 'secondary induction' (EVANS, 1971, p. 378). Classic is the comparison of *Xanthium strumarium* and *Perilla crispa*, worked out by ZEEVAART (1958). In contrast to *Xanthium*, receptors of *Perilla*, in spite of a flowering-reaction, do not receive donor capacity by grafting. This raised the question of the existence of different floral hormones.

1.2. SCOPE OF THE PRESENT EXPERIMENTS

Let us first define some frequently used terms. '*Induction*' will be restricted to '*deblocking of the synthesis of floral hormone*', indicating the process by which an inhibition or blocking of flower formation in vegetative plants is removed, followed by synthesis of floral hormone (WELLENSIEK, 1969). '*Floral hormone*' stays for: '*one or more substances of an unknown composition, synthesized usually in induced leaves, and initiating the formation of floral primordia in the receptive meristems.*' A choice out of the terms '*indirect induction*', '*autocatalytically multiplying floral hormone*' and '*secondary induction*' could not be made since until now no clear evidence about the mechanism of the synthesis of floral hormone in non exogenously induced parts of plants could be demonstrated. It is questionable whether floral hormone can act as an induction factor, or whether it can multiply itself autocatalytically. Therefore, the term '*non-localized synthesis*' is used, indicating that the synthesis is not restricted to the induced part of the plant, contrary to the clear example of *Perilla crispa* with strictly '*localized synthesis*'. The properties of floral hormones were studied by investigation of factors influencing its synthesis, its transmission and its activity, and by trying new graft combinations. The central question is whether other explanations for differences in behaviour of floral hormone can be given than the assumption of the existence of different floral hormones. Comparison of the plants with non-localized synthesis of floral hormone mutually and with other plants was supposed to give useful information. Therefore, the former plants and plants of their families, being the *Compositae*, *Crassulaceae* and *Caryophyllaceae*, were chosen as experimental material.

2. MATERIAL AND METHODS

2.1. PLANT MATERIAL

Crassulaceae:

Bryophyllum daigremontianum (R. HAMET et PERR.) BERG., originating from the Laboratorium voor Tuinbouwplantenteelt, Wageningen. Propagated vegetatively by means of leaf plantlets.

Bryophyllum pinnatum (LAM.) OKEN kindly supplied by Prof. J. TH. HACKMAN, Enschede. Propagated vegetatively by means of cuttings.

Kalanchoë blossfeldiana cv. 'Vulcan' and cv. 'Hendrik van der Dussen' were obtained from commercial sources. The cv. 'Annette' and cv. 'Josine' were kindly supplied by Miss L. LEFFRING, Proefstation Aalsmeer. All these cultivars were propagated vegetatively by means of cuttings.

Kalanchoë jongmansii, HAMET et PERRIER.

Kalanchoë manginii, HAMET et PERRIER.

Both these species were kindly supplied by the Botanic Garden of the University of Leiden. Propagation vegetatively by means of cuttings.

Compositae:

Xanthium strumarium L., from the Laboratorium voor Tuinbouwplantenteelt, Wageningen. This strain was originally obtained from DR. J. A. D. ZEEVAART, U.S.A. Two other strains were kindly supplied by Prof. C. MACMILLAN, U.S.A. The nomenclature is according to SALISBURY (1969). The propagation was generative.

Rudbeckia bicolor NUTT., obtained from a commercial source and propagated generatively.

Caryophyllaceae

Chosen from a collection of 50 species, brought together by WELLENSIEK (unpublished) and determined by Mrs. N. E. NANNENGA-BREMEKAMP of the Herbarium Vadense, Wageningen.

Silene armeria L., originally obtained from DR. A. LANG, U.S.A. The other species are listed in table 39 (p. 60–62). The propagation was generative.

Labiatae

Perilla crispa (THUMB.) TANAKA. The nomenclature is according to ZEEVAART (1969b). Seeds were obtained from a commercial source and the propagation was vegetative by cuttings.

2.2. Growing and grafting

Plants were grown in a greenhouse at approximately 20°C. For LD natural day light was supplemented by MLL 160 W Philips lamps, giving at plant level at least 1750 erg. cm^{-2}. sec^{-1}. The generally used LD conditions were 16 h light and 8 h dark. In order to reduce the influence of possible technical disturbances, the LD conditions for *Xanthium strumarium* were kept at 20 h light and 4 h darkness. Cuttings were made in a mist propagation bench. Temperature experiments were done in a phytotron with TL 55 Philips lamps and a light intensity of about 35000 erg.cm^{-2}. $sec.^{-1}$ at plant level.

The mostly used method of grafting was an ordinary cleft graft in the stem. The place of grafting was surrounded by a spongy rubber band, called stericrepe, made by Beacon and Janis LTD, Southampton Row, London, W.C.I. Another method was a cleft graft in the rosette. In this case the graft partners were held together by ordinary hair grips (curly grips). According to literature, receptor rosettes are often treated with GA_3 for stem elongation and split grafting in the stem is applied in the elongated stem. However, the rosette-grafting technique was developed and used in those cases where the receptor rosette could not be elongated by GA_3 without the risk of partial or complete induction by GA_3 as such. Photo 1 shows a rosette grafting of *Silene armeria*. The grafted plants were kept in a plastic cabinet in a controlled LD or SD room at 20°C with TL 29

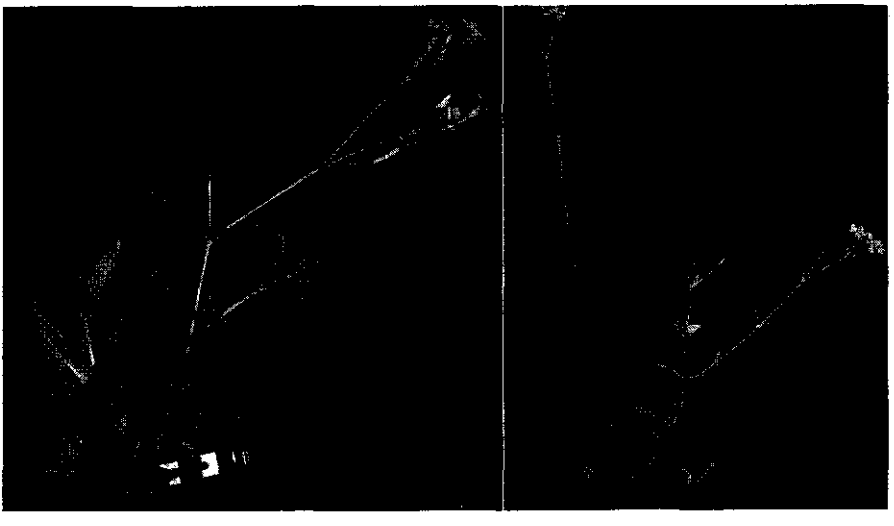


Photo 1. *Rosette-grafting d/r.*
A donor shoot of *Silene armeria* was cleft grafted in a vegetative rosette of the same species. The graft partners were held together by an ordinary hair grip.
Photo left: 3 weeks after the grafting.
right: 7 weeks after the grafting, the receptor is flowering.

Philips lamps of 65 W, resulting in a light intensity of about 8000 erg. cm^{-2}. sec^{-1}. at plant level. In the plastic cabinet the air humidity was kept high by misting twice a day with water, mixed with 1 g/l TMTD to protect the plants against fungi. When the graft union had been established, the plants were hardened off, and put in the LD or SD division of a greenhouse.

3. EXPERIMENTS WITH SOME CRASSULACEAE

3.1. INTRODUCTION

Most of the *Crassulaceae* are natives of Madagascar. Besides the ornamental value of *Kalanchoë blossfeldiana*, the family of the *Crassulaceae* proved to be of great interest for research on the physiology of flowering.

RODENBURG (1939) already noted that short days accelerate flowering of *K. blossfeldiana* and HARDER and his school (1940) confirmed that this species represents a typical short-day plant. On account of the quantitative relation between induction and the number of flowers produced, this plant has been useful in numerous investigations into photoperiodic behaviour and the underlying mechanisms.

DOSTÀL (1949, 1950) discovered the dual daylength requirement of *Bryophyllum crenatum* and *B. tubiflorum* (= *B. verticillatum*). Both species remained vegetative if kept in continuous LD or SD for two years, while the shift LD → SD did result in flower formation. RESENDE (1952) found that *B. daigremontianum*, *K. moçambicana*, *K. rotundifolia* and *Aloë bulbillifera* also needed the shift LD → SD for flower induction and called this new class of photoperiodically sensitive plants long-short-day plants (LSDP).

Soon after gibberellic acid (GA_3) was discovered as a chemical which could induce flowering in a number of cold-requiring plants, BÜNSOW and HARDER (1956) reported thet GA_3 can substitute for the LD but not for the SD requirement in two *Bryophyllum* species.

DOSTÀL (1949, 1950) reported that floral hormone could be transmitted from a donor to a receptor in *B. crenatum* and *B. tubiflorum*. ZEEVAART (1958) extended these observations to *B. daigremontianum*, and RESENDE (1959) proved transmission of flowering between hybrids of different species of *Bryophyllum*. CARR and MELCHERS (1954) succeeded in transmitting the floral hormone from induced plants of *K. blossfeldiana* acting as donors to non-induced receptors. They also could prove transmission of the floral hormone of *Sedum kamtschaticum* as a donor to *K. blossfeldiana* as a receptor. ZEEVAART (1958) reported positive transmission of the floral hormone of *Kalanchoë blossfeldiana* to *Sedum spectabile* and *S. ellacombianum* and from both these long-day plants to *K. blossfeldiana*.

Surveying these results, the question arose whether the family of the *Crassulaceae* has one universal floral hormone, independent from the differences in daylength requirement and growth habit. In the present experiments factors influencing induction, production of floral hormone and reaction on floral hormone were investigated, especially temperature and some new graft combinations. The floral mechanisms of some *Crassulaceae* were compared, particularly the relations between *K. blossfeldiana* and *B. daigremontianum*.

3.2 Influence of temperature on the flowering of some Crassulaceae

3.2.1. *Introduction.*

Harder and Von Witsch (1940) found that a change of temperature per cycle of SD during the induction of *K. blossfeldiana* gives a stronger floral impulse than a constant temperature.

It were Zeevaart and Lang (1962), who discovered that induction of *B. daigremontianum* and *B. crenatum* by LSD as well as by GA_3-treatments in SD, is only possible when a day temperature of 23 °C is combined with a night temperature of 15 °C or less. A night temperature of 19 °C or higher prevented the induction. So it is clear that temperature plays an important role during induction of *K. blossfeldiana*, *B. daigremontianum* and *B. crenatum*.

In the present experiments the need for thermoperiodicity during induction of *K. blossfeldiana* and *B. daigremontianum* is compared.

Harder and Von Witsch (1940) and Rünger (1959) found that in *K. blossfeldiana* the flowering influenced by temperature is light independent. This means that the low temperature can act in the light as well as in the dark period. The question arose whether the reaction of *B. daigremontianum* is similar to *K. blossfeldiana* as found by Harder and Von Witsch, cited above.

B. daigremontianum being a LSDP has two inductive reactions, one in LD and one in SD. The influence of temperature on these two daylength reactions could give more information about these processes.

Another important question will be: What is the influence of the external factors on the flowering after induction and are there differences in comparison with the inductive process? Resende and Viana (1948) already discovered that generative *B. daigremontianum* becomes vegetative when the induced leaves received light of a low intensity. Resende (1965) reported that in LD with warm nights the inflorescences of *B. daigremontianum* tend to revert to vegetative growth. In my first grafting-experiments the generative *B. daigremontianum* donor rootstocks became vegetative. These results show that not only induction, but also the induced state can be influenced by external and unknown factors. The influence of temperature on the induced state of *B. daigremontianum* and *K. blossfeldiana* therefore is investigated.

3.2.2. *GA_3 + SD-induction of B. daigremontianum.*

Experiment 1. Plants grown in SD from plantlets were sprayed, at an age of 4 months, with GA_3 100 ppm once and brought to the phytotron, where they received SD at different temperature combinations. As *B. daigremontianum* can form various degrees of inflorescence development due to the level of induction (Penner, 1960), observations were made not only on flowering as such, but also on floral stage, total lengths of the upper 4 internodes, total lengths of the lateral floral shoots and the plant length. The upper 4 internodes were chosen, because they concern a normally developed inflorescence. The data were obtained 74 days after the beginning of the GA_3 + SD-induction and hence are

an instantaneous observation of the degree of inflorescence development due to different temperature treatments.

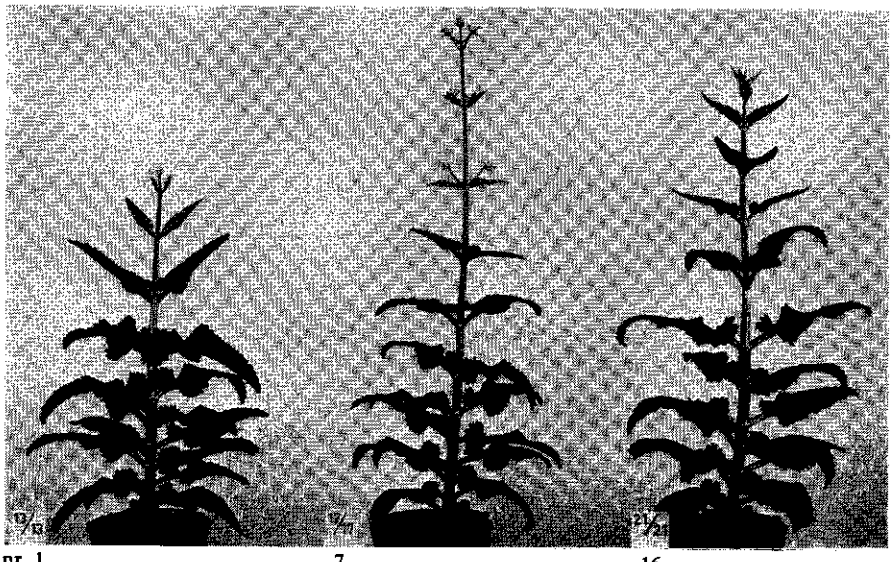
The main results are presented in Table 1 which shows that complete induction + realization (stage 6) are possible when the sum of the products of temperature times hours per 24 hours is 472 or less. A sum of 504 resulted in moderate flowering, whereas sums of 536 and higher did not have any effect. This corresponds with the conclusions of ZEEVAART and LANG (1962): induction is possible when a day temperature of 23 °C is combined with a night temperature of 15 °C or lower, meaning a temperature sum of 424 or lower. In their experiments no flowering occurred when a day temperature of 23 °C or higher was

TABLE 1. The flowering response of *B. daigremontianum* after GA_3 + SD-induction and realization at different temperature treatments.
stage = average stage of the flowering plants, according to PENNER (1960):
stage 0. Plants completely vegetative, no axillary shoots
stage 1. Axillary shoots grown out partially
stage 2. Vegetative inflorescence: axillary shoots fully developed, but no flower buds
stage 3. Inflorescence with a few flower buds; bracts show phyllody
stage 4. No phyllody, but inflorescence with very short internodes
stage 5. Normal inflorescence
stage 6. Normal inflorescence with additional inflorescences arising from the upper leaf pairs
internodes = mean sum of the lengths in cm of the upper 4 internodes
shoots = mean sum of the lengths in cm of the axillary floral shoots
* = no observiations made
Further abbreviations see p. 0
number of plants per treatment: 16

nr.	°l	°d	sum	%	days	stage	inflorescence	
							inter-nodes	shoots
1	13	13	312	100	43	6	20	18
2	21	9	312	100	37	6	*	*
3	17	13	344	100	43	6	24	22
4	25	9	344	100	37	6	*	*
5	13	17	376	100	36	6	29	33
6	21	13	376	100	37	6	37	45
7	17	17	408	100	35	6	29	26
8	25	13	408	100	30	6	43	66
9	13	21	440	100	33	6	17	27
10	21	17	456	100	*	6	*	*
11	17	21	472	100	*	6	*	*
12	25	17	472	100	*	6	*	*
13	4h13 + 4h21	21	472	100	*	6	*	*
14	2h13 + 6h21	21	488	100	*	5	*	*
15	13	25	504	100	43	5	16	17
16	21	21	504	100	69	4	2	0,2
17	17	25	536	0	∾	0	1,5	0
18	25	21	536	0	∾	0	1,5	0
19	21	25	568	0	∾	0	1,5	0
20	25	25	600	0	∾	0	1,5	0

combined with a night temperature of 19 °C or higher, meaning a temperature sum of 488 or higher.

Table 1 also shows that low temperature, tending to decline the temperature sum, works in the light as well as in the dark period. This means that the temperature influence is light independent. It appears that the combination of high day temperature with low night temperature (25°/13°, treatment 8) gives the best result. Comparison of treatments 7 and 8, 17°/17° and 25°/13° respectively, both with a temperature sum of 408, shows a better floral result for the latter.

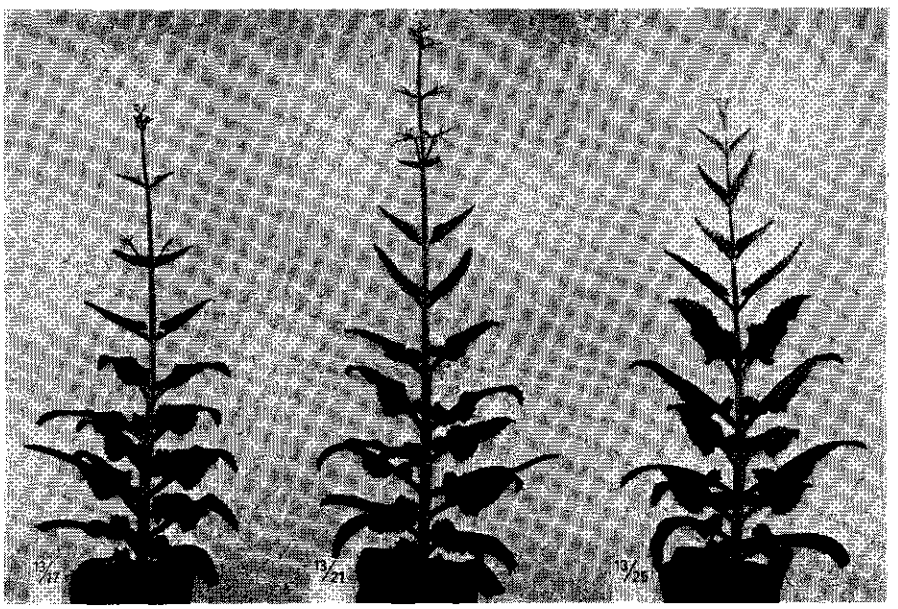


nr. 1 7 16
nr. = treatment number of table 1 (p. 9)

PHOTO 2. *Inflorescence development of B. daigremontianum as a result of GA_3 + SD-induction plus realization at constant temperatures of 13, 17 and 21 °C.*
Photo: 74 days after the beginning of the inductive treatment.

Photos 2, 3 and 4 show the influence of different temperature conditions on the flowering. (p. 10, 11, 12).

Experiment 2. In contrast to experiment 1 induction and realization are separated as well as possible. Similarly to those of experiment 1 plants were sprayed with GA_3 100 ppm once and brought to the phytotron, where they received SD at different temperature conditions for 21 days, followed by LD 21°/13°, where the flowering as the expression of induction plus realization was observed. Hence, besides the part of the realization which already occurred during the SD-treatment, the rest took place under the same temperature con-



nr. 5 9 15
nr. = treatment number of table 1 (p. 9)

PHOTO 3. *Inflorescence development of B. daigremontianum as a result of GA_3 + SD-induction plus realization at a day temperature of 13°C combined with night temperatures of 17, 21 and 25°C.*

Photo: 74 days after the beginning of the inductive treatment.

ditions, so that differences in flowering will mainly be due to the influence of the different temperature treatments during induction.

Table 2 shows that induction is possible when the temperature sum lies between 312 and 568, while even some flowering occurred between the sums of 248 and 312. Comparison with experiment 1 shows that induction is possible between wider margins of temperature than induction + realization. This suggests that mainly the realization of flowering is influenced by temperature. It is remarkable that there are no differences between the floral stages while the lengths of the inflorescences, measured 122 days after the beginning of induction, hardly differed. Treatment 12 with the temperature combination of 9°/21° shows the fewest days till visible flower buds, while 17°/17° at the same temperature sum of 408, results in 6 days later flowering.

Photo 5 demonstrates the results of treatments 14 and 25 of table 2. (p. 14)

Experiment 3. RÜNGER (1959) gave a SD-period of 12 days with a shift of temperature after 6 days. His results are cited in Table 3. Temperatures of 20°C or 25°C during the first period followed by 15°C or 20°C during the second 6 days are the best.

Similar to the experiment of RÜNGER (1959) with *Kalanchoë blossfeldiana,*

nr. 3 6 8
nr. = treatment number of table 1 (p. 9)

PHOTO 4. *Inflorescence development of B. daigremontianum as a result of GA_3 + SD-induction plus realization at a night temperature of 13°C combined with day temperatures of 17, 21 and 25°C.*

Photo: 74 days after the beginning of the inductive treatment.

one change of temperature during the whole inductive treatment of *B. daigremontianum* is tried in the next experiment. Young plants of *B. daigremontianum* obtained from plantlets and grown in the SD division of a greenhouse, were sprayed with GA_3 100 ppm and moved to the phytotron where they received different cycles of SD at temperature conditions presented in Table 4, followed by LD 21°l/13°d. (p. 14)

Treatment 1 demonstrates that 10 cycles of SD at 25°C are not sufficient for induction, while treatment 2 shows that induction is possible at 25°C, so the last 4 cycles of treatment 2 are necessary for induction. Comparison of treatments 2, 6 and 8 shows that induction during the first 10 cycles of SD occurs optimally, in spite of a temperature of 25°C. However, during the following 4 inductive cycles 13°C is better than 25°C, while treatments 3, 4 and 9 show that after induction a temperature of 25° acts negatively.

The conclusion is that hardly induction but rather the resulting processes are influenced by too high a temperature. This corresponds with the results of experiments 1 and 2.

TABLE 2. The flowering response of *B. daigremontianum* after GA_3 + 3 weeks SD-induction at different temperature combinations followed by LD 21 °l/13 °d.

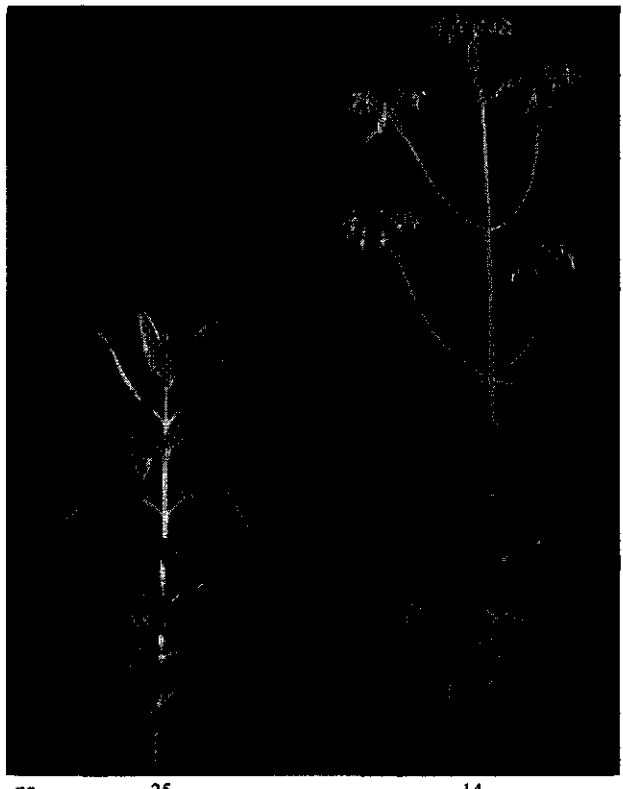
nr.	°l	°d	sum	q	%	days	stage	inflorescence	
								inter-nodes	shoots
1	9	9	216	0/8	0	∞	0	10	0
2	13	9	248	1/8	13	40	6	44	106
3	9	13	280	3/8	38	42	6	33	50
4	17	9	280	7/8	88	38	6	45	95
5	13	13	312	7/8	88	34	6	47	90
6	21	9	312	8/8	100	32	6	44	116
7	9	17	344	8/8	100	31	6	42	96
8	17	13	344	8/8	100	31	6	44	90
9	25	9	344	8/8	100	35	6	43	97
10	13	17	376	8/8	100	32	6	45	81
11	21	13	376	8/8	100	30	6	45	83
12	9	21	408	8/8	100	29	6	43	68
13	17	17	408	8/8	100	35	6	46	72
14	25	13	408	8/8	100	31	6	43	82
15	13	21	440	8/8	100	31	6	44	82
16	21	17	456	8/8	100	35	6	42	84
17	9	25	472	8/8	100	34	6	45	69
18	17	21	472	8/8	100	34	6	41	78
19	25	17	472	8/8	100	35	6	42	84
20	13	25	504	8/8	100	34	6	42	84
21	21	21	504	8/8	100	36	6	41	70
22	17	25	536	8/8	100	37	6	42	78
23	25	21	536	8/8	100	36	6	40	94
24	21	25	568	8/8	100	38	6	40	74
25	25	25	600	2/8	25	42	6	33	38

TABLE 3. Average number of axillary shoots with flowers of *K. blossfeldiana* as a result of one change of temperature after 6 days during an inductive period of 12 cycles of SD (Cited from RÜNGER, 1959).

Temp. in the first period of 6 days	Temp. in the second period of 6 days			
	15	20	25	30
	average number of axillary shoots with flowers			
15	1,6	0,6	0,0	0,0
20	4,7	4,1	0,0	0,0
25	5,9	3,2	2,2	0,0
30	1,6	3,8	0,4	0,0

3.2.3. *LSD-induction of B. daigremontianum.*

B. daigremontianum, needing 2 daylengths for induction, will have 2 daylength dependent processes preceding the synthesis of floral hormone. It is



nr. 25 14
nr. = treatment number of table 2 (p. 13)

PHOTO 5. *Inflorescence development of B. daigremontianum as a result of GA_3 + 21 days SD followed by LD 21°l/13°d.*
Left: SD at 25°l/25°d. Right: SD at 25°l/13°d
Photo: 74 days after the beginning of SD induction.

TABLE 4. Flowering of *B. daigremontianum* as a result of GA_3 + SD treatments, with different cycles of SD and no or one change of temperature, followed by LD 21°l/13°d.

nr.	GA_3 + SD treatment			flowering			
	days 13°	days 25°	days 13°	q	%	days	stage
1	0	10	0	0/8	0	∞	0
2	0	14	0	5/8	63	42	6
3	0	20	0	2/8	25	51	6
4	0	∞	0	0/8	0	∞	0
5	0	0	4	0/8	0	∞	0
6	0	10	4	8/8	100	31	6
7	0	0	10	0/8	0	∞	0
8	0	10	10	8/8	100	31	6
9	10	10	0	0/8	0	∞	0
10	20	0	0	8/8	100	45	6

questionable, when the LD component is available as a precursor, whether a separate SD component is formed or directly the floral hormone.

The inductive circumstances of these two daylength processes could be optimal at different temperature levels. In order to investigate this, the temperature must be altered in light and darkness, in LD as well as in SD. A problem is that with 5 different temperatures $5^4 = 625$ combinations are possible. Therefore a choice had to be made.

Experiment 4. Vegetative plants with 15 pairs of leaves, grown up in SD, were moved to a phytotron where they received 6 weeks of LD followed by SD, both at different temperature conditions. The flowering was observed in SD 76 days after the shift LS → SD and is due to the influence of temperature on induction + realization.

In Table 5, comparison of treatments 1, 2 and 3 shows that at a similar SD-treatment of 21°/13°, the LD-treatments at 13°/21° and 13°/25° have better effects on flowering than 17°/17°. Comparison of the treatments 3, 4, 5 and 6 shows that at the same LD-treatment of 17°/17°, the SD-treatment of 21°/13° is the best, while SD 13°/25° does not give flowering at all. So here is a clear parallelism with the influence of temperature on the GA_3 + SD-induction. It is obvious that the marginal LD-induction of 17°/17° will reduce the marginal temperature sum for SD-induction. Treatments 7, 8 and 9 show that LD-induction occurs very well at temperatures of a sum between 504 and 600. The following SD-induction however is not possible at a sum of 600.

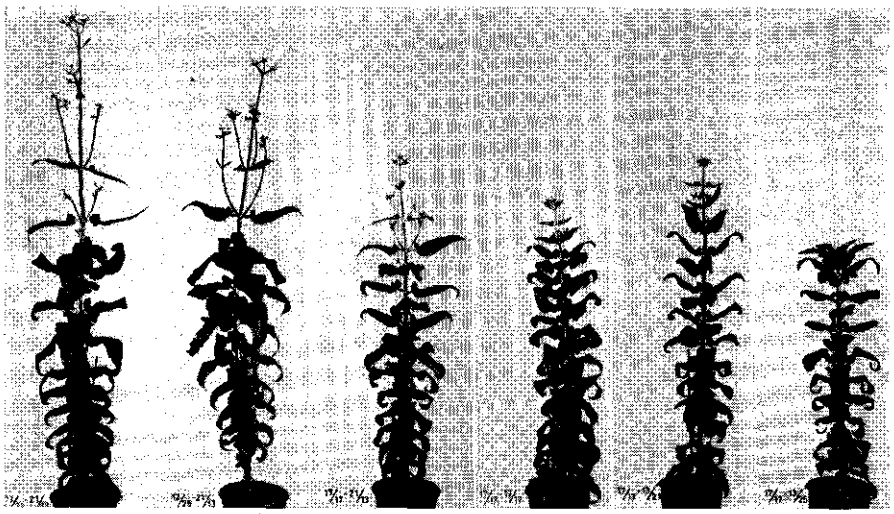
The flowering results are illustrated in photo 6. (p. 16)

3.2.4. *Influence of temperature on the flowering of B. daigremontianum.*

The preceding experiments clearly showed the influence of temperature on induction. However, what is the influence on the other floral processes like inductive state and synthesis and activity of floral hormone?

TABLE 5. The flowering of *B. daigremontianum* as a result of LSD-induction at different temperature combinations.
Number of plants per treatment = 5.
Annotations: internode length = average length of the upper 4 internodes
shoot length = average length of the total floral shoots.

nr.	LD			SD			flowering			plant length	inter-node length	shoot length
	°l	°d	sum	°l	°d	sum	%	days	stage			
1	13	21	376	21	13	392	100	35	6	139	51	110
2	13	25	408	21	13	392	100	40	6	135	47	118
3	17	17	408	21	13	392	100	45	6	102	24	65
4	17	17	408	17	17	408	100	47	3	93	18	22
5	17	17	408	13	21	440	80	41	4	95	17	25
6	17	17	408	13	25	504	0	∞	0	79	3	0
7	25	13	504	25	13	408	100	*	6	*	*	*
8	25	25	600	25	13	408	100	*	6	*	*	*
9	25	25	600	25	25	600	0	∞	0	*	*	*



nr. = treatment number of table 5

PHOTO 6. *Inflorescence development of B. daigremontianum as a result of LSD induction plus realization at different temperature combinations in LD and SD.*
Photo: 76 days after the shift LD → SD.

Experiment 5. Flowering plants, 6 weeks after the beginning of GA_3 + SD-induction, received different temperature treatments in SD.

Table 6 shows that floral development is influenced by temperature. Treatments 6 and 7 show that flowering plants can revert to the vegetative stage when the temperature sum per 24 hours is higher than 536.

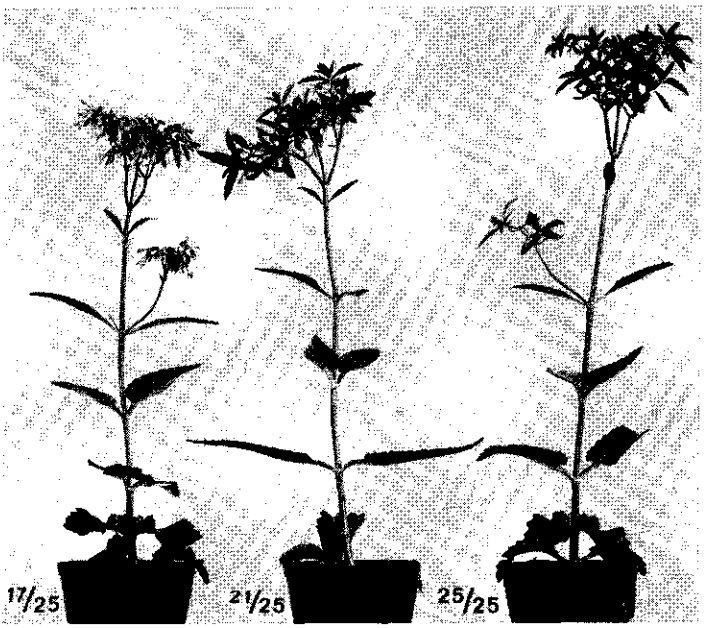
Photo 7 shows the results.

Experiment 6. Old generative plants, 5 months after GA_3 + SD-induction, received different temperature treatments in SD or in LD.

Table 7, treatments 1–8, shows that in SD a temperature sum above 472 stops

TABLE 6. The flowering-stage of generative *B. daigremontianum* after 2 months of temperature treatment in SD.

nr.	°l	°d	sum	stage
1	25	13	408	6
2	27	17	472	6
3	13	25	504	6
4	25	21	536	6
5	17	25	536	6
6	21	25	568	3
7	25	25	600	2



nr. 5 6 7
nr. = treatment number of table 6

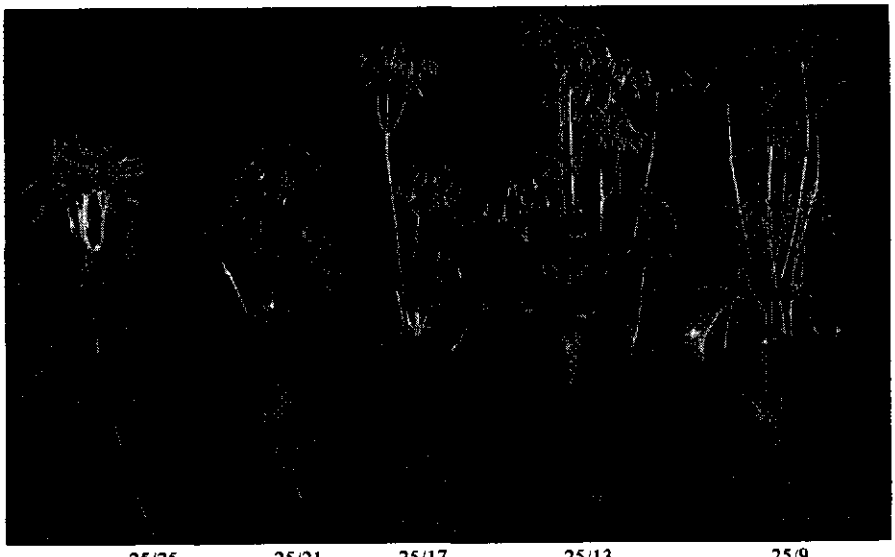
PHOTO 7. *Flowering-stages of B. daigremontianum after 7 weeks of temperature treatment in SD.* Photo: 49 days after the beginning of the temperature treatment.

the flowering of old generative plants. Treatments 9–13 show that in LD a temperature sum of 472 is already too high for the continuing of flowering.

Photo's 8 and 9 show the results.

TABLE 7. The flowering-stage of old generative plants of *B. daigremontianum* after 2 months of temperature treatment in SD or LD

nr.	daylength	°l	°d	sum	stage
1	SD	17°C	9°C	280	6
2	SD	17	13	344	6
3	SD	25	9	344	6
4	SD	25	13	408	6
5	SD	17	17	408	6
6	SD	25	17	472	6
7	SD	25	21	536	3
8	SD	25	25	600	0
9	LD	25	9	472	2
10	LD	25	13	504	2
11	LD	25	17	536	0
12	LD	25	21	568	0
13	LD	25	25	600	0

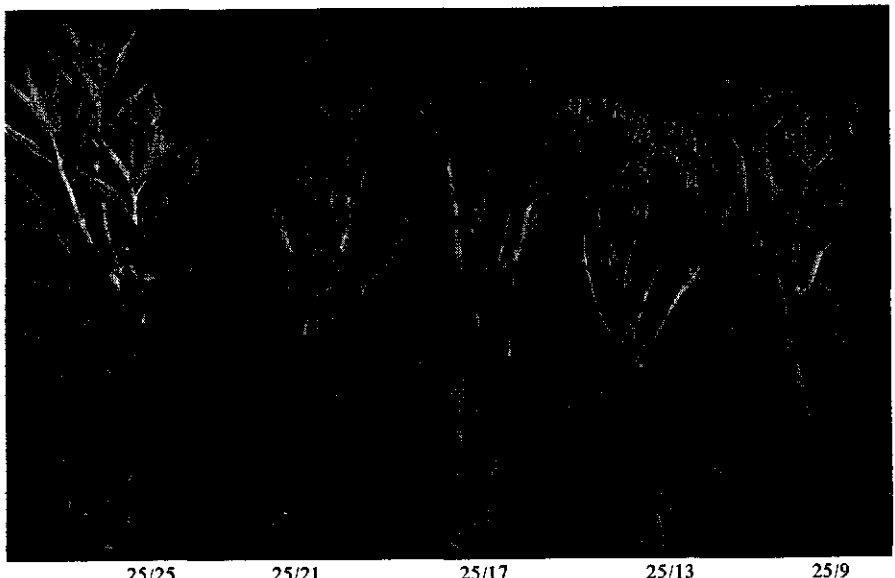


	25/25	25/21	25/17	25/13	25/9
nr:	8	7	6	4	3

nr. = treatment number of table 7

PHOTO 8. *Flowering-stages of old generative plants of B. daigremontianum after 2 months of 25°C during the light period of SD, combined with different temperatures (25, 21, 17, 13 and 9°C) during the dark period.*

Photo: 64 days after the beginning of the temperature treatment.



	25/25	25/21	25/17	25/13	25/9
nr:	13	12	11	10	9

nr. = treatment number of table 7

PHOTO 9. *Flowering-stages of old generative plants of B. daigremontianum after 2 months of 25°C during the light period of LD, combined with different temperatures (25, 21, 17, 13 and 9°C) during the dark period.*

Photo: 64 days after the beginning of the temperature treatment.

3.2.5. *Influence of temperature on the inductive state of B. daigremontianum studied by grafting.*

As too high a temperature sum stops the flowering of a generative plant, it is questionable what happens to the inductive state, being the capacity of producing floral hormone. Also, what happens with the donor capacities?

Experiment 7. Vegetative shoots were grafted on generative rootstocks, 7 weeks after the beginning of GA_3 + SD-induction in 21°/13°. The grafted plants $^{r}/_{d}$ received LD or SD at different temperature combinations and the flowering result of the receptors was observed.

Table 8 shows that transmission of flowering only occurs during a temperature sum of 552 or lower, both under LD and SD-conditions.

Photo 10 shows the results of grafting at SD 25°/25° and 25°/13°.

Experiment 8. The results of the preceding experiment raise the question: is the reaction of the growing-point to floral hormone suppressed by temperature or are the floral hormone and its synthesis destructed?

Young generative plants, 7 weeks after the beginning of GA_3 + SD-induction, received 2 or 4 weeks LD at 21°/13° or 25°/25°. After this temperature treatment the plants were grafted in the combinations r^{-}/d in LD 21°/13°.

Table 9 shows the flowering of the receptors. It appears that 2 weeks of LD 25°/25° is too short to destruct the donor capacity of the rootstocks. A treatment of 4 weeks of LD 25°/25°, however, is long enough to make the influence of the temperature irreversible, resulting in no flowering of the receptors.

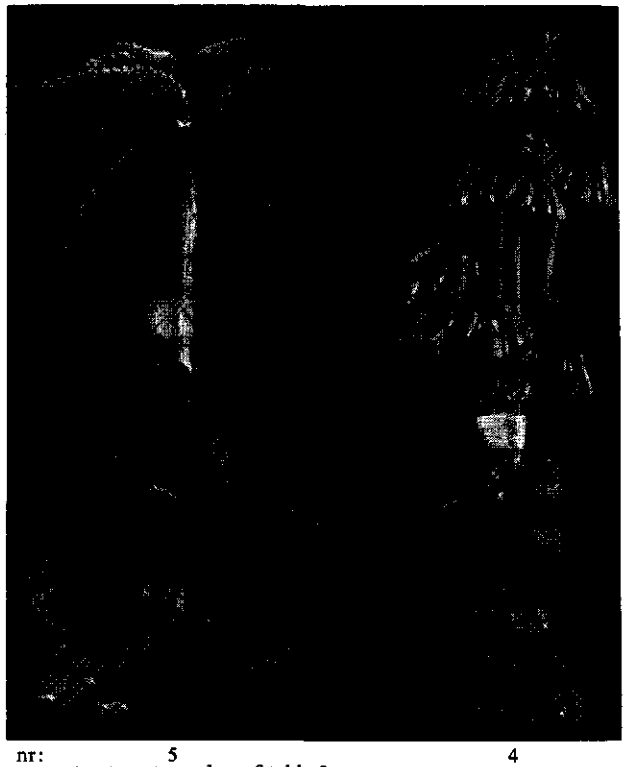
It is obvious that a high temperature sum stops the activity of floral hormone, produced before the temperature treatment, and moreover destructs the inductive state.

Experiment 9. The question arises whether re-induction is possible of the leaves from which the inductive state was destructed by a high temperature sum. Plants, 8 weeks after the beginning of GA_3 + SD-treatment, received temperature treatments. In order to determine the inductive state, the donor capacity was tested by grafting the combination r_1/d.

The receptors of grafting 1 of Table 10 show that the donor capacity is suppressed by 25°/25° (treatments 2 and 3). Next the receptors were removed to prevent induction of these shoots by GA_3. The table shows that the axillary

TABLE 8. The flowering of receptor shoots of *B. daigremontianum*, grafted on donor rootstocks and grown at different temperature conditions.

					flowering		
nr.	daylength	°l	°d	sum	q	%	days
1	SD	25	13	408	16/16	100	24
2	SD	$4^{h}13 + 4^{h}25$	25	552	16/16	100	23
3	SD	25	25	600	0/16	0	∞
4	LD	25	13	504	14/15	93	22
5	LD	25	25	600	0/16	0	∞



nr: 5 4
nr. = treatment number of table 8

PHOTO 10. *Transmission of flowering impulse of B. daigremontianum at different temperature conditions.*
Left: LD 25°l/25°d Right: LD 25°l/13°d
Photo: 47 days after grafting in LD.

TABLE 9. Flowering of receptors of *B. daigremontianum*, grafted on donors which received 2 or 4 weeks of temperature treatment before grafting.

nr.	donor before grafting	flowering		
		q	%	days
1	2 weeks LD 21/13	10/10	100	28
2	2 weeks LD 25/25	10/10	100	30
3	4 weeks LD 21/13	9/9	100	29
4	4 weeks LD 25/25	0/10	0	∾

shoots of the donors do not flower under the favourable circumstances of 25°/13° when 25°/25° preceded. So the suppressing action of 25°/25° is irreversible. When however GA_3 was added to the leaves which were in an inductive state before, the axillary shoots flowered. The second grafting r_2/d confirms that re-induction by GA_3 occurred.

TABLE 10. The flowering reaction of receptors of *B. daigremontianum* grafted on rootstocks which were temperature treated and re-induced.

nr.	r_1/d during 31 days SD				re-induction /d			r_2/d in LD 25/13		
	temp.	flowering of r_1			GA_3 + SD	flowering of/d		flowering of r_2		
		q	%	days		q	%	q	%	days
1	25/13	13/16	81	27	–	–	–	–	–	–
2	25/25	0/16	0	∞	SD 25/13	0/16	0	0/10	0	∞
3	25/25	0/16	0	∞	GA_3 + SD 25/13	16/16	100	5/8	63	34

3.2.6. *SD-induction of Kalanchoë blossfeldiana.*

Other *Crassulaceae* were tried in order to find out parallelisms with *Bryophyllum* about the influence of temperature on the flowering process. Although a similar experiment was already done by RÜNGER (1959), the influence of temperature on induction of *K. blossfeldiana* was tested under the same conditions as *B. daigremontianum.*

Experiment 10. Plants of *K. blossfeldiana* cv. 'Van der Dussen', obtained from cuttings, received SD-induction at different temperature conditions. After 6 weeks the plants were moved into the LD-division of a greenhouse for the observation of flowering.

Table 11 shows, that the reaction on the temperature sum is only quantitative, resulting in 100% flowering under all circumstances, with differences in number of days till the first opening flower. Similarly to *B. daigremontianum* the combination of 25°/13° is the best, resulting in 77 days till the first opening flower, differing highly significantly from 25°/25°. In another experiment *K. blossfeldiana* cv. '*Vulcan*' flowered normally under permanent conditions of SD 25°/25°, so induction as well as realization are possible in 25°.

TABLE 11. The flowering of *K. blossfeldiana* cv. 'Van der Dussen' as a result of SD-induction at different temperature combinations.
days = average number of days till the first opening flower
Number of plants per treatment = 9.

nr.	°l	°d	sum	%	days
1	25	13	408	100	77
2	25	17	472	100	90
3	13	25	504	100	81
4	25	21	536	100	89
5	17	25	536	100	83
6	21	25	568	100	102
7	25	25	600	100	105

3.2.7. *Influence of temperature on the flowering of K. blossfeldiana.*

Experiment 11. Analogous to *B. daigremontianum* flowering plants of *K. blossfeldiana* cv. 'Van der Dussen' received different temperature treatments in LD. A light temperature of 25°C was combined with dark temperatures of 9, 13, 17, 21 and 25°C. Although there was a variation of temperature sums from 472 to 600, no differences in flowering occurred within 2 months.

3.2.8. *GA_3 + SD-induction of Bryophyllum pinnatum.*

Experiment 12. Similar to *B. daigremontianum* the influence of temperature on GA_3 + SD-induction of *B. pinnatum*, another LSDP, was tested. Plants obtained from cuttings were sprayed with GA_3 and received SD at different temperatures in the phytotron.

Table 12 shows that only the extremely low and high temperature sums of 312 (21°/9°) and 600 (25°/25°) did not give 100% flowering.

TABLE 12. The flowering of *B. pinnatum* after GA_3 + SD-induction at different temperature conditions

nr.	°l	°d	sum	flowering	
				q	%
1	21	9	312	14/16	87
2	21	13	392	16/16	100
3	25	13	408	16/16	100
4	13	21	440	16/16	100
5	21	17	456	16/16	100
6	25	17	472	16/16	100
7	21	21	504	16/16	100
8	25	21	536	16/16	100
9	25	25	600	4/16	25

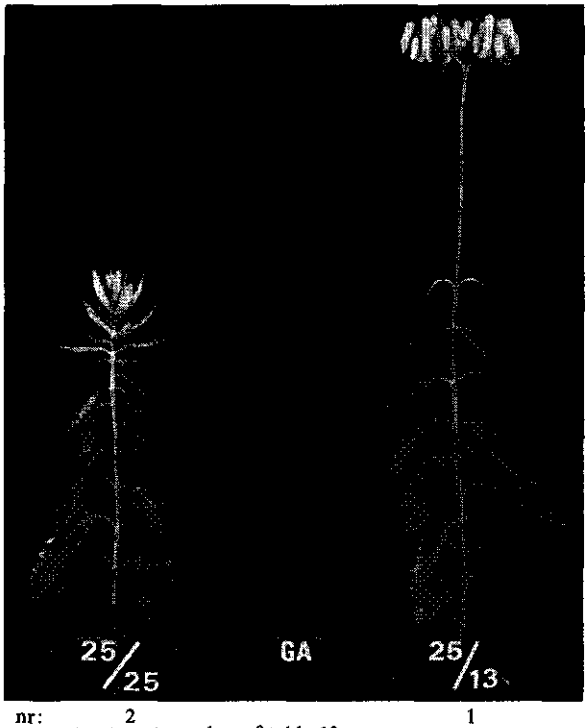
3.2.9 *GA_3 + SD-induction of Kalanchoë jongmansii.*

Experiment 13. Plants grown up from cuttings in LD were moved to the phytotron and received different inductive treatments.

Table 13 shows that GA_3-treatment is necessary for induction in SD although LD-treatment preceded. It is possible that *K. jongmansii* is a LSDP like *B.*

TABLE 13. The flowering of *K. jongmansii* as a result of GA_3 + SD-induction at different temperatures.

nr.	°l	°d	sum	GA-treatment	flowering	
					q	%
1	25	13	408	−	0/16	0
2	25	13	408	+	16/16	100
3	25	25	600	+	0/16	0



nr: 2 1
nr. = treatment number of table 13

PHOTO 11. *Flowering of K. jongmansii as a result of SD + GA_3-induction plus realization at different temperature conditions.*
Photo: 74 days after the beginning of the inductive treatment.

daigremontianum and *B. pinnatum*, but that rejuvenation occurred by the cutting-procedure. More exact research on the daylength type is required. Treatments 2 and 3 show that induction is possible at 25°/13° and not at 25°/25°. This is similar to *B. daigremontianum*.

Photo 11 illustrates the results.

3.2.10. *SD-induction of Kalanchoë manginii.*

Experiment 14. Plants grown up from cuttings in LD were moved to the phytotron and received SD at different temperature combinations.

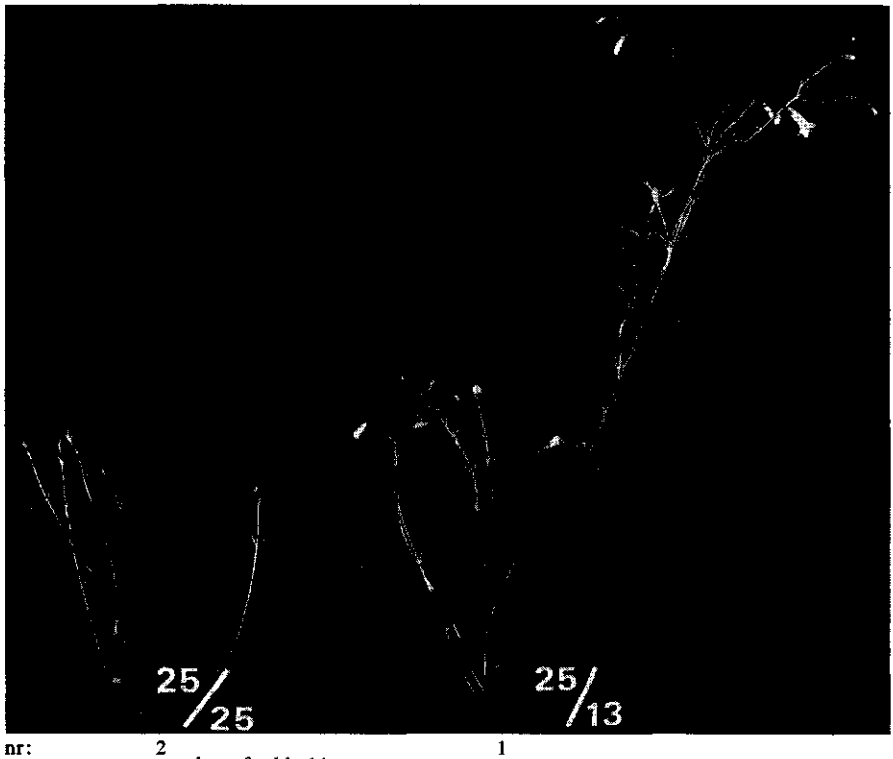
Table 14 shows that no GA_3 is necessary for induction in SD. This means that *K. manginii* is a SDP or a LSDP which was old enough at the shift LD → SD.

Table 14 also shows that induction is possible at 25°/13° and not at 25°/25°. This is similar to *B. daigremontianum* and *K. jongmansii*.

Photo 12 shows the results.

TABLE 14. The flowering of *K. manginii* after temperature treatments in SD.

nr.	°l	°d	sum	flowering	
				q	%
1	25	13	408	16/16	100
2	25	25	600	0/16	0



nr: 2 1
nr. = treatment number of table 14

PHOTO 12. *Flowering of K. manginii as a result of SD induction plus realization at different temperature conditions.*
Photo: 74 days after the beginning of the SD induction.

3.3. THE FLORAL HORMONE OF BRYOPHYLLUM DAIGREMONTIANUM

3.3.1. *Indirect induction and juvenility of B. daigremontianum.*

ZEEVAART (1962) discovered that a vegetative, juvenile shoot of *B. daigremontianum* can be brought into flowering by grafting on a generative rootstock.

Hence, the apex being able to react to floral hormone, the juvenility of *B. daigremontianum* must be caused by the absence of the floral hormone under otherwise inductive circumstances. This absence of floral hormone can be brought about by the incapability of young leaves to be photo-induced or by the incapability to produce the floral hormone. As already mentioned before (p. 2), ZEEVAART and LANG (1962) found that vegetative receptor rootstocks of *B. daigremontianum*, brought into flowering by grafting with a *B. daigremontianum* donor, can act as donors to other vegetative scions in a next grafting: 'indirect induction'. When a juvenile shoot, brought into flowering by grafting, is able to act as donor in a next grafting, this would mean that a juvenile shoot can be brought to multiplication of floral hormone, resulting in donor capacity. In this case juvenility would only exist of the inability to react to photo-induction.

Experiment 15. From young plants, grown from plantlets, shoots were cut off, just when the fourth pair of leaves was developed. These shoots, with the third and fourth pair of leaves, were grafted on GA_3 + SD induced rootstocks. Just when the first floral bud became visible, the shoots were cut off and re-grafted on vegetative defoliated rootstocks. The apical and lateral inflorescence of the shoots were removed to prevent apical dominance to the lateral buds of the rootstock. Unfortunately, the lateral buds of the rootstocks released very slowly and did not flower. Even when in a next experiment the rootstocks were decapitated 4 weeks before the grafting, to release the lateral buds sooner, no flowering occurred. The question was put whether the downward movement of the floral hormone of *B. daigremontianum* goes more difficultly than the upward movement.

Experiment 16. Following Table 15, juvenile shoots r_1, brought into flowering by grafting on d, were regrafted on vegetative, defoliated rootstocks r_2, which were decapitated 4 weeks earlier. After 2 weeks, vegetative defoliated shoots r_2 were grafted on the same juvenile shoot. The result was a generative juvenile shoot with at least 2 pairs of leaves as interstock between a vegetative, defoliated rootstock and a vegetative defoliated shoot.

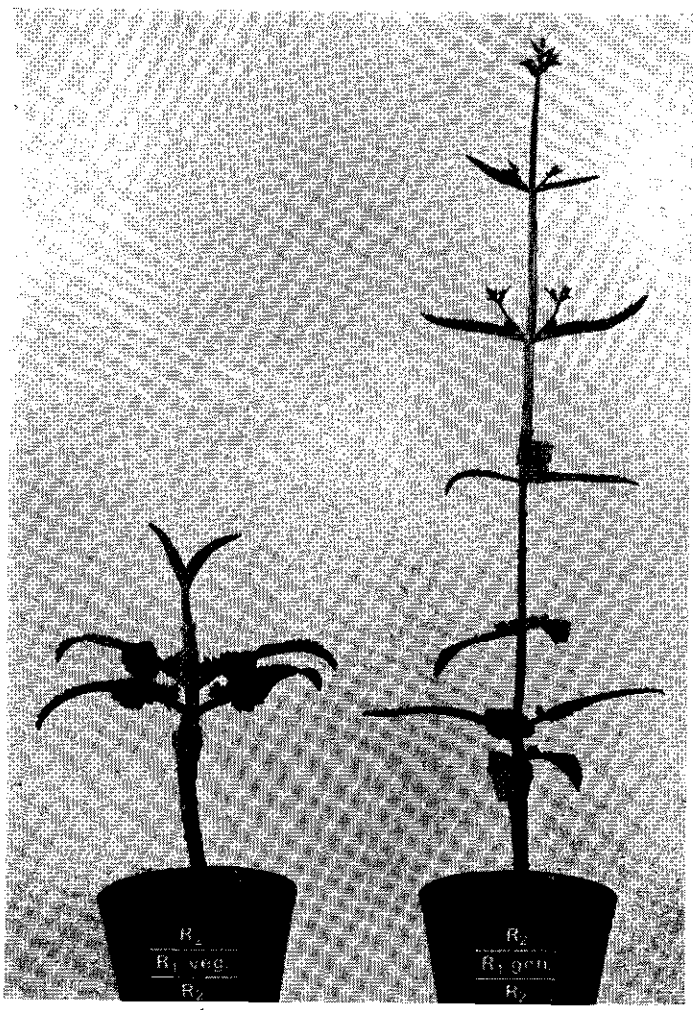
Table 15 shows that juvenile shoots are able to act as donor, so that in this case the juvenility is not a matter of non-production of floral hormone, but an

TABLE 15. Flowering of receptors r_1 of *B. daigremontianum* grafted on vegetative or generative rootstocks (grafting 1) and flowering of receptors r_2, grafted with r_1 as interstock (grafting 2).

nr.	grafting 1	flowering of r_1			grafting 2	flowering of r_2		
		q	%	days		q	%	days
1	$\frac{r_1^+}{r_1^+}$	0/15	0	∞	$\frac{r_2^-\ \text{veg.}}{r_1^+\ \text{veg.}}$	0/12	0	∞
						–	–	–
					r_2^- veg.	0/12	0	∞
2	$\frac{r_1^+}{d^+}$	14/15	93	27	$\frac{r_2^-\ \text{veg.}}{r_1^+\ \text{gen.}}$	14/14	100	38
						–	–	–
					r_2^- veg.	1/14	7	82

inability to react to photo-induction. An explanation for the poor result of experiment 15 and the poor flowering of the rootstocks of the present experiment is, that the downward movement of the floral hormone of *B. daigremontianum* indeed proceeds very difficultly and slowly. This phenomenon is also found in other experiments with *B. daigremontianum*. Table 15 also shows that the number of days till visible flower bud is lower in grafting 1 than in grafting 2. An explanation is that in grafting 1 the donor with 6 pairs of well developed leaves contained more floral hormone than r_1^+ generative of grafting 2 with 2 pairs of small leaves.

Photo 13 illustrates the results.



nr: 1 2
nr. = treatment number of grafting 2 of table 15

PHOTO 13. 63 days after the last grafting.

3.3.2. *The result of LD and SD induction of B. daigremontianum as studied by a shift of day length.*

B. daigremontianum being a LSDP can be induced to flowering by LD, followed by SD. BÜNSOW and HARDER (1956) and PENNER (1960) found that GA_3 can replace LD-treatment of *B. crenatum* as well as *B. daigremontianum*. PENNER moreover discovered that GA_3 can induce flowering in *B. crenatum* in LD, however only when the plants were at least 6 months and received SD till no more than 5 days before the GA-treatment. PENNER supposed that *B. crenatum* produces, when old enough, a substance in SD and another substance in LD. When these substances will come together, flowering occurs. PENNER concluded that the SD substance is produced also when no LD treatment preceded. When the plants are shifted from SD to LD, the production of the LD substance will proceed slowly and the SD substance will be broken off by LD before enough LD substance is made. GA_3 however can replace the LD substance. If given before the SD substance is broken off, there will be flowering.

The question arose whether *B. daigremontianum*, like *B. crenatum*, produces a flower forming substance in SD, independent whether LD preceded or not.

Experiment 17. Plants of 1 year with 15 pairs of leaves and grown in SD received 6 weeks SD at the temperature combinations 25°/13°, 25°/17°, 13°/25°, 25°/21°, 21°/25°, 25°/25°. Next the plants were sprayed with GA_3 100 ppm and moved to LD 21°/13°. None of the 4 plants per treatment flowered.

May be *B. daigremontianum* can be brought into flowering when there are some cycles of SD between GA-treatment and the shift to LD. When the number of these necessary SD-cycles is lower than the normal minimum number of 15, necessary for SD-induction, it is obvious that SD before LD is active in *B. daigremontianum* too.

Experiment 18. Vegetative plants of about 1 year with 14 pairs of leaves and grown in SD were exposed to 4 weeks SD at different temperatures. Next the plants were sprayed with GA_3 100 ppm and after 0, 1, 5, 10 or 15 cycles of SD 21°/13° moved to LD 21°/13°. Only the plants with 15 cycles of inductive SD after the GA_3 treatment flowered, independent of the preceding temperature treatment.

The conclusion is that no SD induction occurs in *B. daigremontianum* without preceding GA_3 or LD.

3.3.3. *Effect of LD and SD as studied by grafting.*

It is possible that LD-induction of *B. daigremontianum* results in the production of a LD substance and SD results in the production of a SD substance. Are these substances transmissable and can they be brought together by grafting, resulting in a floral hormone?

RÜNGER obtained negative results by giving different day lengths to two pairs of leaves standing upright above each other. Nevertheless, grafting of two parts of plants from different day lengths will be of interest.

Experiment 19. Shoots of adult SD plants were grafted on adult LD rootstocks. The graftings were placed in a LSD cabinet (described by WELLENSIEK

and ELINGS, 1967), where the scions received SD and the stocks LD. Although the temperature conditions were favourable, being 22°/15°, none of the 10 graft combinations produced flowers within 3 months. Neither did the reverse graft combination LD/SD. The conclusion is that the existence of graft transmissable substances, produced under LD- or under SD-conditions, could not be demonstrated.

3.3.4. *The role of GA_3 during the production of floral hormone.*

ZEEVAART and LANG (1963) found that CCC, applied to *B. daigremontianum* during the SD-treatment after the shift LD → SD, suppresses the flower formation. As GA-treatment could nullify the CCC effect by causing flowering, ZEEVAART and LANG concluded that CCC prevented the activity of GA, produced by the plant under LD-induction.

The questions were asked: Is GA an induction factor for *B. daigremontianum*, producing an inductive state, or is GA a permanently necessary building-substance in the synthesis of floral hormone? Does a flowering plant only continue to flower, when the production of floral hormone continues?

When GA is a precursor of floral hormone, and when continued production of floral hormone is a condition for continued flowering, application of CCC to flowering plants might have a disturbing effect on the flowering.

Experiment 20. Generative plants of *B. daigremontianum*, induced by GA_3 + SD, were treated with CCC by administering to the roots 100 ml 4000 ppm solution 3 × with intervals of 5 days. During and after the treatment the flowering-pattern was compared with untreated control plants. However, during 4 months no differences could be observed. It is possible that the quantity of applied CCC was not sufficient to nullify the amount of applied GA_3, but since there are absolutely no differences with the controls, it is likely that GA_3 does not play a role in the continuation of flowering.

3.4. SD-INDUCTION OF AN EARLY AND A LATE CV. OF K. BLOSSFELDIANA

For one cv., under certain conditions of light and temperature, a range of inductive cycles can be given, resulting from aborting buds to 100% flowering. Hence *K. blossfeldiana* is a SDP with a quantitative flowering-reaction after sub-optimal SD-induction.

In *experiment 21* the inductive ranges of an early and a late cultivar were compared. Plants of the early cv. 'Vulcan' and the late 'Van der Dussen' were grown up from cuttings in LD. Inductive treatment was given in the SD-division of a greenhouse. Table 16 shows that the inductive range of the early cv. 'Vulcan' lies between 7 and 14 cycles of SD.

The inductive range of the late cv. 'Van der Dussen' is demonstrated in Table 17 and lies between 21 and 28 cycles of SD. So there is a difference of about 14 cycles of SD-induction between the early and the late variety.

Photos 14 and 15 illustrate these results.

nr: 2 9
nr. = treatment number of table 16

PHOTO 14. *K. blossfeldiana 'Vulcan' after 1 (left) or 2 (right) weeks of SD induction.*
Photo: 92 days after the beginning of the induction.

nr: 2 9
nr. = treatment number of table 17

PHOTO 15. *K. blossfeldiana 'Van der Dussen' after 2 (left), 3 (middle) or 4 (right) weeks of SD induction.*
Photo: 92 days after the beginning of the induction.

TABLE 16. The flowering of early *K. blossfeldiana* cv. 'Vulcan' after different cycles of SD.

nr.	SD-cycles	flowering				
		q	%	aborting buds	stopping flowering	continuous flowering
1	6	0/10	0	–	–	–
2	7	3/10	30	3/10	–	–
3	8	5/10	50	5/10	–	–
4	9	10/10	100	10/10	–	–
5	10	10/10	100	10/10	–	–
6	11	10/10	100	5/10	5/10	–
7	12	10/10	100	–	5/10	5/10
8	13	10/10	100	–	3/10	7/10
9	14	10/10	100	–	–	10/10[1]

[1] Average number of days till visible bud was 21.

TABLE 17. The flowering of late *K. blossfeldiana* cv. 'Van der Dussen' after different cycles of SD

nr.	SD-cycles	flowering				
		q	%	aborting buds	stopping flowering	continuous flowering
1	20	0/10	0	–	–	–
2	21	2/10	20	–	2/10	–
3	22	2/10	20	–	2/10	–
4	23	5/10	50	–	5/10	–
5	24	6/10	60	–	5/10	1/10
6	25	8/10	80	–	5/10	3/10
7	26	10/10	100	–	6/10	4/10
8	27	10/10	100	–	3/10	7/10
9	28	10/10	100	–	–	10/10[1]

[1] Average number of days till visible bud was 33.

3.5. GRAFTCOMBINATIONS BETWEEN K. BLOSSFELDIANA AND B. DAIGREMONTIANUM

3.5.1. *Graft combinations with B. daigremontianum under different temperature conditions.*

ZEEVAART (1958) demonstrated the identity of the floral hormones of the Crassulaceae *Kalanchoë blossfeldiana, Sedum ellacombianum* and *Sedum spectabile*. In the present experiments graft combinations of *K. blossfeldiana* and *B. daigremontianum* are investigated to find out the relationship between their floral hormones. *K. blossfeldiana* was chosen as a plant with early and late culti-

vars, while grafting of *Kalanchoë* with *Bryophyllum* presents the combination of a plant without non localized production of floral hormone and a plant with that property.

Experiment 22. Vegetative shoots of the early cv. 'Vulcan' and the late cv. 'Van der Dussen' of *K. blossfeldiana* were grafted on generative *Bryophyllum* (*K.*r/*B.*d) to find out whether the floral hormones of *B. daigremontianum* and *K. blossfeldiana* are identical and to compare the floral reaction of the early and late cv. of *Kalanchoë*. The reverse combination *B. daigremontianum* receptor on *K. blossfeldiana* donor-rootstock was made too (*B.*r/*K.*d). In order to find out the influence of temperature on these graft combinations, different conditions were given during 6 weeks of LD in the phytotron, followed by LD-treatment under greenhouse conditions.

In Table 18, treatments 10, 12 and 16 show that *B. daigremontianum* is able to bring *K. blossfeldiana* into flowering, while the controls 9, 11 and 15 remain strictly vegetative. Comparison of treatments 2, 10 and 14 shows a slow flowering reaction of only the early cultivar of *Kalanchoë* during the next greenhouse temperature conditions, so that 6 weeks of 25°C in light and dark will reduce the level of floral hormone of the *B. daigremontianum* donor. This would suggest that early *K. blossfeldiana* needs less floral hormone than late *K. blossfeldiana* or *B. daigremontianum*.

Treatments 6 and 8 show that neither in 25°/25° nor in 25°/13° *K. blossfeldiana* is able to bring *B. daigremontianum* into flowering. An explanation for this might be that the sum of temperature of LD 25°/13° of 504 is too high for *B. daigremontianum* to react to the floral hormone of *K. blossfeldiana*. ZEEVAART

TABLE 18. The influence of dark temperature on the transmission of flowering-capacity of some graft combinations of *B. daigremontianum* and the early and late cv. of *K. blossfeldiana*.

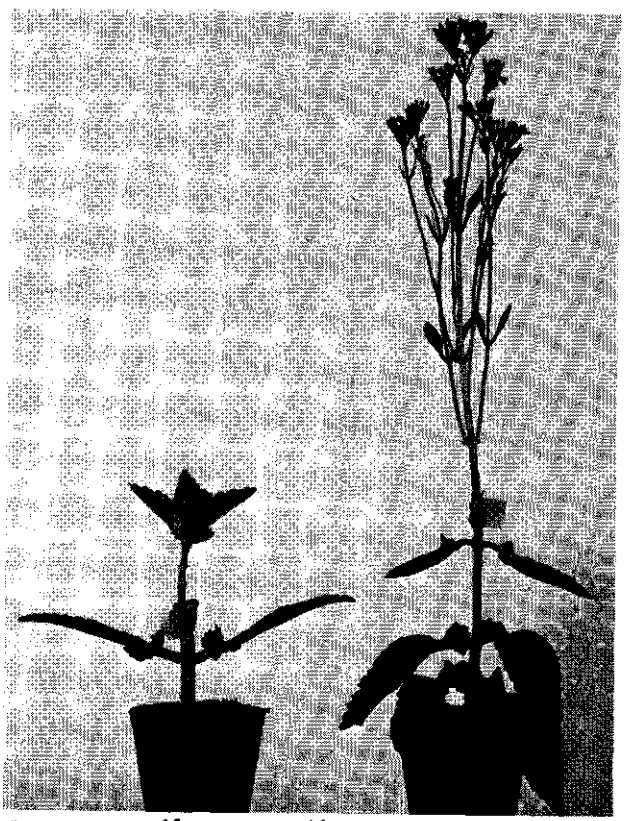
nr.	combination	°l	°d	flowering q	flowering %	flowering days
1	*B.*r/*B.*r	25°C	25°C	0/10	0	∞
2	*B.*r/*B.*d	25	25	0/15	0	∞
3	*B.*r/*B.*r	25	13	0/10	0	∞
4	*B.*r/*B.*d	25	13	14/15	93	21,6
5	*B.*r/*K.*early r	25	25	0/10	0	∞
6	*B.*r/*K.*early d	25	25	0/15	0	∞
7	*B.*r/*K.*early r	25	13	0/10	0	∞
8	*B.*r/*K.*early d	25	13	0/15	0	∞
9	*K.*early r/*B.*r	25	25	0/10	0	∞
10	*K.*early r/*B.*d	25	25	12/14	86	74,0
11	*K.*early r/*B.*r	25	13	0/10	0	∞
12	*K.*early r/*B.*d	25	13	12/12	100	20,3
13	*K.*late r/*B.*r	25	25	0/10	0	∞
14	*K.*late r/*B.*d	25	25	0/14	0	∞
15	*K.*late r/*B.*r	25	13	0/9	0	∞
16	*K.*late r/*B.*d	25	13	12/13	92	20,1

(1958) also found that it is difficult to bring *B. daigremontianum* into flowering by a *K. blossfeldiana* donor.

Photo 16 shows the results of treatments 15 and 16, while photo 17 clearly demonstrates the influence of the temperatures of treatments 16 and 14.

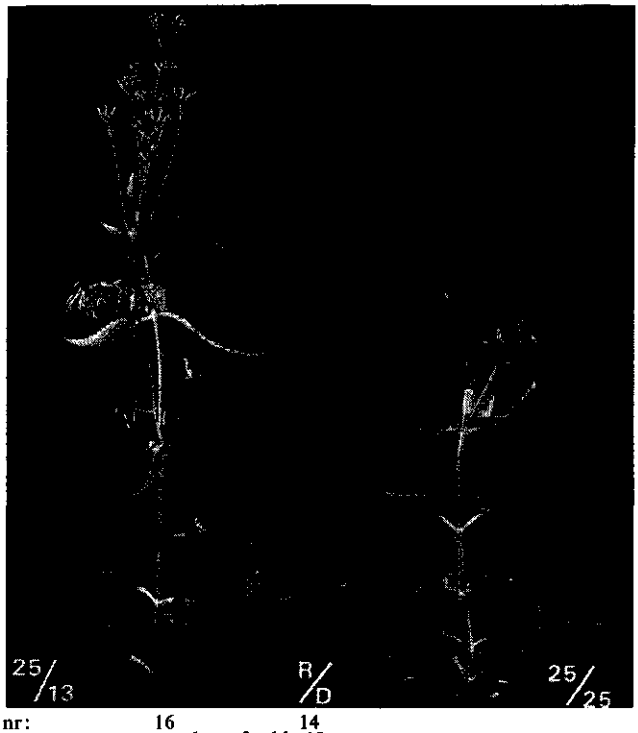
Experiment 23. Following experiment 22, vegetative shoots of *B. daigremontianum* were grafted on generative rootstocks of the early cv. 'Annette' of *K. blossfeldiana* in LD 21°/13° (*B.*r_1^+/*K.*d^+). As a control *B. daigremontianum* was grafted on vegetative rootstocks of *K. blossfeldiana* (*B.*r_1^+/*K.*r^+). Also the reverse combinations *K./B.* were made.

Table 19, left part, treatment 2 shows that flowering of *B.*r_1^+ occurred after upward movement of the floral hormone, while in treatment 4 the downward movement of the floral hormone did not result in flowering, confirming former experiments. The flowering reaction of treatment 2, however, was very slow and



nr: 15 16
nr. = treatment number of table 18

PHOTO 16. *K. blossfeldiana as receptor grafted on B. daigremontianum receptor (control, left) or on B. daigremontianum donor (right) at 25°l/13°d* in *LD.*
Photo: 72 days from grafting.



nr: 16 14
nr. = treatment number of table 18

PHOTO 17. *K. blossfeldiana as receptor on B. daigremontianum donor at 25°l/13°d (left) and 25°l/25°d (right) in LD.*
Photo: 66 days after grafting.

the floral stage was moderate.

In grafting 2, the flowering receptors (r_1^+ gen.) of treatment 2 of grafting 1, were regrafted as an interstock between vegetative, defoliated pieces of *B. daigremontianum* (r_2^- veg.). As a control the vegetative receptors of treatment 1 of grafting 1 (r_1^+ veg.) were grafted identically. Table 19, right part, shows that the generative *B. daigremontianum* receptors have no donor capacities.

The conclusion of this experiment is that *K. blossfeldiana* is hardly able to bring *B. daigremontianum* into flowering and cannot induce donor capacities to *B. daigremontianum*.

3.5.2. *Graft combinations of B. daigremontianum with different periods of grafting-contact.*

Experiment 24. Another approach to the problem of differences in the amount of floral hormone between early and late cultivars of *K. blossfeldiana* has been to graft vegetative shoots of the early cv. 'Vulcan' and of the late 'Van der Dussen' on generative *B. daigremontianum* rootstocks in LD 21°/13°. The

TABLE 19. The flowering of *B. daigremontianum* receptors grafted with receptors or donors of *K. blossfeldiana* in LD 21 °l/13 °d.

nr.	grafting 1	flowering				grafting 2	flowering		
		q	%	days	stage		q	%	days
1	$\frac{B.r_1^+}{K.r^+}$	0/10	0	∞	0	$\frac{B.r_2^-\text{ veg.}}{B.r_1^+\text{ veg.}}$	0/10	0	∞
						$B.r_2^-$ veg.	0/10	0	∞
2	$\frac{B.r_1^+}{K.d^+}$	6/10	60	77	4	$\frac{B.r_2^-\text{ veg.}}{B.r_1^+\text{ gen.}}$	0/6	0	∞
						$B.r_2^-$ veg.	0/6	0	∞
3	$\frac{K.r^+}{B.r^-}$	0/10	0	∞	0				
4	$\frac{K.d^+}{B.r^-}$	0/10	0	∞	0				

K. blossfeldiana receptors were defoliated till the appearance of the first floral bud. After different periods of grafting-contact these receptors were sidegrafted on vegetative rootstocks of the cv. 'Josine' of *K. blossfeldiana.*

Table 20 shows that the early cv. 'Vulcan' needs less grafting-contact for flowering than the late cv. does. However, there are no differences in days till visible flowering between early and late. Moreover no differences in floral stages between any of the flowering receptors were observed.

Hence, if a shoot obtains sufficient floral hormone for flowering, the duration till visible flower buds will be equal for the early and late cv. None of the re-grafted flowering receptors was able to bring the vegetative *K. blossfeldiana* 'Josine' rootstocks into flowering. Even 3 months after the regrafting there were

TABLE 20. Flowering of receptors of an early and a late cultivar of *K. blossfeldiana,* grafted on *B. daigremontianum* donors with different durations of graft contact, followed by regrafting on vegetative *K. blossfeldiana* cv. 'Josine.'

nr.	cultivar	duration *K.*r/*B.*d	flowering		
		in days	q	%	days
1	early	7 d.	0/14	0	∞
2	early	14 d.	11/13	85	27,4
3	early	21 d.	13/14	93	22,5
4	early	∞	5/5	100	24,4
5	late	7 d.	0/15	0	∞
6	late	14 d.	2/15	13	28,5
7	late	21 d.	7/15	47	19,7
8	late	∞	5/5	100	23,6

flowering shoots on vegetative rootstocks. These flowering shoots formed vegetative lateral shoots. So *B. daigremontianum* was not able to bring about non localized synthesis of floral hormone in *K. blossfeldiana*!

3.6. Conclusions

1. SD-induction + realization of *B. daigremontianum* by GA_3 + SD as well as by LSD, was only obtained when the sum of products of temperature times hours per 24 hours did not exceed a certain value (Exp. 1 and 4).
2. Low temperature treatment of *B. daigremontianum* to reduce the temperature sum is active in the light- as well as in the dark period. (Exp. 1 and 4).
3. Hardly induction but rather the following realization is inhibited by too high a temperature. (Exp. 2 and 3).
4. Similar cases of qualitative influence of temperature on induction + realization were found in *Kalanchoë jongmansii* and *K. manginii*. (Exp. 13 and 14). Experiments 10 and 12 with *K. blossfeldiana* and *B. pinnatum* respectively, however, show a more quantitative influence of temperature. It is likely that the marginal level of the temperature sum for these plants is higher than for the former *Crassulaceae*, and is not reached in the present experiments. The negative results of Rünger (1959) with *K. blossfeldiana* at 30°C confirm the last supposition. (Exp. 10, 12, 13 and 14).
5. Temperature sums between 392 and 600 do not prevent LD-induction of *B. daigremontianum*. It is likely that for LD-induction a period per 24 hours at a temperature above 17°C is preferable. (Exp. 4).
6. Flowering of *B. daigremontianum* is stopped by too high a temperature sum. The marginal level of this temperature sum depends on the flowering-condition of the plant. Also the donor capacity of a flowering *B. daigremontianum* plant could be destructed by high temperature. (Exp. 5, 6 and 7).
7. It is likely that the influence of a high temperature sum on the flowering process is a destruction of floral hormone and inductive state, both formed before the temperature treatment. (Exp. 5, 6 and 8).
8. The destruction of the floral state is irrevisible when the temperature treatment exceeds a certain duration, resulting in a vegetative plant. (Exp. 8).
9. A generative plant of *B. daigremontianum*, which becomes vegetative by temperature treatment can be induced again via the leaves which were in an inductive state before. (Exp. 9).
10. The floral state of *K. blossfeldiana* cannot be influenced by the temperature combinations which were used. In contrary to *B. daigremontianum* there is a quantitative influence of temperature on induction while no influence on the realization could be demonstrated. (Exp. 11).
11. Juvenility of *B. daigremontianum* means the period of development during which the leaves cannot be photo-induced by the shift LD → SD. Juvenile leaves, however, are capable to reproduce the floral hormone after grafting with a donor. (Exp. 16).

12. SD-induction without preceding LD-treatment could not be demonstrated by the shift SD $\rightarrow GA_3$ + LD or SD $\rightarrow GA_3$ + unsufficient SD $\rightarrow$ LD. (Exp. 17 and 18).
13. No transmissable substance after SD- or LD-induction could be demonstrated by grafting parts of plants from SD and LD on each other. (Exp. 19).
14. GA-induced, flowering plants of *B. daigremontianum* were not influenced by CCC, applied to the roots. This could mean that GA_3 does not play a role in the continuation of flowering. (Exp. 20).
15. Vegetative shoots of *K. blossfeldiana* can be brought into flowering by grafting on a generative rootstock of *B. daigremontianum*. This suggests identity of the floral hormones of the LSDP *B. daigremontianum* and the SDP *K. blossfeldiana*. (Exp. 22).
16. The flowering of *K.* caused by grafting on a *B.* rootstock is abundant. Nevertheless, the floral hormone of *B.*, a plant with the property of non localized synthesis of floral hormone, cannot bring about this capacity into the *K.* receptors. Following conclusion 15 it is likely that the differences between *B.* and *K.*, concerning non localized synthesis of floral hormone is caused by internal differences between these plants, and not by different floral hormones. (Exp. 24).
17. The graft combination *B.* receptor on *K.* donor gives a slow flowering-reaction of the *B.* receptors and a low floral stage. As conclusion 15 suggests identity of the floral hormones of *B.* and *K.*, the moderate reaction of the *B.* receptor suggests a lower level of floral hormone in a flowering *K.*, compared with a flowering *B.* (Exp. 23).
18. Moderately flowering *B.* receptors, after grafting with *K.*, were not able to act as donors in a next grafting. So *K.* was not able to bring about synthesis of floral hormone in *B.* (Exp. 23).
19. The early cv. 'Vulcan' of *K.* needs 14 cycles of SD-induction less than the late cv. 'Van der Dussen' for complete flowering. (Exp. 21). However, in contrast to SD-induction, both cultivars flowered simultaneously, when grafted on *B.* donor rootstocks. On the other hand, for becoming generative the early cv. needs shorter grafting-contact with *B.* donor than the late one. This means that the early and late cv. will have different threshold values of the floral hormone for a flowering reaction. It is likely that the rate of the floral reaction is influenced by the amount of floral hormone. Therefore, the number of days till visible flower bud of the late and early cv. can be equal, provided sufficient floral hormone is available. (Exp. 21 and 24).
20. When the donor capacity of *B.* is lowered by temperature treatment, only the early cv. of *K.* becomes generative, while the *B.* receptors as well as the late *K.* receptors remain vegetative. This result is in accordance with conclusion 19, that the early cv. of *K.* needs less floral hormone for flowering than the late one. (Exp. 22).

4. THE FLORAL HORMONE OF XANTHIUM STRUMARIUM

4.1. FLORAL HORMONE AND FLORAL DEVELOPMENT

4.1.1. *The floral development of Xanthium strumarium.*

Many investigations about the flowering of *Xanthium* have already been done. SALISBURY (1969) cited about 200 papers. Still questions about the role of the floral hormone in the transition from vegetative to generative development remain.

Already HAMNER and BONNER (1938) concluded that the development of floral primordia into mature flowers proceeds much more slowly in a plant which has been induced by one long dark period than in one given a succession of long dark periods. They observed the terminal buds microscopically at a certain period after the inductive treatment and characterized the magnitude of flowering as: strictly vegetative, inflorescence primordia, flower primordia, or macroscopical flowers and fruits. NAYLOR (1941) presented an extensive study about the daily development of the male and female flowers as a result of different cycles of SD induction. SALISBURY (1955, 1963) supposed that the rate of development of the *Xanthium* flower (specifically the male flower at the stem apex) seems to be primarily a function of the amount of floral hormone which arrives at the tip. He established a series of 8 arbitrary morphogenetic stages of development of the bud. LINCOLN et al. (1956) assumed that the transverse diameter of the staminate inflorescence at the time of dissection is a direct function of the relative amount of the floral initiating stimulus, that has reached the apical bud. A quantitative measure of the effectiveness of the various treatments was established by assigning numerical values to recognizable stages in the ontogeny of the terminal staminate inflorescence. The values were raised one unit with each 0,25 mm increase in diameter above 1 mm. SALISBURY (1969) summarized most of the floral stage systems used for *Xanthium*, which have in common that they are based on the rate of floral development.

A remaining question is whether the microscopically different floral stages develop into permanently distinguishable plants, or only represent a difference in the rate of development. NAYLOR (1941) already concluded that increasing the number of inductive cycles seems to stimulate the production of carpellate more than of staminate inflorescences. According to LINCOLN et al. (1956, 1958) the different floral stages are due to different amounts of floral hormone, and the continuation of flowering is caused by storage or continued synthesis of floral hormone in the young leaves. It remains questionable whether a low level of floral hormone, resulting in a low microscopical floral stage, means a prolonged synthesis of floral hormone at a low level.

OKUDA (1953) reported that he got deviations from normal flowering of *Xanthium canadense*, when he brought plants into flowering by grafting with some LD *Compositae*.

The flowering rootstocks of *Xanthium strumarium*, as a result of grafting with *Calendula officinalis* (VAN DE POL, 1971) were not able to serve as a donor within 3 months after the appearance of the first floral bud. Moreover, 15 months after the grafting these plants were still alive, while formation of stems and leaves with axillary buds occurred in combination with generative development, being the formation of male and female flowers. Simular forms of development were obtained by short grafting-contact with a *Xanthium* donor. Completely flowering plants, as a result of sufficient induction or plenty grafting-contact with a donor of *Xanthium* itself or of *Helianthus annuus*, show generative development with little or no vegetative development. When all the present meristems have formed flowers and the flowers are ripening, the plant dies.

The conclusion is, that the floral pattern of *Xanthium strumarium* can show different balances of vegetative and generative development.

4.1.2. *The inflorescence of Xanthium strumarium.*

Xanthium is a monoecious plant. The terminal meristem always forms a staminate capitulum due to induction, while the axillary reproductive buds form one pistillate flower or an axis with usually three flowers. These female flowers have an involucre. The male capitulum, originating from the top meristem, always appears before the axillary female flowers. Often some buds from the axils of the involucral bracts of this male capitulum form axes with one or more male capitulums. Frequently, axillary buds from the leaves will become branches with a terminal staminate capitulum and axillary pistillate flowers (SALISBURY, 1969).

The question was put: What factors are influencing the development of an axillary bud into female flowers or into a shoot with a final male capitulum and female flowers in the lower axils?

Experiment 25. Plants of 5 weeks from sowing in LD, at a size of about 8 nodes, were moved to SD. After 1 week of SD treatment the plants were moved back to LD where the floral development was observed 4 weeks later.

Table 21 shows the place and number of the male capitulums and the female flowers. It appears that the final meristem always forms a male capitulum with sometimes a second one developed from the lowest male flower bud. It is remarkable that with one exception the 4 axils below the final meristem form female flowers only. Since the plants had about 8 nodes at the beginning of the SD-induction, these highest 4 axillary buds are formed during and after induction. The axillary buds from about the sixth until the lowest bud, which were already formed before induction, all form shoots with a male capitulum on the top followed by female flowers in the axils of small leaves.

It would be preferable to reserve the term inflorescence of *Xanthium* for a shoot with a final male capitulum, with eventual side-capitulums, and female flowers in the axils of the lower leaves. Remarkable is the appearence of bi-sexual capitulums in the transitory zone of axils 4, 5 and 6 which can be described as a female flower with male flowers in the axils of the prickles of the involucrum. Besides these cases, similar ones in other material were occasionally

TABLE 21. The average place and number of male capitulums and female flowers and the average lengths of internodes and axillary shoots of *X. strumarium* as a result of 7 days SD induction; n = 10.
axil number 0 = the top meristem.

axil number	male capitulums	female flowers	bisexual flowers	internode length	shoot length
0	1,3	0	0	0,2 cm	0 cm
1	0	1,6	0	0,2	0
2	0	2,1	0	0,3	0
3	0	2,5	0	0,4	0
4	0	2,2	0,1	0,8	0
5	0,2	2,9	0,1	1,5	0,3
6	0,5	2,7	0,4	2,0	1,0
7	1,0	3,5	0	2,5	2,0
8	1,0	4,5	0	3,0	5,0
9	1,0	4,6	0	6,0	2,0
10	1,0	4,6	0	4,0	1,0
11	0,5	1,9	0	9,0	0
12	0,1	0,5	0	8,0	0
13	0,1	0,3	0	5,0	0
Total	6,7	33,9	0,6		

found directly below the final male capitulum. So it appears that bisexual capitulums are formed at places where male as well as female flowers can be formed. The existence of bisexual capitulums confirms the old statement of FARR (1915), that a bur is a modified capitulum.

Table 21 also shows the lengths of the internodes and axillary shoots. It appears that the length of the main inflorescence, being the sum of the internodes between axil numbers 0 and 5 is very small. This means hardly any vegetative development during the formation of the flowers. It is a common phenomenon that the lengths of the axillary shoots, being inflorescences, increase in downward direction, with exception of the lowest ones. These differences in inflorescence can be explained by the general condition of the plant during the flower formation. The main inflorescence of this well induced plant will be formed during a high endogenous level of floral hormone. Since no new leaves are formed after the induction, the present leaves grow older and the synthesis of floral hormone will diminish. The later developing axillary inflorescences will have the disposal of less floral hormone and show a stronger vegetative development during the flower formation, resulting in an increase of the shoot lengths. The lowest inflorescences are formed when the plant has almost died, and they do not reach full development, so their lengths cannot be compared.

4.1.3. *The influence of different cycles of SD on the appearance of male capitulums and female flowers.*

From the effect of 2 or 4 cycles of SD induction, HAMNER and BONNER (1938) concluded that the rate of development of floral primordia depends on induc-

tion. The rate of development of the inflorescence as a result of induction is investigated in the next experiment.

Experiment 26. Plants of 6 weeks from sowing in LD were transferred to SD and after 1 to 5 days removed back to LD where the rates of appearance of male capitulums and female flowers were observed.

Table 22 shows that the flowers appear sooner when more cycles of SD preceded. The female flowers always appear later than the male capitulums. However, the differences decline rapidly after longer induction.

The differences between the numbers of days till the appearance of male and female flower buds can be considered as a measurement for the rate of development of the inflorescence.

TABLE 22. Number of days from beginning of SD till the appearence of male capitulums and female flowers of *X. strumarium* in LD after different cycles of SD induction; n = 10.

SD	♂	♀	♀ minus ♂
0	∾	∾	–
1	37 d.	86 d.	49 d.
2	31	61	30
3	29	39	10
4	28	34	6
5	24	29	5

4.1.4. *Floral stages at different balances between vegetative and generative development.*

As mentioned before, many floral stage systems were used to distinguish the rate of flower development as a result of inductive treatments. A flowering *Xanthium* plant almost never reverts to the vegetative state, even if the plant never receives an inducing treatment again. Observation of plants, which were brought into flowering by different periods of grafting-contact or by grafting with other donors than *Xanthium*, showed that different balanced stages between vegetative and generative development occur. Are these balances caused by continuous synthesis of floral hormone at different levels? Before entering into this question, the next classification and Photo 18 demonstrate the representative floral stages of *Xanthium*, which could be distinguished macroscopically.

Floral stages at different balances between vegetative and generative development.

Stage 0. Vegetative development.
Formation of leaves with axillary buds and internodes. No or very late branching by apical dominance.

Stage 1. Slow male flowering with strong vegetative development.
Continuously growing male capitulum at the top, with bracts which grow into green leaves, and stay closer together than normal leaves of a vegetative shoot. The buds of the male flowers in the axils of the bracts do not develop or slowly. Sometimes they form a shoot with leaves, with a male capitulum at the top. The axillary buds below the capitulum do not develop, or very late, since the apical dominance of the continuously growing capitulum goes on. So the vegetative development is so strong that the plant does not surpass the formation of the final male capitulum.

Stage 2. Male and female flowering with vegetative development.
A final male capitulum with flowers which produce pollen. The bracts are or are not developed. Sometimes the lowest buds of the capitulum form a male capitulum or an axis with some male capitulums or even a female flower. By the flowering of the final capitulum the apical dominance is stopped and the axillary buds below the capitulum grow out and form shoots with leaves with a final male capitulum or a final female flower. The female flower is usually surrounded by a rosette of leaves. The many newly formed axillary buds form shoots with leaves again, with a final male or female flower. This stage results in an endlessly growing and flowering bushy plant with a small number of flowers.

Stage 3. Normal flowering without vegetative development.
A small final male capitulum with quickly developing flowers. The bracts are not developed. Sometimes the lowest male buds form a second male capitulum. The axillary buds below the capitulum form 1 female flower or an axis with 3 female flowers, with a few small leaves or none at all. The lowest axillary buds

PHOTO 18. *Floral stages of X. strumarium at different balances between vegetative and generative development.*
From left to right:
0 = vegetative development.
1 = slow male flowering with strong vegetative development.
2 = male and female flowering with vegetative development.
3 = normal flowering without vegetative development.

form inflorescences, also being a small shoot with a final male capitulum and female flowers in the lower axils. Since all meristems become flowers and no new buds are formed, this stage means the end of the plant.

The stages described will be used in the following.

4.2. Floral stages as a result of grafting

4.2.1. *Different periods of grafting-contact in the combination Xanthium d/r.*

Experiment 27. Plants of 5 weeks from sowing in LD were grafted above the first pair of leaves with donor shoots, which had received 4 weeks of SD. After different periods of grafting-contact the donors were removed.

Table 23 shows the results at 5 months after the grafting. It appears, that the figures about the flowering-quotient, percentage and stage, rise with increasing duration of the grafting-contact, while the numbers of days till visible flower bud decrease. Since during a longer graft-contact more floral hormone will arrive in the receptor, it is likely that the rate of the flowering-reaction and the floral stage are determined by the amount of floral hormone.

Table 23. Flowering of receptor rootstocks of *X. strumarium* after different periods of grafting-contact of the combination d/r–.

nr.	duration d/r–	flowering			
	in days	q	%	days	stage
1	0	0/18	0	∞	0
2	8	8/14	57	42	1,2
3	10	9/14	64	36	1,3
4	12	11/12	92	32	1,4
5	14	13/13	100	32	2,4
6	16	15/15	100	32	2,6
7	18	17/17	100	31	3,0

4.2.2. *Rudbeckia bicolor as a donor for Xanthium strumarium.*

Okuda (1953) demonstrated transmission of flowering-impulse by approach grafting of a donor of *Rudbeckia bicolor* and a receptor of *Xanthium canadense.* Although he applied permanent grafting-contact, his average floral percentage did not exceed 46%. In the next experiment the donor capacity of *R. bicolor* was tested.

Experiment 28. Shoots of *R. bicolor*, induced in LD, with at least 4 leaves and a just visible flower bud, were grafted on defoliated rootstocks of *Xanthium strumarium*, which had an age of 5 weeks from sowing in LD.

Table 24 shows that the control rootstocks of *X.* in grafting 1 remained

strictly vegetative. The donor shoots of *R. bicolor* in grafting 1, treatment 2, were only capable to bring 20% of the *X.* rootstocks into flowering, although the growing together of donors and receptors was satisfactory. Moreover, the flowering was late and at stage 2, while no synthesis of floral hormone could be demonstrated in grafting 2.

As *R. bicolor*, like *Calendula officinalis* can only bring about limited flowering in *X.* receptors, in spite of long grafting-contact, it is likely that the level of floral hormone of a flowering *R. bicolor* is low. (p. 38)

TABLE 24. Flowering of receptor rootstocks of *X. strumarium* after grafting with donors of *R. bicolor*. Shoots of receptors in grafting 1 were tested on synthesis of floral hormone in grafting 2.

nr.	grafting 1	flowering				grafting 2	flowering	
		q	%	days	stage		q	%
1	$\frac{X.r}{X.r_1^+}$	0/15	0	∞	0	$\frac{X.r_1^+ \text{ veg.}}{X.r_2^- \text{ veg.}}$	0/15	0
2	$\frac{\textit{Rudbeckia}\ d}{X.r_1^+}$	3/15	20	64	2	$\frac{X.r_1^+ \text{ gen.}}{X.r_2^- \text{ veg.}}$	0/3	0

4.2.3. *The donor capacity of shoots from Xanthium plants, flowering at stage 2.*

We have seen that no donor capacity could be proved of the *X.* rootstocks which were brought into flowering by grafting with *Calendula officinalis*, *Rudbeckia bicolor* or by short grafting-contact with generative *X.* shoots. However, a *X.* plant, flowering at stage 2, shows big differences in the floral development of its individual shoots. The question arose whether shoots with only a male capitulum contain a different level of floral hormone in comparison with shoots with only a female top flower.

Experiment 29. In an attempt to demonstrate possible differences in levels of floral hormone, shoots with a male capitulum and shoots with a female flower were grafted on receptor shoots.

Table 25 shows that some male as well as some female flowering shoots were able to bring about flowering in the receptors. The low flowering-percentages, the many days till visible flower bud, and the low floral stages suggest low levels of floral hormone in both donor types. Comparison of combinations 2 and 3 shows a somewhat better floral reaction, when a female flowering shoot was used as donor. This could mean that a female flowering shoot contains a somewhat higher level of floral hormone than a male flowering shoot. The small number of flowering receptors, however, does not allow definite conclusions.

TABLE 25. Flowering of receptor rootstocks of *X. strumarium* after grafting with male or female flowering donor shoots.

nr.	graft combination	flowering			
		q	%	days	stage
1	r/r	0/24	0	∞	–
2	♂ d/r	2/24	8	105	1
3	♀ d/r	2/12	17	60	1

4.3. FACTORS INFLUENCING THE TRANSMISSION OF FLORAL HORMONE BY GRAFTING

4.3.1. *Introduction*

Grafting is a radical action with many factors influencing the results. It is likely that many unexpected and contradictory effects are caused by unknown influences.

OKUDA (1953) concluded that some unknown factors would be responsible for the abnormal fruits formed by grafted plants of *Xanthium canadense*. HAMNER and BONNER (1938) investigated the influence of leaves and buds on the transmission of flowering of two-branched plants of *Xanthium*. They found an inverse relationship between the amount of LD leaf tissue and the magnitude of the flowering-response of the receptor branch. Young LD leaves were found to be favourable for the flowering-reaction of the receptor, while removal of buds and young leaves from the SD-donorshoot had a favourable influence on the flowering of the receptor. LINCOLN et al. (1956) extended this work and concluded that young and rapidly growing leaves and buds cause an area of carbohydrate deficit. They explained the influence on flowering by assuming that the flow of carbohydrates carries floral hormone with it.

In the present work some factors, influencing the transmission of floral hormone by grafting of *Xanthium*, were studied. Experiment 27 already showed the influence of the duration of grafting-contact on the transmission of flowering. Shortening the necessary duration of grafting-contact is important, since in most cases the optimal condition of the donor only exists for a short time.

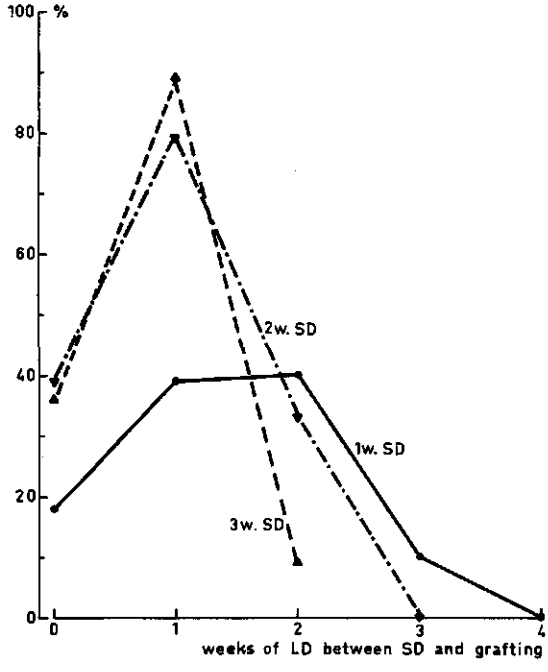
4.3.2. *The influence of SD induction and LD treatment on the donor capacity.*

Experiment 30. Xanthium plants at an age of 4 weeks from sowing in LD when the fourth leaf was just developing, received different cycles of SD induction. In an effort to separate induction and synthesis of floral hormone, the different cycles of inductive treatment were followed by different periods of LD at about 20 °C. Top shoots from these induced plants were cut off and grafted on receptor rootstocks with a contact of 3 weeks.

Table 26 shows that the best flowering % is obtained after treatment 12, where the donor received 3 weeks of SD followed by 1 week of LD. Comparing the influences of the LD periods following the SD induction, it is remarkable that

TABLE 26. Flowering of receptor rootstocks of *X. strumarium* after 3 weeks grafting-contact with donors, pretreated differently.

nr.	donor pretreatment		flowering		
	weeks SD	weeks LD	q	%	days
1	0	5	0/16	0	♀
2	1	0	3/17	18	55
3	1	1	5/13	39	39
4	1	2	4/10	40	54
5	1	3	1/10	10	38
6	1	4	0/10	0	♀
7	2	0	5/13	39	24
8	2	1	11/14	79	33
9	2	2	4/12	33	31
10	2	3	0/6	0	♀
11	3	0	4/11	36	27
12	3	1	8/9	89	41
13	3	2	1/11	9	32
14	4	0	6/11	55	36
15	4	1	7/11	64	33
16	5	0	3/8	38	33



GRAPH. 1. The flowering-percentages (ordinate) of receptor rootstocks of *X. strumarium* after grafting with donors, induced by 1, 2 or 3 weeks of SD, followed by 0 to 4 weeks of LD before grafting. (Table 26)

always 1 week of LD has a favourable effect on the flowering %. To demonstrate this more clearly, the relevant data are composed in Graph 1, which also shows that more LD treatment than one week has a negative influence on the flowering %, with the exception of 2 weeks LD after 1 week SD. An explanation for this influence of LD could perhaps be that the donors received more light energy in LD than in SD, because the experiment was done in a greenhouse in summer.

4.3.3. *The influence of temperature on the synthesis of floral hormone.*

SALISBURY (1963) concluded that low night and day temperatures are unfavourable for flowering of *Xanthium*, whether they are given before the SD period or after it. He assumes that the best temperature conditions will depend strongly on the species used. SALISBURY found for his *Xanthium* strain in growth chambers with adequate light intensities, that the flowering response was still increasing with increasing day and night temperatures up to 27°C, the highest temperature tested. The preceding experiment 30 showed that 2 or 3 weeks of SD were almost sufficient for complete induction, while 1 week of following LD proved to be favourable. When the effect of this last week is due to an influence on the synthesis of floral hormone, the question arises, what other environmental factors are able to influence this synthesis.

Experiment 31. In order to find out the influence of temperature on the synthesis of floral hormone, different temperatures were given during one week in LD or SD to plants which had received 2 weeks of SD. The top shoots of these plants were cut off after the temperature treatment and grafted on vegetative rootstocks. After 12 days of grafting-contact the donor shoots were removed.

Table 27 shows the unexpected results. It appears that the level of floral hormone 2 weeks after the beginning of induction was hardly sufficient for a transmission of flowering within 12 days. Only when these 2 weeks were filled up with SD, normal flowering occurred.

Comparison of treatments 1, 2 and 3, 4, 5 shows that instead of a flower promoting effect of LD as found in experiment 30 a flower inhibiting effect occurred at the higher temperatures.

TABLE 27. Flowering of receptor rootstocks of *X. strumarium* after 12 days of grafting-contact with donors which received 1 week of LD or SD at different temperatures after 2 weeks of ordinary SD induction.

nr.	temperature donor after 2 weeks of SD	flowering q	flowering %
1	LD 9°C	7/19	37
2	LD 13°	7/19	37
3	LD 17°	0/20	0
4	LD 21°	0/20	0
5	LD 25°	0/20	0
6	SD 17°	9/10	90
7	SD 21°	16/18	89
8	SD 25°	18/18	100

4.3.4. *The influence of the surface-active chemical dimethylsulfoxyde on the transmission of floral hormone.*

Several investigators concluded that at least a tissue connection and in most cases a vessel connection is required for transmission of flowering by grafting (ZEEVAART 1958). Therefore the question was asked whether a surface-active chemical could raise the membrane permeability of especially the walls of the cells around the grafting-place.

Experiment 32. Vegetative rootstocks with 2 branches were grafted with a donor shoot on one branch. During the 3 weeks of grafting-contact the graft combinations were misted weekly with solutions of dimethylsulfoxyde (DMSO) in order to try to promote the transmission of flowering from the donor to the receptor and eventually from the grafted branch to the ungrafted branch.

Table 28 does not show clear differences with the controls. So an influence of DMSO on the transmission of flowering could not be demonstrated.

TABLE 28. The flowering of 2-branched receptor rootstocks of *X. strumarium* after weekly spraying with D.M.S.O. during 3 weeks of grafting-contact with a donor.

nr.	DMSO	flowering of grafted branch			flowering of second branch		
	%	q	%	days	q	%	days
1	0	24/27	89	26	24/27	89	30
2	0,1	25/26	96	25	25/26	96	29
3	1	27/31	88	26	22/31	71	28

4.3.5. *The influence of the mature leaves of the receptor on the growth of the axillary shoots and the transmission of flowering from a grafted donor-shoot.*

HAMNER and BONNER (1938) and later LINCOLN et al. (1956) convincingly proved that the presence of mature leaves on the receptor branch of a two-branched *Xanthium* plant, inhibits the transmission of flowering from the donor branch. In the present experiments usually a donor shoot was grafted on a receptor rootstock and the flowering of the upper axillary shoot of the receptor was observed. However, when the receptor rootstocks were completely defoliated at the moment of grafting with a donor shoot, the axillary shoots developed slowly and often many of the rootstocks died. When the mature leaves of the rootstocks were removed when the axillary buds were developing, less mortality occurred. Moreover it was remarkable, that when a shoot of *Xanthium* was used as a donor and the mature leaves were removed at the moment of grafting, more rootstocks survived than when a less or non related donor, like *Calendula officinalis* or *Perilla crispa*, was used.

According to these observations it is likely that a *Xanthium* rootstock dies due to a lack of substances produced in its own leaves. DE STIGTER (1956)

extensively studied this problem in *Cucurbitaceae*. The differences in mortality between the graftings of *Xanthium* on *Xanthium* and another donor on *Xanthium* could be explained by differences in rate of growing together of the grafting-partners and/or differences in leaf substances between the donors. Besides the necessity of surviving of the rootstocks, the influence of defoliation on the rate of development of the axillary buds will be of interest for the reaction on floral hormone. Although not directly related to the present subject, it seems worth while to investigate the influence of the presence of a leaf on the development of the corresponding axillary bud.

Experiment 33. Plants of 3 weeks from sowing in LD were decapitated above the first pair of leaves, in order to release the axillary buds. The first pair of leaves was removed at different periods after decapitation. Table 29 shows that the growth of the axillary shoots is strongly influenced by the corresponding leaves, especially during the first week of development. Photo's 19 and 20 clearly demonstrate this.

For grafting-experiments with a donor, this means that the point of time of defoliation of the receptor determines the vegetative development which interacts with the floral hormone.

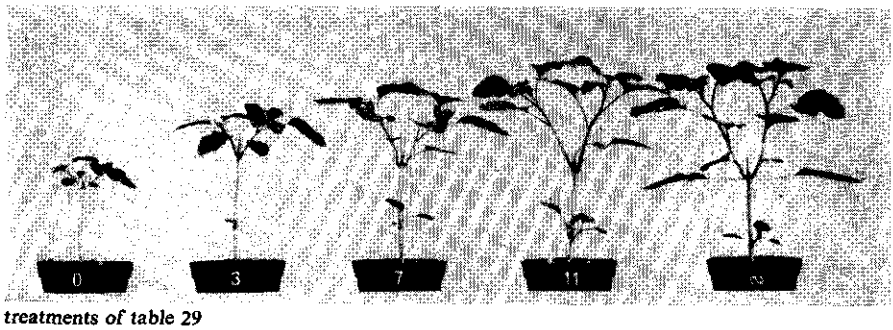
TABLE 29. The average lengths of the axillary shoots from the first leaf pair of *X. strumarium* in mm as a result of decapitation with removal of the first leaf pair at different moments; n = 10.

days between decapitation and defoliation	days between decapitation and observation				
	21	28	35	42	49
0	17	37	56	83	115
3	13	38	60	93	123
7	38	68	96	125	159
11	53	83	109	147	167
∞	61	96	118	153	178

The next questions are: Does a connection between the leaf surface and the growth of the axillary buds exist? Is the influence of a leaf restricted to the corresponding bud?

Experiment 34. Plants at an age of 3 weeks from sowing in LD were decapitated above the first pair of leaves, in order to release the axillary buds. The surface of the first pair of leaves was reduced and the lengths of the axillary shoots were measured at some intervals from 2 weeks after the decapitation.

Table 30 shows the results. It is remarkable that the effects of treatments 2 and 3, in spite of transverse or longitudinal halving of the leaves, are absolutely equal. Comparison of treatments 1 and 4 shows that the axillary bud of the right leaf of treatment 4 is influenced by the left leaf. Comparison of treatments



treatments of table 29

PHOTO 19. *Influence of the first pair of leaves of X. strumarium on the growth of their axillary shoots.*

Decapitation above the first pair of leaves.
From left to right defoliation after 0, 3, 7, 11 or ∞ days after decapitation.
The growth of the axillary shoots of the first pair of leaves and even of the cotyledons increases at longer presence of the first pair of leaves.
Photo: 28 days after decapitation.

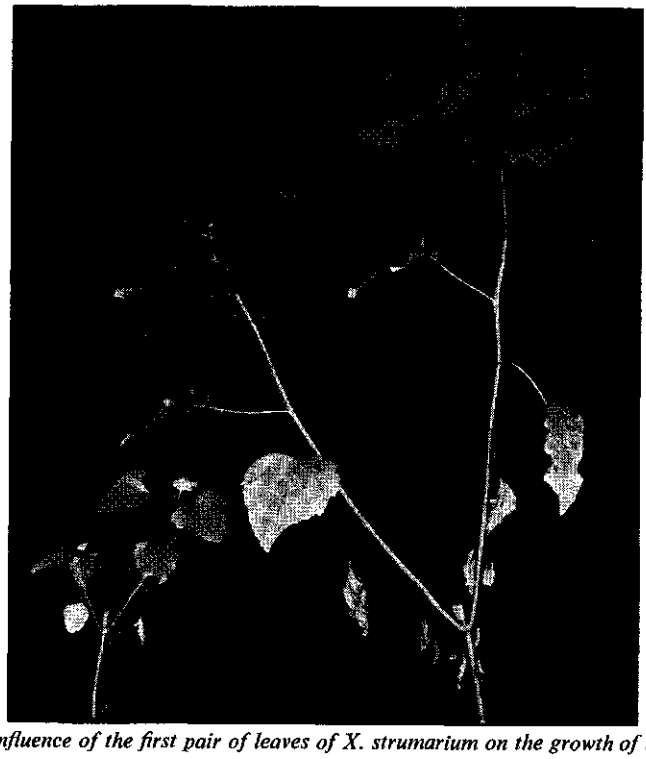


PHOTO 20. *Influence of the first pair of leaves of X. strumarium on the growth of their axillary shoots.*

Left: decapitated above the first pair of leaves and defoliated.
Right: decapitated above the first pair of leaves without defoliation.
Photo: 77 days after decapitation.

TABLE 30. The lengths of the axillary shoots from the first pair of leaves of *X. strumarium* in mm as a result of decapitation with differently reduced leaf surfaces; n = 10.
− = removed completely.
+ = intact.

nr.	leaf surface	days between decapitation and observation		
		14	21	28
1	left leaf −	3	12	32
	right leaf −	3	12	32
2	transversely halved:			
	left leaf	20	48	78
	right leaf	20	48	78
3	longitudinally halved:			
	left leaf	20	48	78
	right leaf	20	48	78
4	left leaf +	18	49	71
	right leaf −	17	39	60
5	left leaf +	32	74	109
	right leaf +	32	74	109

4 and 5 shows that the influence of the left leaf on the right axillary bud of treatment 4 reduced the influence of the left leaf on its own axillary bud.

The growth of the shoots of treatment 4 approaches the growth at treatments 2 and 3. The conclusion is that the total leaf surface of the plant determines the growth of the released axillary buds. The influence of the leaves is transversely translocated.

This induced the question whether also vertical translocation occurs.

Experiment 35. Plants of 5 weeks from sowing in LD with the fourth leaf just fully developed, were decapitated above the fourth leaf in order to release the axillary buds. The leaves were removed in different combinations as shown in Table 31. It appears that the highest buds, which were the soonest released by decapitation, show the strongest growth, with only small differences between the third and the fourth, staying closely together.

So the total leaf surface per plant determines the growth of the axillary shoots in sequence of releasing, independent from the place of the leaves.

Experiment 36. Plants at an age of 5 weeks from sowing in LD were grafted with donor shoots above the first pair of leaves. The donor shoots were cut from plants which had received 4 weeks of SD. The leaves of the receptor root-stocks were cut off at 0, 3 or 8 days after the grafting. The donor shoots were removed after 12 days of grafting-contact.

In Table 32 comparison of treatments 1 and 2 shows that the number of

TABLE 31. The average lengths of the axillary shoots of *X. strumarium* in mm after decapitation above the fourth leaf, with variously reduced leaves; n = 10.
Leaves: − = removed; + = present.

nr.	leaves		days between decapitation and observation	
			21	28
1	cotyledons	−	0	0
	first pair	−	0	0
	third	−	0	22
	fourth	−	0	17
2	cotyledons	+	0	0
	first pair	−	1	4
	third	−	10	31
	fourth	−	12	34
3	cotyledons	−	0	0
	first pair	+	6	15
	third	−	16	54
	fourth	−	8	34
4	cotyledons	−	0	0
	first pair	−	11	15
	third	+	40	74
	fourth	+	48	85
5	cotyledons	+	0	0
	first pair	+	10	12
	third	+	48	120
	fourth	+	71	135

surviving rootstocks is higher when the leaves are on the receptor for some days. The number of days till visible flower bud increases with longer presence of the receptor leaves. The number of days till visible bud of treatment 2 is hardly higher than of treatment 1, while the flowering % of the former is better. The conclusion is that it is preferable to defoliate the receptor rootstocks 3 days after the grafting, although it is not sure whether 3 days are optimal. Presence of the mature leaves of the receptor for a longer period inhibits the rate of transmission of flowering.

TABLE 32. Surviving and flowering of receptor-rootstocks of *X. strumarium* after 12 days of grafting-contact, during which the receptors were defoliated at different times.

nr.	defoliated after	surviving		flowering		
	days	q	%	q	%	days
1	0	24/32	67	16/24	67	30
2	3	31/32	97	24/31	77	32
3	8	30/32	94	22/30	73	39

The next question is: When the presence of leaves is required for some days in order to release the axillary buds more rapidly, has decapitation before the grafting the same or a better effect, because then the leaves can be removed at the grafting and do not have a possible inhibiting influence.

Experiment 37. Plants of 5 weeks from sowing in LD were decapitated above the first pair of leaves at different times. At 8 days after the decapitation of the first group the plants were grafted with donor shoots from plants which had received 4 weeks of SD. After 12 days of grafting-contact, the donors were removed.

Table 33 shows that, like in the preceding experiment, the number of days till visible flower bud increases with a longer period between decapitation and grafting, or: with a stronger growth of the axillary shoots. The absolute values of the figures of the flowering-results of tables 32 and 33 cannot be compared since the experiments were taken at different seasons.

TABLE 33. Flowering of receptor rootstocks of *X. strumarium*, which were decapitated at different times, after 12 days of grafting-contact.

nr.	days between decapitation and grafting	flowering		
		q	%	days
1	0	39/42	93	25
2	3	35/36	97	28
3	8	25/29	86	30

4.3.6. *The influence of the age of the receptor on the transmission of flowering.*

Experiment 38. A range of plants at ages from 3 to 7 weeks from sowing in LD were grafted with donor shoots. The number of available rootstocks of the different ages was variable. The rootstocks were defoliated and the graft places were chosen as high as possible. The donors were cut from plants which had received 4 weeks of SD, and were removed after 20 days of grafting-contact.

Table 34 shows that the flowering-result is better, the younger the receptor. ZEEVAART (1969a) proved that the grafting-place of *Bryophyllum* is important for the rate of transmission of flowering. Young tissue in the top of the stem proved to be better than the more woody tissue in the lower parts of the stem. He concluded that differences in rate of growing together, due to differences between the tissues at the grafting-places, were responsible for these results. However, in the present experiment the graft-places were chosen in the young tissues below the apical meristem. Therefore it is not likely that this factor alone is responsible for the big differences. It is presumable that other factors, the root system included, play a role. For instance the influence of a donor shoot on a small rootstock will be of more importance than the influence on a big rootstock on account of the relative differences in the sizes of the graft partners.

TABLE 34. Flowering of receptor rootstocks of *X. strumarium* of different ages at grafting after 20 days of grafting-contact.

nr.	receptor age in weeks	flowering		
		q	%	days
1	3	18/18	100	17
2	4	23/24	96	20
3	5	7/9	78	26
4	6	4/11	36	36
5	7	3/7	43	38

The next question is: What is the influence of the height of the grafting-place?

Experiment 39. Plants of 7 weeks from sowing in LD were defoliated and grafted with donor shoots at different heights. The donor shoots were cut from plants which had received 4 weeks of SD. After 20 days of grafting-contact the donors were removed.

In table 35 it is remarkable that relatively few of the rootstocks which were grafted above the cotyledons survived. Evidently referring to experiment 33 on p. 48, the slow development of the axillary buds of the cotyledons will be responsible for this. Furthermore, as far as the rootstocks survived, the flowering-results are variable and no clear influence of the grafting-place could be demonstrated.

TABLE 35. Surviving and flowering of receptor rootstocks of *X. strumarium* after 20 days of grafting-contact with the graft places at different heights.

nr.	graft-place above:	surviving rootstocks		flowering		
		q	%	q	%	days
1	cotyledons	8/18	44	6/8	75	33
2	first leaf pair	15/18	83	8/15	53	36
3	third leaf	13/18	72	8/13	61	31
4	fourth leaf	13/18	72	5/13	38	32
5	fifth leaf	13/18	72	6/13	46	29

4.4. INTERACTION OF INDUCTION AND FLORAL HORMONE

As discussed before, the mature leaves of a receptor inhibit the transmission of flowering from a donor (exp. 36, p. 50). This inhibiting action can be explained by the supplying of the receptor buds with assimilates, which interferes with the flow of assimilates with floral hormone from the donor. Moreover, it

is possible, that the mature leaves are a sink of floral hormone, or even form inhibiting substances.

The question arose: Can the inhibiting activity of mature receptor leaves be diminished by limited induction, hardly sufficient for a flowering reaction as such? It is clear, that the inhibiting influence due to assimilate synthesis will remain. Experimental evidence is available that 1 cycle of SD given to a whole plant gives a slow flowering-reaction, while 1 SD given to a reduced number of leaves hardly yields any flowering.

A next question is whether the floral hormone from a grafted donor will cooperate with floral hormone, synthesized by the receptor leaves. A problem will be to let the action of both types of floral hormone coincide at the receptive meristem.

Experiment 40. Plants of 5 weeks from sowing in LD received 0, 1, 2 or 3 cycles of SD and were grafted one week later above the first pair of leaves with a donor shoot which had received 4 weeks of SD. The rootstocks were not defoliated. The donor shoots were removed after 12 days of grafting-contact.

The results are presented in Table 36. Comparing the effects of SD alone (treatments 1, 2, 3, 4) and of grafting alone (treatment 5) with their combined effects (treatment 6, 7, 8), shows that there is an interaction between the result of induction and 12 days of grafting-contact. Comparison of treatments 2, 5 and 6 shows that the result of 1 SD given to the relatively old first pair of leaves can only be demonstrated in treatment 6. This means, that the interaction technique might be useful in order to demonstrate induction-effects which cannot be observed as such.

TABLE 36. The flowering of receptor rootstocks of *X. strumarium* which were exposed to 0, 1, 2 or 3 cycles of SD-induction, not followed ('0') or followed by grafting with donor shoots, grafting-contact 12 days ('12 d.').

nr.	SD	grafting d/r+	flowering			
			q	%	days	stage
1	0	0	0/9	0	∾	0
2	1	0	0/9	0	∾	0
3	2	0	2/9	22	21	1
4	3	0	7/9	78	16	1
5	0	12d.	4/9	44	28	1,4
6	1	12d.	6/9	67	23	2,1
7	2	12d.	7/9	78	23	2,4
8	3	12d.	8/8	100	18	3,0

4.5. COMPARISON OF AN EARLY, A MIDDLE AND A LATE LINE

4.5.1. *Material*

Preceding experiments showed that the used line of *X. strumarium*, for which

the critical dark period could be fixed to 8.33 h., only needs one cycle of SD (8 h. light/16 h. dark) to become just generative, while 3 cycles of SD are already sufficient for a normal flowering-result. In this connection the used line is not always suitable for experiments with sub-optimal induction, for example interaction experiments. A later flowering line of *X. strumarium* would then be preferable. McMillan (1970, 1971) has collected lines of *X. strumarium* with different critical dark periods. Seeds of lines with critical dark periods of 8.00 and 11.00 were obtained from him. Considering that our own line with a critical dark period of 8.33 lies between 8.00 and 11.00, the 3 lines were called early, middle and late. Since the numbers of seed of the early and late line were limited, only some preliminary experiments could be done.

4.5.2. *SD induction of middle and late.*

Experiment 41. Plants of middle and late, 5 weeks from sowing in LD, were moved to SD and with intervals of one week were moved back to LD. Table 37 shows that the middle line is optimally induced after 1 week of SD treatment. However, the late line does not show a weak flowering-response before 2 weeks of SD. Although 3 and 4 weeks of SD give plants flowering normally (stage 3), the flowering-quotient remains incomplete. Only continuous SD results in a complete flowering.

Photo 21 shows the differences between treatments 2 and 7 with 1 week of SD.

Table 37. The flowering of a middle and a late line of *X. strumarium* after different numbers of SD-treatment during increasing numbers of weeks.

nr.	line	SD	flowering			
		in weeks	q	%	days	stage
1	middle	0	0/6	0	∞	0
2	middle	1	6/6	100	18	3
3	middle	2	6/6	100	18	3
4	middle	3	6/6	100	18	3
5	middle	4	6/6	100	18	3
6	late	0	0/6	0	∞	0
7	late	1	0/6	0	∞	0
8	late	2	3/6	50	30	1
9	late	3	2/6	33	21	3
10	late	4	3/6	50	21	3
11	late	∞	6/6	100	30	3

nr: 2 7
nr. = treatment number of table 37

PHOTO 21. *The flowering-response to 1 week of SD of a middle and a late line of X. strumarium.*
Left: middle – generative.
Right: late – vegetative.
Photo: 5 weeks from the beginning of induction.

4.5.3. *Grafting of early, middle and late.*

The evidence of interspecies grafts (VAN DE POL, 1971) and the results of the present experiment 22 on an early and a late cv. of *Kalanchoë blossfeldiana* (p. 31) induced the questions whether differences in the level of floral hormone between early, middle and late lines of *X. strumarium* exist and influence their flowering and non localized synthesis of floral hormone.

Experiment 42. Plants of the 3 lines, 5 weeks from sowing in LD, were decapitated above the first pair of leaves and grafted 2 days later with donor shoots of the middle line from plants which had received 5 weeks of SD. The receptors were defoliated. The donors were removed after different periods of grafting-contact.

Table 38 shows, that 10 days of grafting-contact are enough for an optimal floral stage of the early line; the middle line needs 14 days, while the late one requires more than 20 days. Shoots of the flowering receptors were cut off and grafted on vegetative rootstocks, in order to test their donor capacity. It appears that only the optimally flowering receptors of treatments 2, 8, 9 and 16 were able to act completely as a donor, as far as the test goes. The conclusion is that longer grafting-contact is necessary for a flowering-reaction and non localized synthesis of floral hormone, as the receptor needs more SD induction.

Photo 22 shows the differences between the 3 lines, 2 months after the grafting.

TABLE 38. Flowering of receptor rootstocks of an early, middle and late line of *X. strumarium* after different periods of grafting-contact with middle donors in the combinations d/r_1^-. Shoots of r_1 were tested on synthesis of floral hormone in r_1/r_2.
r_1 = receptor rootstocktype.
contact = duration of grafting-contact of d/r_1^-, in days.
r_1/r_2 = next grafting of r_1 on middle r_2.

nr.	r_1	contact	flowering r_1				flowering r_2 of r_1/r_2			
			q	%	days	stage	q	%	days	stage
1	early	0	0/5	0	∾	0	0/5	0	∾	0
2	early	10	5/5	100	21	3	5/5	100	65	3
3	early	12	3/3	100	18	3	–	–	–	–
4	early	14	1/1	100	17	3	–	–	–	–
5	middle	0	0/5	0	∾	0	0/5	0	∾	0
6	middle	10	2/4	50	23	2	0/2	0	∾	0
7	middle	12	5/5	100	28	2	1/5	20	82	2
8	middle	14	4/4	100	23	3	4/4	100	74	2
9	middle	16	4/4	100	27	3	3/3	100	74	3
10	middle	18	3/3	100	30	3	–	–	–	–
11	late	0	0/5	0	∾	0	0/5	0	∾	0
12	late	10	0/5	0	∾	0	0/5	0	∾	0
13	late	14	3/4	75	33	2	0/3	0	∾	0
14	late	17	5/5	100	31	2	0/5	0	∾	0
15	late	20	5/5	100	31	2	0/5	0	∾	0
16	late	∾	4/4	100	29	3	4/4	100	76	2

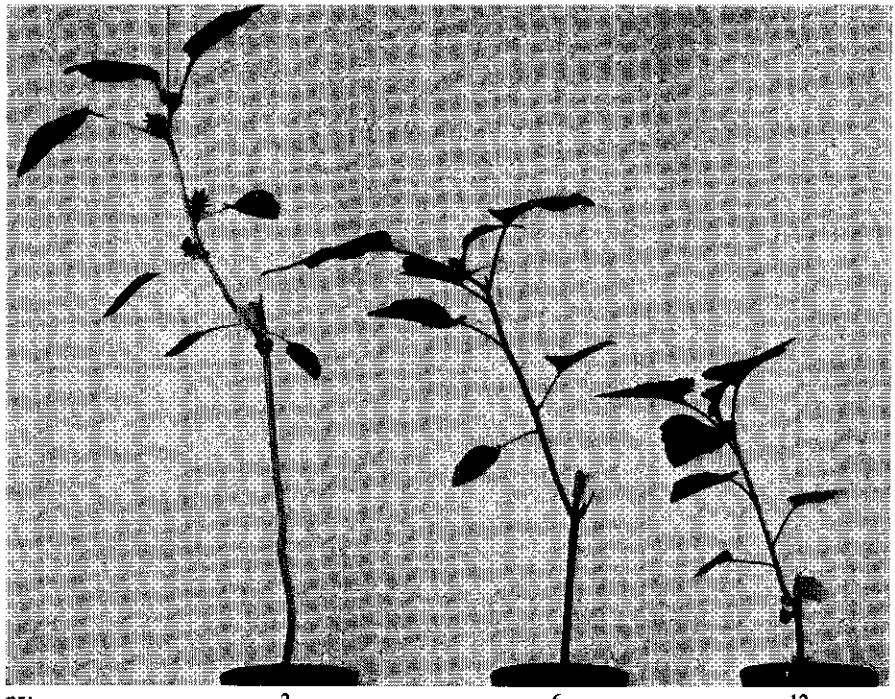


PHOTO 22. *Flowering of 3 lines of X. strumarium as receptors after 10 days of grafting-contact.*
Left: early line, normal flowering, stage 3.
Middle: middle line, only male flowering, stage 2.
Right: late line, no flowering, stage 0.
Note the differences in internode lengths between the lines.
Photo: 2 months after the grafting.

4.6. CONCLUSIONS

21. *Xanthium strumarium* can show different balances between vegetative and generative development (4.1.1.).
22. Meristems, which were formed before complete induction, form a shoot with a final male capitulum and female flowers in the lower axils. It is preferable to reserve the term inflorescence of *Xanthium* for such a flowering shoot. (Exp. 25).
23. Bisexual capitulums were found at places where a male capitulum as well as a female flower could be formed. (Exp. 25).
24. Four floral stages at balances of vegetative and generative development were distinguished: 0 = vegetative;
    1 = male only + strong vegetative development;
    2 = male and female with vegetative development;

3 = male and female with little or no vegetative development. (p. 40).

25. Differences between the numbers of days till the appearance of male and female flower buds are a function of the number of inductive cycles and can be considered as a measurement for the rate of development of the inflorescence. (Exp. 26).
26. Grafting-experiments showed that it is likely that the different balances of vegetative and generative development implicate different, constant levels of floral hormone. (Exp. 27, 28 and 29).
27. *Rudbeckia bicolor* can only bring about limited flowering in *Xanthium*, in spite of long grafting-contact. The few flowering receptors were not able to act as donor in a next grafting. It is likely that the level of floral hormone of *Rudbeckia* is low in contrast to *Helianthus annuus* or *Xanthium* (Exp. 28).
28. Only some shoots of plants of *Xanthium* in stage 2 were able to transmit flowering by grafting. (Exp. 29).
29. The duration of grafting-contact of d/r determines the continuous level of the balance between vegetative and generative development. It is possible that continuous synthesis of floral hormone in *Xanthium* can be initiated at different levels. (Exp. 27).
30. LD treatment of the donor after SD and before grafting proved to be important for the donor condition; 1 week LD after the SD-induction was optimal in the present experiment. (Exp. 30).
31. No influence of the surface active chemical dimethylsulfoxyde (DMSO) on the transmission of flowering could be demonstrated (Exp. 32).
32. The growth of axillary shoots was strongly influenced by the total leaf surface per plant, especially during the first week of development. (Exp. 33, 34 and 35).
33. Defoliation of the receptor stimulated the rate of the flowering-response. However, it is preferable to defoliate some days after the grafting to prevent dying of the rootstocks by too slow a development of the axillary buds. (Exp. 36).
34. Decapitation before the grafting had the same influence on the releasing of the buds and growth of the axillary shoots as delaying of defoliation after the grafting. (Exp. 37).
35. Three weeks old receptor rootstocks proved to be better than older ones for transmission of flowering. (Exp. 38).
36. No clear influence of the height of the grafting-place on the transmission of flowering could be demonstrated. (Exp. 39).
37. Interaction between induction of the receptors and transmission of flowering by grafting could be demonstrated. (Exp. 40).
38. A late line needed more than 1 week of SD for any flowering-reaction in contrast to a middle line with a complete flowering-reaction already after 1 week of SD. (Exp. 41).
39. Longer grafting-contact was necessary for a flowering-reaction and for non localized synthesis of floral hormone, as the receptor needed more SD induction. (Exp. 42).

5. EXPERIMENTS WITH CARYOPHYLLACEAE

5.1. INTRODUCTION

WELLENSIEK (1966) demonstrated the great persistence of the floral hormone of *Silene armeria* and mentioned as possible mechanism autocatalytical multiplication of the floral hormone. In the present experiments other *Caryophyllaceae* were tried in order to study whether a transmissable floral hormone could be demonstrated and whether graft combinations with *S. armeria* could give more information about the nature of the persistence of the floral hormone.

5.2. INDUCTION

Experiment 43. A group of 16 *Caryophyllaceae* was selected from a collection of 50 species, brought together by WELLENSIEK (unpublished). After about 8 weeks from sowing in SD, the conditions for flower induction were investigated. The plants received: permanent SD or LD, and in some cases 3 × GA_3 sprays 100 p.p.m., or 16 weeks 2°C in SD followed by ordinary SD or LD.

Table 39 summarizes the results. It appears that most of these *Caryophyllaceae* are qualitative LDP. In species 1 and in species 14 for 9%, the requirement of LD for induction could be replaced by GA_3. Species 4 can be considered as an LDP with requirement for low temperature or GA_3. Species 2 and 15 have low temperature requirement and then are DNP, while species 3 looks like the same type but with the *possibility of replacing low temperature by GA_3*.

TABLE 39. Flowering of caryophyllous species after SD, LD, GA_3 or 2°C treatment. + = applied; − = not applied.

nr.	species	SD	LD	GA_3	2°C	flowering		induction
						q	%	by
1	*Silene cucubalis* WIBEL	+	−	−	−	0/20	0	
		−	+	−	−	20/20	100	LD
		+	−	+	−	20/20	100	SD + GA_3
		+	−	−	+	4/20	20	2° + SD
2	*Silene italica* PERS.	+	−	−	−	0/15	0	
		−	+	−	−	0/15	0	
		+	−	+	−	0/15	0	
		−	+	+	−	0/15	0	
		−	+	−	+	15/15	100	2° + LD
		+	−	−	+	15/15	100	2° + SD

nr.	species	SD	LD	GA_3	2°C	flowering		induction
						q	%	by
3	*Silene nutans* L.	+	−	−	−	0/15	0	
		−	+	−	−	0/15	0	
		+	−	+	−	4/15	27	GA_3 + SD
		−	+	+	−	14/15	93	GA_3 + LD
		−	+	−	+	7/15	47	2° + LD
		+	−	−	+	10/15	67	2° + SD
4	*Silene otites* S.M.	+	−	−	−	0/15	0	
		−	+	−	−	7/15	47	LD
		+	−	+	−	0/15	0	
		−	+	+	−	15/15	100	GA_3 + LD
		−	+	−	+	15/15	100	2°C + LD
		+	−	−	+	3/15	20	2°C + SD
5	*Silene gallica* KOCH	+	−	−	−	0/15	0	
		−	+	−	−	15/15	100	LD
6	*Silene nocturna* L.	+	−	−	−	0/15	0	
		−	+	−	−	15/15	100	LD
7	*Silene bosniaca* BECK	+	−	−	−	0/22	0	
		−	+	−	−	22/22	100	LD
8	*Silene maritima* WITH.	+	−	−	−	2/13	15	SD
		−	+	−	−	13/13	100	LD
9	*Silene annulata* HORT. ex FENZL	+	−	−	−	0/13	0	
		−	+	−	−	13/13	100	LD
10	*Silene brachypetala* HORT. ex FENZL	+	−	−	−	0/13	0	
		−	+	−	−	13/13	100	LD
11	*Silene quinquevulnera* L.	+	−	−	−	0/22	0	
		−	+	−	−	22/22	100	LD
12	*Silene echinosperma* BOISS. et HELDER	+	−	−	−	0/22	0	
		−	+	−	−	22/22	100	LD
13	*Silene alba* (MILLER) E. H. L. KRAUSE	+	−	−	−	0/22	0	
		−	+	−	−	21/22	95	LD
14	*Melandrium album* (MILL.) GARCKE	+	−	−	−	0/22	0	
		−	+	−	−	22/22	100	LD
		+	−	+	−	2/22	9	GA_3 + SD
		+	−	−	+	20/22	90	2°C + SD

nr.	species	SD	LD	GA_3	2°C	flowering		induction
						q	%	by
15	*Melandrium rubrum* (WEIG.) GARCKE	+	−	−	−	0/22	0	
		−	+	−	−	0/22	0	
		+	−	+	−	0/22	0	
		+	−	−	+	22/22	100	2° + SD
		−	+	−	+	22/22	100	2° + LD
16	*Dianthus sinensis* c.v. 'Bravo'	+	−	−	−	22/22	100	SD
		−	+	−	−	22/22	100	LD

It is likely that flowering responses between 0 and 100% are caused by genetic differences in the unselected material, as will be demonstrated for *Melandrium album* on p. 70.

5.3. GRAFTING-EXPERIMENTS

Experiment 44. The most promising species of the available *Caryophyllaceae* were selected and tried in grafting-experiments. For the graft combination r/d always a split grafting in the stem was made, while for the reverse combination d/r mostly a split-grafting in the rosette was made, as described in 'Methods', p. 5.

Moreover, it is possible that for many plants an interaction exists between GA_3 and other inductive factors.

Table 40 shows a summary of the graft combinations with at least 2 months of grafting-contact. The control graftings of the combinations without transmission of flowering are not mentioned; all are negative. It is remarkable that most of the graft combinations did not show any transmission of flowering. Besides combinations 33 and 34 with the already known case of *S. armeria*, only a part of the receptors of treatments 16, 19 and 20 flowered. Although the numbers of the flowering plants are small, the strictly vegetative controls of combinations 15 and 18 allow the provisional conclusion that a flowering plant of *S. echinosperma* contains a transmissable floral hormone which is able to bring vegetative shoots of both *S. echinosperma* and *S. armeria* in the combination r/d into flowering. Moreover, 2 rootstocks of *S. armeria* reacted on downward movement of floral hormone from donor shoots of *S. echinosperma* (nr. 20).

The successful combinations of treatments 16 and 19 are demonstrated in photo's 23 and 24.

The promising graft combinations with *S. echinosperma*, treatments 15, 16 and 17, were planned to be repeated in *experiment 45*. However, all the plants,

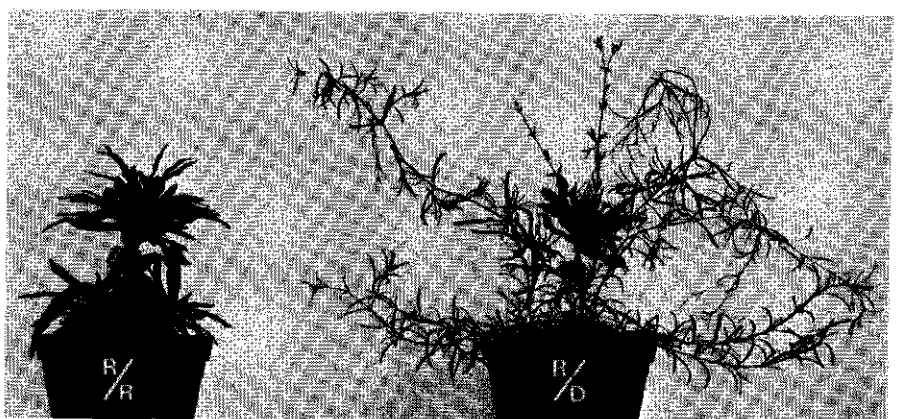
TABLE 40. Flowering response of some Caryophyllaceae receptors after at least 2 months of grafting-contact with donors of the same family.
s = cleft grafting in the stem.
r = cleft grafting in the rosette.

nr.	combination	grafting-method	flowering	
			q	%
1	*S. cucubalis* r / *S. cucubalis* d	s	0/20	0
2	*S. cucubalis* d / *S. cucubalis* r	r	0/20	0
3	*S. armeria* r / *S. cucubalis* d	s	0/20	0
4	*S. cucubalis* d / *S. armeria* r	r	0/20	0
5	*S. nutans* r / *S. nutans* d	s	0/6	0
6	*S. nutans* d / *S. nutans* r	r	0/6	0
7	*S. nutans* r / *S. armeria* d	s	0/5	0
8	*S. otites* r / *S. otites* d	s	0/8	0
9	*S. otites* d / *S. otites* r	r	0/7	0
10	*S. armeria* d / *S. otites* r	r	0/8	0
11	*S. armeria* r / *S. gallica* d	s	0/9	0
12	*S. gallica* r / *S. armeria* d	s	0/9	0
13	*S. armeria* r / *S. nocturna* d	s	0/12	0
14	*S. nocturna* d / *S. armeria* r	s	0/10	0
15	*S. echinosperma* r / *S. echinosperma* r	s	0/12	0
16	*S. echinosperma* r / *S. echinosperma* d	s	7/10	70
17	*S. echinosperma* d / *S. echinosperma* r	s	0/11	0
18	*S. armeria* r / *S. armeria* r	s	0/12	0
19	*S. armeria* r / *S. echinosperma* d	s	5/12	42
20	*S. echinosperma* d / *S. armeria* r	s	2/10	20
21	*M. album* r / *M. album* d	s	0/50	0
22	*M. album* d / *M. album* r	r	0/31	0
23	*M. album* r / *S. armeria* d	s	0/28	0
24	*S. armeria* d / *M. album* r	r	0/10	0
25	*S. armeria* r / *M. album* d	s	0/30	0
26	*M. album* d / *S. armeria* r	r	0/14	0
27	*M. album* r / *S. cucubalis* d	s	0/7	0
28	*S. cucubalis* d / *M. album* r	r	0/9	0
29	*M. rubrum* d / *S. armeria* r	s	0/6	0
30	*S. armeria* r / *D. sinensis* d	s	0/3	0
31	*D. sinensis* d / *S. armeria* r	s	0/12	0
32	*S. armeria* r / *S. armeria* r	r	0/51	0
33	*S. armeria* r / *S. armeria* d	s	48/55	87
34	*S. armeria* d / *S. armeria* r	r	71/75	95

grown in SD became generative before the grafting. So *S. echinosperma*, which reacted in experiments 43 and 44 as a qualitative LDP, behaved now as a DNP, although the same seed lot was used. A possible explanation for this unexpected reaction is that experiments 43 and 44 were done in winter with low light intensity, while experiment 45 was done in summer. Since *S. echinosperma* seems to be of interest for research on the floral hormone, especially in combination with *S. armeria*, more research into the inductive circumstances is

nr: 15 16
nr. = treatment number of table 40

PHOTO 23. *S. echinosperma after grafting on a vegetative rootstock (R/R, not flowering) or on a generative rootstock (R/D, flowering).*
Photo: 75 days after the grafting.



nr: 18 19
nr. = treatment number of table 40

PHOTO 24. *S. armeria after grafting on vegetative S. armeria (R/R, in rosette) or on generative S. echinosperma (R/D, weak flowering shoots from the rosette).*
Photo: 81 days after grafting.

wanted. Selection for a late line might be a solution for the problem of undesired flowering.

The flowering-response of *S. armeria* due to grafting with a donor of *S. echinosperma* (treatment 19 of table 40), was slow and weak, as could be seen in photo 24. So it was no surprise that these old, weak flowering shoots of *S. armeria* were not able to act as a donor in a next grafting on vegetative rootstocks of *S. armeria*.

5.4. SILENE ARMERIA

5.4.1. *The mechanism of GA_3 in flower formation.*

Experiment 46. The donor activity of plants which were brought into flowering by GA_3 in SD was demonstrated preliminary by WELLENSIEK (1966). This problem could be extensively studied since WELLENSIEK (1970b) selected lines of *S. armeria*, like N_4, which can be brought into flowering by GA_3 in SD. Another line, L_1, could not be brought into flowering by GA_3. In order to investigate whether floral hormone was present in GA-treated plants of N_4, flowering shoots were cut off and grafted via the rosettegrafting method on vegetative rosette plants of L_1.

Table 41 shows the results. Although some flowering control plants in treatment 1 occurred, probably due to a technical error, the figures of treatment 2 convincingly demonstrate, that a GA_3 treated N_4 is a good donor for L_1. So GA_3 induces the synthesis of a floral hormone in N_4.

TABLE 41. Flowering of receptor rootstocks of the late line L_1 of *S. armeria* after rosettegrafting with L_1r (control) or with donors of the GA_3-induced line N_4 of *S. armeria*.

nr.	combination	flowering		
		q	%	days
1	L_1r / L_1r	3/21	14	94
2	N_4d / L_1r	17/18	94	60

5.4.2. *The influence of the roots and the lower part of the rosette on the inhibition for flowering of the shoots of a vegetative plant.*

As mentioned before, several lines of *S. armeria* were selected. Some of them could not be induced to flowering by GA_3, for example L_1. GA_3 applied to L_1, gave stem elongation, and when the influence of GA_3 had been finished, the plants formed again a rosette, but now on a stem. When cut off and placed on small bottles with water, most of these rosettes elongated and even flowered for about 30%, although they were always under non-inductive circumstances. Since the rosettes with roots remained vegetative, even although the lower

leaves were removed, the questions arose: Does an inhibiting influence exist on flowering, arising from the roots, and is there interaction between an inductive treatment and removal of the roots?

Experiment 47. Plants of the line L_1, 8 weeks from sowing in SD, were misted 3 times weekly with GA_3-100 p.p.m. The plants elongated and 5 weeks after the last GA_3-treatment, when the activity of GA_3 had dimished and rosettes on stems had been formed, 0 or 2 cycles of CL were given. The next day the rosettes were not or were cut off and placed on water. The results are presented in table 42. Comparison of treatments 1 and 3 shows the generally observed phenomenon of some flowering under non inductive circumstances as a result of removal of the roots. The limited reaction can perhaps be explained by assuming genetical differences for the inhibiting influence of the roots. Comparison of treatments 2 and 4 shows that the small number of flowering plants induced by 2 cycles of CL can be increased by removal of the roots. The relatively large numbers of days till visible flower bud points to slow development on water. The conclusion is that interaction between CL and removal of the roots might be possible. However, the small numbers do not allow definite conclusions.

TABLE 42. The flowering of rosettes of *S. armeria* 'L_1' after 0 or 2 cycles of CL, not followed or followed by removal of the roots, or: with roots (+) and without roots (—) respectively.

nr.	CL	roots	flowering		
			q	%	days
1	0	+	0/9	0	∞
2	2	+	2/12	17	40
3	0	—	3/12	25	53
4	2	—	5/10	50	55

WELLENSIEK (1967) demonstrated that GA_3 is a partial induction factor for the line L_1. This means, that the inductive influence of GA_3 can only be demonstrated in an interaction experiment. In order to investigate an eventual interaction between GA_3 and removal of the roots, the next experiment was done.

Experiment 48. Plants of 8 weeks from sowing in SD received 3 weekly GA_3 treatments. Directly after the last GA_3 misting, the shoots were not or were cut off and placed on water or on one of 3 concentrations of GA_3. Table 43 shows the results. Comparison of treatments 1 and 2 shows that also in this experiment removal of the roots resulted in a partial flowering-reaction. Although GA_3 was applied until the removal of the roots, no complete flowering appeared. Comparison of treatments 2, 3, 4 and 5 shows that GA_3 in the absorption medium had no influence on the flowering-reaction. The conclusion

TABLE 43. Flowering-reaction of *S. armeria* 'L_1' after misting with GA_3, not followed or followed by removal of the roots, or: with roots (+) and without roots (−) respectively and placing of the shoots on water or one of 3 concentrations of GA_3.

nr.	roots	medium	flowering	
			q	%
1	+	−	0/10	0
2	−	water	19/49	39
3	−	GA_3 50 p.p.m.	3/10	30
4	−	$GA_3$100 p.p.m.	3/10	30
5	−	$GA_3$200 p.p.m.	3/10	30

is, that no interaction between GA_3 and removal of the roots could be demonstrated.

5.4.3. *The floral hormones of Silene armeria and Perilla crispa.*

In 1. 'General Introduction', it has already been mentioned that *Xanthium strumarium*, *Bryophyllum daigremontianum* and *Silene armeria* have the property of non-localized synthesis of floral hormone, contrary to *Perilla crispa* with strictly localized synthesis. However, ZEEVAART (1958) convincingly proved that induced leaves of *P. crispa* posses a very persistent synthesis of floral hormone, since they were able to transmit flowering in a series of successive graftings. The question was put: Are the differences between the former group and *P. crispa* caused by differences between the floral hormones or by differences between the endogenous conditions of the plants. In the latter case the floral hormones can be identical, but the reactions of the plants are different. This would mean that *P. crispa* forms a floral hormone after SD induction only. In order to investigate the reaction of *S. armeria* on the floral hormone of *P. crispa*, a grafting-experiment was done.

Experiment 49. Plants of *S. armeria* 'L_1' were stem-elongated by GA_3. When the activity of GA_3 had stopped, the shoots were cut off and grafted on generative rootstocks of *P. crispa* which had received 3 weeks of SD. The flowers of the *P. crispa* rootstocks were regularly removed, while the newly formed leaves remained since they were induced by the permanent SD conditions and were considered as new sources of floral hormone synthesis. However, after 5 weeks the rootstocks began to die, while the receptor shoots were still in a good condition. According to the method of WELLENSIEK (1970a) the shoots were cut off and regrafted on vegetative rootstocks of *S. armeria* 'L_1'. When these graft combinations had grown together, they were hardened off and placed in the SD division of a greenhouse.

In the control grafts *S. armeria* r/*P. crispa* r the shoots of *S. armeria* had to be exposed to SD, the *P. crispa* rootstocks to LD, since otherwise they would be induced to flower bud formation by photoperiodic action. These conditions could be achieved in the 'daylength cabinet', described by WELLENSIEK and

ELINGS (1967). However, the shoots of *S. armeria* could not be supplied with sufficient water and wilted.

Another control was the grafting of *S. armeria* r_1 shoots on vegetative rootstocks r_1 of the same species, followed by regrafting the r_1 shoots on vegetative rootstocks r_2 after 5 weeks.

Table 44 shows that the shoots of the control treatment with only grafting-contact with *S. armeria* remained strictly vegetative, while treatment 2 shows that the 5 weeks of grafting-contact with *P. crispa* in grafting 1 resulted in 71% flowering in grafting 2. The number of days till visible flower bud demonstrates, that the flowering-reaction of the receptors was very slow. Still all generative receptors reached the stage of complete flowering, about 5 months from grafting 1. However, the rootstocks were old on that moment and showed hardly any development. May be due to this condition of the rootstocks, no transmission of flowering from the generative receptors r_1 to the vegetative rootstocks r_2 could be observed.

Photo 25 demonstrates the flowering of *S. armeria* after grafting with *P. crispa*.

TABLE 44. Flowering of shoots of *S. armeria* 'L_1' after 5 weeks of grafting-contact with rootstocks of vegetative *S. armeria* 'L_1', or generative *Perilla crispa*, followed by regrafting on vegetative rootstocks of *S. armeria* 'L_1'.
days = average number of days till visible flower bud from the date of grafting 1.

nr.	grafting 1	grafting 2	flowering		
			q	%	days
1	*S. armeria* r_1	*S. armeria* r_1	0/10	0	∾
	S. armeria r_1	*S. armeria* r_2	0/10	0	∾
2	*S. armeria* r_1	*S. armeria* r_1	10/14	71	92
	P. crispa d	*S. armeria* r_2	0/14	0	∾

5.4.4. *Graft combinations between S. armeria, B. daigremontianum and X. strumarium.*

WELLENSIEK (1970a) clearly demonstrated transmission of flowering from *X. strumarium* to *S. armeria*, although numerous attempts with the reverse combination completely failed (personal communication). He points to the possibility of identity of the floral hormones of these plants, which have the property of non-localized synthesis of floral hormone in common. Another plant with this property is *B. daigremontianum*.

The question arose: Is there identity between the floral hormones of *S. armeria*, *B. daigremontianum* and *X. strumarium*? Many graft combinations were made between *X. strumarium* receptors and donors and *B. daigremontianum* donors and receptors, however, without flowering-reaction of the receptors.

PHOTO 25. *Transmission of flowering from a generative rootstock of P. crispa to a grafted vegetative shoot of S. armeria.*
Grafting 1: *left*: *S. armeria* r_1/*S. armeria* r_1 (control), *right*: *S. armeria* r_1/*P. crispa d.*
Photo: 4 weeks from grafting 1.
Grafting 2: shoots regrafted after 5 weeks of grafting contact in grafting 1.
Left: *S. armeria* r_1/*S. armeria* r_2 (r_1 = control-shoot of grafting 1).
Right: *S. armeria* r_1/*S. armeria* r_2 (r_1 had 5 weeks of grafting-contact with *P. crispa*).
Photo: 12 weeks from regrafting on r_2.

Experiment 50. The graft combination of *S. armeria* r/*B. daigremontianum* d seemed to be promising. After 5 weeks of grafting-contact the shoots were still alive. However, neither shoots kept on *B. daigremontianum* nor shoots, regrafted on vegetative rootstocks of *S. armeria*, showed any flowering-reaction.

5.4.5. *The influence of the surface-active chemical dimethylsulfoxyde on the transmission of floral hormone.*

Experiment 51. Plants of *S. armeria* 'L_1', 8 weeks from sowing in SD, were grafted with donor shoots of L_1, which were cut from plants which had received 3 weeks of LD. The grafting-method was split grafting in the rosette. Before and after the grafting a series of DMSO treatments was applied. Although high amounts of DMSO were given, no toxic effect could be observed.

Table 45 shows that 3 weeks of donor-contact of d/r is too short for the slow downward transmission of floral hormone to obtain good flowering. DMSO did not improve this transmission at all.

5.4.6. *Inoculation experiments with sap from flowering plants.*

Certain properties of floral hormone show similarity with some virusses. For instance: transmission by grafting and the eventual capacity of autocatalytical multiplication of the floral hormones of some plants (WELLENSIEK, 1966). A remarkable property of virusses is transmission by inoculation.

TABLE 45. Flowering of rootstocks of *S. armeria* 'L_1' after 3 weeks of grafting-contact with a donor, while different mistings with dimethylsulfoxyde (DMSO) were applied.

nr.	combination	DMSO	misting	flowering		
		in %		q	%	days
1	d/r	0	—	6/24	25	40
2	d/r	5	r, 3 d. before grafting	3/17	18	43
3	r/r	5	r, 4 h. before grafting	0/21	0	∞
4	d/r	5	d/r, 2 × a day	4/23	17	36
5	r/r	1	r/r, 2 × a day	0/21	0	∞
6	d/r	1	d, daily	2/18	11	38
7	d/r	0	d, daily	1/16	6	69
8	d/r	1	d, daily	0/16	0	∞
9	d/r	0	r, daily	2/16	12	68
10	d/r	1	r, daily	1/18	6	69

Experiment 52. Inoculation techniques, used in virology, were tried to transmit flowering from flowering plants of *S. armeria* to vegetative ones. However, none of these treatments resulted in flowering of the receptors. The conclusion is, that the floral hormone of *S. armeria* immediately loses its persistence, once outside the plant.

5.5. MELANDRIUM ALBUM

5.5.1. *Introduction.*

In experiment 43 of 5.2. *M. album* proved to be a qualitative LDP in which low temperature treatment of 2°C could replace the LD action. Moreover, induction by misting with GA_3 was effective for some plants. The general impression was that *M. album* could be a *Caryophyllaceae*, like *S. armeria*, of interest for the research of the flowering-process. However, in experiment 44 of 5.3. no transmission of flowering by grafting could be demonstrated. In the next experiments some selections are described and the existence of a transmissable floral hormone was studied intensively.

5.5.2. *Induction experiments.*

Experiment 53. Plants of 8 weeks from sowing in SD received 1, 2 or 3 weeks of LD and were moved back to SD. The earliest flowering male and female plants of the group of 1 week LD were selected and placed in LD where they were crossed and yielded the seeds of the line early I. Similarly the crossing of the latest flowering plants of the group, which received 3 weeks of LD, resulted in the line late I.

Both lines, early I and late I, were misted with GA_3 100 p.p.m. All the plants reacted with stem elongation.

Table 46 shows that the plants of the line early I reacted to GA_3 with flower-

TABLE 46. Flowering of *M. album* lines early I and late I after misting GA_3 100 p.p.m. weekly, varying times, in SD.

nr.	line	GA_3	flowering	
			q	%
1	early I	0 ×	0/60	0
2	early I	1 ×	3/72	4
3	early I	2 ×	58/78	74
4	early I	3 ×	60/63	95
5	late I	0 ×	0/60	0
6	late I	3 ×	0/60	0

ing better at increasing amounts of GA_3, while late I did not react to the highest amount of GA_3 at all. The flowering plants of treatment 2, being 2 males and one female, were crossed and yielded the seeds of the line early II, which was used in the following experiments, where the uniformity of the new selection early II and the inheritance of the sensitivity for GA_3-induction were studied.

Experiment 54. The lines early II and late I were inbred and crossed. The seeds of the new generations of early II and late I and of their hybrid were sown in SD. From 8 weeks after sowing the plants were treated with GA_3.

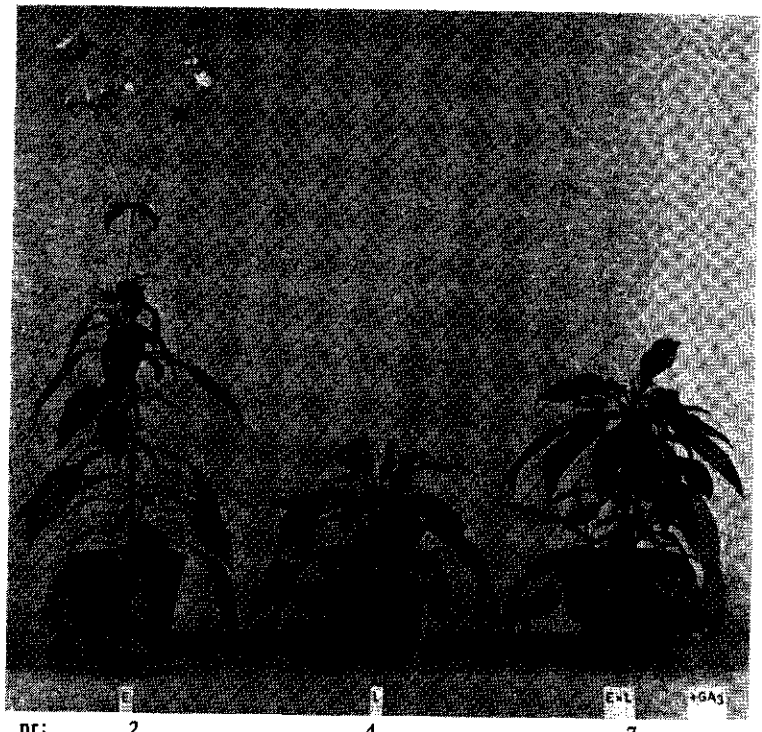
Table 47 shows that early II flowered uniformly after one treatment with GA_3. Late I did not flower at all after 4 weekly treatments with GA_3, while under these conditions the F_1, early × late, flowered for 46%. The conclusion is that the F_1 of early × late, concerning the flowering-reaction after treatment with GA_3, lies between the rapidly flowering early II and the non flowering late I.

Photo 26 illustrates these results.

Experiment 55. The lines early II, late I and their F_1 were sown in LD. After varying numbers of weeks of LD the plants were moved back to SD. The

TABLE 47. Flowering of 2 lines of *M. album* and their F_1 after misting GA_3 100 p.p.m. weekly, varying times, in SD.

nr.	line	GA_3	flowering		
			q	%	days
1	early II	0 ×	0/25	0	∞
2	early II	1 ×	25/25	100	18
3	late I	0 ×	0/25	0	∞
4	late I	1 ×	0/25	0	∞
5	late I	4 ×	0/25	0	∞
6	early × late	0 ×	0/25	0	∞
7	early × late	1 ×	0/25	0	∞
8	early × late	4 ×	11/24	46	33



nr: 2 4 7
nr. = *treatment number of table 47*

PHOTO 26. *Flowering-response of an early (E) and a late (L) line and their* F_1 *(E* × *L) of M. album after 1 misting with* GA_3 *100 p.p.m.*

Only E (left) flowered. The stem elongation of E × L (right) after the GA_3 treatment was stronger than of L.

Photo: 44 days after the GA_3 treatment.

flowering-results are presented in table 48. Comparison of treatments 2, 7 and 12 shows that within 5 weeks from sowing only early II can be induced for 35%. Treatments 3, 8 and 13 show that the numbers of LD, necessary for a flowering-reaction of F_1, are intermediate between early II and late I. Moreover, comparison of the treatments with 95% flowering shows that F_1 with 7 weeks, treatment 14, lies between early with 6 weeks, treatment 3, and late I with more than 8 weeks, treatment 10.

Experiment 56. Plants of 8 weeks from sowing in SD received varying numbers of cycles of LD in order to investigate the minimum number of LD for a 100 percent flowering in SD of the lines early II, late I and their F_1.

Table 49 shows that 7 or less cycles of LD are sufficient for 100 percent flowering of early II, while late I needs more than 13 cycles and the F_1 flowers completely after this treatment. The F_1 is variable, but tends to be intermediate.

TABLE 48. Flowering of 2 lines of *M. album* and their F_1 in SD after varying numbers of weeks in LD from sowing.

nr.	line	LD in weeks	flowering	
			q	%
1	early II	4	0/20	0
2	early II	5	7/20	35
3	early II	6	19/20	95
4	early II	7	19/20	95
5	early II	8	19/20	95
6	late I	4	0/20	0
7	late I	5	0/20	0
8	late I	6	0/20	0
9	late I	7	19/24	79
10	late I	8	19/24	79
11	F_1	4	0/20	0
12	F_1	5	0/20	0
13	F_1	6	8/20	40
14	F_1	7	19/20	95
15	F_1	8	20/20	100

TABLE 49. Flowering of 2 lines of *M. album* and their F_1 after growing in SD for 8 weeks, followed by varying numbers of cycles of LD.

nr.	line	LD cycles	flowering		
			q	%	days
1	early II	7	5/5	100	15
2	early II	9	5/5	100	14
3	early II	11	5/5	100	12
4	early II	13	5/5	100	12
5	late I	7	1/5	20	42
6	late I	9	0/5	0	∞
7	late I	11	3/5	60	40
8	late I	13	1/5	20	41
9	F_1	7	1/5	20	45
10	F_1	9	3/5	60	26
11	F_1	11	3/5	60	24
12	F_1	13	5/5	100	20

5.5.3 *Grafting-experiments.*

The preceding experiments showed, that the line late I cannot be induced to flowering by GA_3-treatment, although stem elongation takes place.

Experiment 57. Rootstocks and shoots of these vegetative elongated rosettes were grafted, by means of an ordinary cleft graft, with shoots and rootstocks of flowering plants respectively. Although many variations within the graft technique and material used were tried, never a transmission of flowering was

observed. Even when the early line was used as a receptor, by means of the rosette grafting without GA_3 treatment, no flowering of the receptor appeared. The general phenomenon was that the donors, which were induced in LD, became vegetative under the following, non-inductive SD circumstances. This induced the question whether diminishing of the donor capacity by SD was responsible for the non transmission of floral hormone to the receptor.

In a next experiment graft combinations of a late receptor shoot on an early donor rootstock and the reverse were sprayed with GA_3 in SD. By this treatment the late receptors remained vegetative, while the early donors could be permanently induced and kept flowering. Different treatments were tried, like the use of young vegetative, just induced donors or donors already flowering at the moment of grafting. Also the removal of donor buds and flowers and receptor leaves was varied. However, no flowering of the receptors occurred. The impossibility to demonstrate a transmissable floral hormone of *M. album* could not be explained. It was asked whether graft combinations of *M. album* with the related *Silene armeria* could give more information. Maybe the very persistent floral hormone of *S. armeria* could bring *M. album* into flowering or perhaps a receptor of *S. armeria* was more sensitive to react to the eventual floral hormone of *M. album* than *M. album* itself.

All possible combinations of *S. armeria* as receptor or donor and *M. album* as receptor or donor were tried. However, in spite of good growing together of all these graft combinations, none of the receptors showed any flowering reaction.

5.6. Conclusions

40. The inductive circumstances of 16 *Caryophyllaceae* were investigated. They proved to be DNP or LDP with in some cases a requirement of low temperature or reaction on GA_3.
    No SD response was found. In 2 species the requirement of LD could be replaced by GA_3.
    Since the inductive treatments were abundant, it is likely that flowering-responses between 0 and 100% are caused by genetic differences. (Exp. 43).
41. Out of 34 graft combinations besides the well known case of *S. armeria*, only *S. echinosperma* gave transmission of flowering, while moreover *S. echinosperma* could bring *S. armeria* into flowering. This suggests identity between their floral hormones. In general, transmission of flowering by grafting was an exception rather than a rule. (Exp. 44).
42. Donor capacity of the line N_4 of *S. armeria*, brought into flowering by GA_3, demonstrated that GA_3 acts like another induction factor via the synthesis of floral hormone. (Exp. 46).
43. Shoots cut from vegetative, GA_3-elongated plants of *S. armeria* 'L_1', flowered for about 30%, when placed on water under non inductive circumstances. This points to the possibility of a flower inhibiting influence

arising from the roots. CL and removal of the roots had some promotive effect, but the combination of GA_3 + removal of the roots had not. It is possible that genetic variability is responsible for the limited flowering-reaction after removal of the roots. (Exp. 47 and 48).

44. *S. armeria* could be brought into flowering by grafting on the SDP. *P. crispa* Although the flowering-reaction of *S. armeria* was very slow and the flowering receptors were not able to act as donor, the possibility exists of identity of the floral hormones of *S. armeria* and *P. crispa.* (Exp. 49).
45. No transmission of flowering between the graft combinations of *Silene armeria* and *Bryophyllum daigremontium* could be demonstrated. (Exp. 50).
46. No influence of the surface active chemical dimethylsulfoxyde (DMSO) on the transmission of flowering by grafting could be demonstrated. (Exp. 51.)
47. Transmission of flowering by inoculation of *S. armeria* with sap from flowering plants of this species could not be demonstrated. (Exp. 52).
48. *M. album* is a qualitative LDP. Two lines could be selected, early II and late I, of which early II could be induced to flowering by 1 treatment of GA_3, while late I remained vegetative after 4 treatments. (Exp. 54).
49. The F_1 of early II $\times$ late I was intermediate concerning the response to GA_3-induction. (Exp. 54).
50. Even in extended experiments transmission of flowering could not be demonstrated by grafting of *M. album* with *M. album* and *M. album* with *S. armeria* in all possible combinations. (Exp. 57).

6. GENERAL DISCUSSION

6.1. THE FLOWERING-PROCESS

The flowering of plants is preceded by the change of the active growing-points from the vegetative to the generative stage. In most plants this change will be due to an endogenous signal, being the result of a chain of processes, called the *floral chain* in the following discussion. This floral chain is often called *the induction of flowering* (EVANS, 1969). Another concept is to reserve the term *induction* for the initial stages of the floral chain, resulting after complete induction in the *inductive state.*

There are plants in which induction occurs without any specific exogenous signal after the completion of eventual juvenility. Other plants, however, need such a signal, for example thermo-induction, photo-induction or chemo-induction.

In several plants the existence of a floral hormone could be proved. In those cases induction can be considered as the deblocking of the synthesis of floral hormone. The term *deblocking* implicates the hypothesis that no synthesis of floral hormone occurs before induction. However, when induction would be a raise of the level of already present floral hormone, the term *acceleration* would be better. The floral chain of plants with a floral hormone can be roughly divided in: induction and realization, the latter subdivided in transport and action of floral hormone as initiation of flower buds in the receptive meristems. This presentation will be too simple, and more processes between induction and visible flowering may occur. However, the absence of more concrete knowledge may justify the use of a simple scheme. Study of separate factors influencing the individual processes of the floral chain may give more information. Most of the preceding experiments were done with that purpose and some of their results justify a further discussion.

6.2. THE INFLUENCE OF TEMPERATURE ON THE FLORAL CHAIN OF SOME CRASSULACEAE

In the case of *Bryophyllum daigremontianum* no flowering occurred after the shift LD $\rightarrow$ SD or after GA_3 + SD when the average temperature per 24 hours was about 23 °C or higher (exp. 1). Differentiation of the influence of temperature on the individual processes of the floral chain resulted in the conclusions that not the LD induction was influenced by the investigated temperatures (exp. 4), while comparison of experiments 1 up to and including 9 demonstrates that not the SD induction, but the resulting inductive state and the already synthesized floral hormone are destructed by too high a temperature.

Because both the inductive state and the floral hormone are destructed by

too high a temperature, it might be possible that for a continued inductive state under non inductive circumstances the presence of floral hormone is required for its own continued synthesis like in a chain reaction mechanism. In this case, destruction of the inductive state is only indirectly influenced by temperature. Experiment 9 demonstrated that temperature treatment, when long enough, can destruct the inductive state completely while renewed induction of the formerly induced leaves is possible. This points to the hypothesis that high temperature causes a reblocking of induced leaves.

Since normal flowering occurs when the temperature during induction was at a level, destructive for floral hormone, it is not likely that synthesis of floral hormone takes place before the stage of complete induction.

The conclusion of experiment 1 was that the inhibitory action of temperature is determined by a sum of the products of temperatures times hours per 24 hours. This means that moderate temperature, in light as well as in darkness, can nullify the influence of high temperature. No further data are available to explain this phenomenon.

RÜNGER (1959) demonstrated that the related *Kalanchoë blossfeldiana* has a requirement for thermoperiodicity during the SD induction, which could be fullfilled by the combination of 2 temperatures per 24 hours or by dividing the SD induction into 2 periods with different temperatures. The hypothesis of RÜNGER was that 2 processes with different temperature optima are necessary for complete induction. Experiment 10 confirmed these results about the influence of temperature during induction, while in experiment 11 no influence of temperature on the continued flowering could be demonstrated. Experiments 12, 13 and 14 showed that also the floral chain of the *Crassulaceae Bryophyllum pinnatum*, *Kalanchoë jongmansii* and *K. manginii* is temperature sensitive.

The general conclusion is that temperature influences somewhere the floral chain of the investigated *Crassulaceae*, while the cases of *B. daigremontianum* and *K. blossfeldiana* demonstrate that in individual species different processes of the floral chain may be influenced.

6.3. FLORAL HORMONE

Transmission of flowering – read: of the impulse to flowering – between graft-partners of different photoperiodic response types or even of different families, suggests a more universal floral hormone. This could be confirmed by the next new graft combinations:

SDP *K. blossfeldiana* r/LSDP *B. daigremontianum* d (exp. 22)
DNP *H. annuus* d/SDP *X. strumarium* r
DNP *C. officinalis* d/SDP *X. strumarium* r } (VAN DE POL 1971).
LDP *Caryophyllaceae S. armeria* r/SDP *Labiatae P. crispa* d (exp. 49).

It was questionable whether the LSDP *B. daigremontianum* synthesized 2 components of floral hormone, one in LD and one in SD, as supposed by PENNER (1960). The negative result after the shift SD + GA_3 → LD demon-

strates that no SD induction occurs before LD induction (exp. 17). ZEEVAART (1967) demonstrated that although the level of endogenous GA of a plant kept in SD is quite low compared to a plant in LD, it will be the shift LD → SD that increases the endogenous level once more. ZEEVAART and LANG (1963) discovered suppression of floral initiation by CCC, when applied during the SD treatment after the shift LD → SD. Their conclusion was that *B. daigremontianum* needs a high GA level for production of floral hormone in SD. An explanation for the negative results of the experiments in which a LD- and a SD part of a plant were connected (RÜNGER, 1960, and exp. 19) might be that in the LD part of a plant the GA level is still too low to be active in the SD part when transported. RÜNGER did not obtain flowering in the combination $\frac{LD + GA_3.}{SD}$ Difficult downward movement of GA_3 could be an explanation for this negative result. It is a pity that the reverse combination $\frac{SD}{LD + GA_3}$ was not made.

The conclusion for *B. daigremontianum* is that there are no 2 daylength components of the floral hormone, but that one leaf needs both daylengths in the sequence LD + SD for complete induction. Applied GA_3 in SD must then be considered as an induction factor, which replaces the requirement for LD, as was demonstrated by ZEEVAART and LANG (1962).

Experiment 46 also demonstrated that GA_3 in *S. armeria* induces to the synthesis of floral hormone, like other induction factors.

6.4. TRANSPORT OF FLORAL HORMONE

All experiments, in which the influence of the duration of grafting-contact on the transmission of flowering was tested, demonstrate that a minimal period is necessary (EVANS, 1969). The reason will be that a graft combination without sufficient growing together of the partners will not give a translocation of intact floral hormone. The negative results of floral hormone extraction and inoculation experiments with *S. armeria* (exp. 52) would suggest that even persistent floral hormones are directly inactivated outside the plant. The conclusion is that floral hormone can only remain active during the transport from the leaves to the meristems, when the plant can protect it against destructing external influences.

In many cases it could be demonstrated that translocation of floral hormone will accompany the flow of carbohydrates. LINCOLN et al. (1956) demonstrated with *X. strumarium* that factors influencing the translocation of carbohydrates, for instance the creation of a carbohydrate-deficient condition, will also influence the transport of floral hormone. They concluded that an inverse relationship exists between the amount of LD leaf tissue of a receptor shoot and the magnitude of the flowering-response. A unit area of LD leaf tissue would be able to negate a definite quantity of hormone. Experiment 36 demonstrated that defoliation of a grafted receptor stimulated the rate of the flowering-response.

An explanation for the inhibiting action of LD leaves is that they form a sink of floral hormone. Experiments 33, 34 and 35 demonstrated that the presence of receptor leaves has a strong influence on the growth of axillary shoots. When defoliation means a lowering of the vegetative development, it might be easier for the floral hormone to promote the generative development.

Grafting-experiments demonstrated that many factors can influence the transmission of floral hormone. Experiment 16 demonstrated that the floral hormone of *B. daigremontianum* prefers upward movement. RESENDE and VIANA (1948) discovered that inverted generative plants of *B. daigremontianum* could flower from the root pole, while usually the basic buds form vegetative shoots. This means that the movement of the floral hormone of *B. daigremontianum* has a negative geotrophism.

Experiment 38 demonstrated that young receptor rootstocks of *X. strumarium* can react much more easily to floral hormone than old ones. In this case the age of the receptor tissue may influence the transport of floral hormone.

6.5. FLORAL HORMONE AND FLORAL INITIATION

The term floral initiation is used for the change of a meristem from the vegetative to the generative stage. EVANS (1969 p. 457) prefers the term *evocation* for this process, adopted from embryology. However, to my opinion *initiation* indicates the process satisfactorily and has the advantage of being generally used.

In literature many negative flowering-reactions in interspecies grafting-experiments were explained by assuming the existence of different floral hormones. In this reasoning flower development of the meristems of one type of plant could only be initiated by one specific type of floral hormone. Like different keys belong to different locks, different floral hormones implicate different types of meristems. However, comparison of the cases of *K. blossfeldiana* early and late (exp. 24) and *X. strumarium* early, middle and late (exp. 42) demonstrates that early lines need less floral hormone for a flowering-reaction than the late ones. This could mean that the threshold values for floral hormone of the meristems will be lower for the early lines than for the late ones. It remains unanswered why a low requirement for induction and a low requirement for floral hormone are combined. Moreover, the question cannot be answered whether the endogenous level of floral hormone of a flowering early plant remains lower than of a flowering late plant.

The good donor capacity of *B. daigremontianum* towards *K. blossfeldiana* (exp. 22) in contrast to the donor capacity of *K. blossfeldiana* for *B. daigremontianum* (exp. 23) suggests a lower level of floral hormone in *K. blossfeldiana* than in *B. daigremontianum*. Experiment 29 showed that a moderately flowering plant of *X. strumarium* with a low level of floral hormone is not able to act as a donor. So the inability of some plants to transmit flowering by grafting is caused by too low a level of floral hormone. The influence of temperature on the flowering of *Crassulaceae* and the factors influencing the transmission of flowering of

Xanthium demonstrated that many factors can influence the transmission of flowering.

Experiment 44 demonstrated that a flowering-reaction after grafting a vegetative and a generative part of a plant is an exception rather than a rule. The case of *M. album* (exp. 57) showed that in spite of many efforts the existence of a graft-transmissable floral hormone could not be proved. However, it is not necessary to assume that plants without demonstrable transmission of flowering do not have a floral hormone, because many negative results can be ascribed to factors, mentioned in the foregoing, and to other still unknown factors.

6.6 Floral hormone and flower development

Tukey et al. (1954) define the function of floral hormone as: 'initiation of the formation of floral primordia, or promotion of their development.' Experiment 21 demonstrated that after marginal induction the flower buds of *K. blossfeldiana* abort, while the plant reverts to the vegetative state. The explanation is that marginal induction results in synthesis of a small, disappearing amount of floral hormone, while the presence of sufficient floral hormone is necessary for flower initiation as well as for flower development.

Rünger (1959) demonstrated that the number of flowers of *K. blossfeldiana* depends on the inductive treatment. In this case the conclusion might be that the level of floral hormone, which will depend on the inductive treatment, determines the number of flowers. This could also be observed in *X. strumarium* where the level of floral hormone determined the level of the balance between vegetative and generative development (exp. 27, 28 and 29).

6.7. Floral hormone and continued flowering

An important question in the physiology of flowering is: Why does a plant stop flowering? In the case of *B. daigremontianum* destruction of floral hormone by too high a temperature is the cause of the reversion of the meristematic development from generative to vegetative. This is an indication that the presence of floral hormone is a condition for continued flowering. This conclusion is confirmed by the flowering-pattern of *B. daigremontianum* under optimal temperature conditions. In this case the lateral buds, releasing in downward direction, form inflorescences until the induced leaves become old and will stop synthesis of floral hormone. Then the later releasing lateral meristems form vegetative shoots.

It is remarkable that the 3 known plants with non localized synthesis of floral hormone will continue to flower under non inductive but otherwise favourable circumstances. It is unknown whether in other plants with continued flowering under non inductive circumstances non localized synthesis of floral hormone occurs.

The term '*non localized synthesis of floral hormone*' was already mentioned in the introduction, p. 3. As early as 1938 HAMMER and BONNER called 'indirect induction' the continuous flowering of a receptor of *X. strumarium* due to contact with a donor. ZEEVAART and LANG (1962) reserved this term for the phenomenon that shoots of some plants, brought into flowering by grafting, can act as a donor in a next grafting. EVANS (1971) called this '*secondary induction*'. It is likely that these authors meant to indicate that floral hormone as such can act as an induction factor. In order to distinguish this form of induction from the normal exogenous induction, they used the terms *indirect* or *secondary*. The term *endogenous induction* would indicate more clearly what process is meant, distinguishing it from exogenous induction. ZEEVAART (1969) found that only generative plants of *B. daigremontianum* with leaves can act as a donor in a grafting experiment. So here only the leaf can act as source of floral hormone. However, no arguments are available to decide whether the multiplication of floral hormone in non exogenously induced tissue occurs via true induction or via autocatalytical multiplication (WELLENSIEK, 1966). Therefore, the term *non-localized* synthesis of floral hormone is preferred, indicating the contrast to the mechanism with strictly *localized* synthesis in *P. crispa*.

Although the flowering of *K. blossfeldiana*, caused by grafting on *B. daigremontianum*, was abundant, no synthesis of floral hormone in the *K. blossfeldiana* receptors could be demonstrated (exp. 24). It is therefore likely that the differences between *B. daigremontianum* and *K. blossfeldiana*, concerning the phenomenon of localized synthesis of floral hormone, are caused by differences in internal conditions between these plants and not by different floral hormones. Also the case of *S. armeria*, brought into flowering by *P. crispa* (exp. 49), points to this explanation.

6.8. GENERAL CONCLUSION

The functions of floral hormone in the flowering-process can be flower initiation, promotion of flower development and continuation of flowering.

Since differences in flowering-reactions can be explained by different levels of the floral hormone and/or by differences in internal conditions of the plant in which the floral hormone has to act after formation or arrival, the existence of one universal floral hormone remains possible.

7. SUMMARY

The factors, influencing the synthesis and action of floral hormones, and possible differences between floral hormones in different plants were studied. The experimental results are summarized in the conclusions 1–20, on pages 35–36 ('*Crassulaceae*'); 21–39 on pages 58–59 ('*Xanthium strumarium*') and 40–50 on pages 74–75 ('*Caryophyllaceae*').

General conclusions are as follows:

1. The processes leading to flower formation of plants with a floral hormone can be roughly divided in induction and realization, the latter subdivided in synthesis, transport and action of floral hormone as initiation of flower buds in the receptive meristems. These processes are called the 'floral chain'.
2. In the long-short-day plant *Bryophyllum daigremontianum* neither the LD, nor the SD induction were influenced qualitatively by temperature, but the resulting inductive state and the already synthesized floral hormone were destructed by too high a temperature.
3. In the short-day plant *Kalanchoë blossfeldiana* the induction was influenced by temperature, so that in individual species different processes of the floral chain may be influenced.
4. In the LSDP *B. daigremontianum* no 2 separate components of the floral hormone are formed.
5. The transmission of floral hormone by grafting is influenced by the direction of movement of the hormone in *B. daigremontianum* and by the age of the receptor in the SDP *Xanthium strumarium*. Presence of the leaves in *X. strumarium* strongly stimulates the growth of the axillary shoots and reduces the flowering-reaction, while defoliation of receptors promotes the transmission of flowering.
6. In *K. blossfeldiana* and *X. strumarium* early lines need less floral hormone for a flowering-reaction than late ones. The former have a lower threshold value of the hormone.
7. The good donor capacity of *B. daigremontianum* towards *K. blossfeldiana* in contrast to the reverse combination suggests a lower level of floral hormone in *K. blossfeldiana* than in *B. daigremontianum*.
8. Also in *X. strumarium* different levels of floral hormone, corresponding with different floral stages and different donor capacities, could be distinguished.
9. The factors influencing the flowering of a receptor, grafted with a donor, mentioned sub 5, 6, 7 and 8, make it questionable to assume that plants without demonstrable transmission of flowering do not have a floral hormone, while negative results of interspecies graftings need not be caused by differences in floral hormones. New successful combinations between graft partners of different photoperiodic response types suggest a more universal floral hormone.

10. The action of floral hormone is not restricted to floral initiation. In *K. blossfeldiana* the presence of sufficient floral hormone is also necessary for flower development, while in *K. blossfeldiana* and in *X. strumarium* the number of flowers depend on the level of floral hormone. In *B. daigremontianum* the presence of floral hormone is a condition for continued flowering.
11. The term 'non-localized synthesis of floral hormone' was introduced for the phenomenon that receptors of some species brought into flowering by grafting, can act as a donor in a next grafting. It is likely that the difference between *B. daigremontianum* and *K. blossfeldiana* concerning localized synthesis of floral hormone is caused by differences in internal conditions between these plants rather than by different floral hormones. Also the case of *Silene armeria*, brought into flowering by *Perilla crispa*, suggests this explanation.
12. Since differences in flowering-reactions can be explained by different levels of floral hormone and/or by differences in internal conditions of the plants in which the floral hormone has to act, the existence of one universal floral hormone remains possible.

8. SAMENVATTING

BLOEI-INDUCTIE, BLOEIHORMONEN EN BLOEI

Faktoren, die de synthese en de werking van bloeihormonen beïnvloeden en mogelijke verschillen tussen bloeihormonen van verschillende planten werden onderzocht.

De algemene conclusies zijn als volgt:

1. De processen die leiden tot bloemknopvorming van planten met een bloeihormoon kunnen globaal worden verdeeld in inductie en realisatie, het laatste onderverdeeld in synthese, transport en werking van het bloeihormoon. Deze werking van het bloeihormoon bestaat uit bloei-initiatie in ontvankelijke meristemen. Deze processen worden de 'bloeiketen' genoemd. Vele van de hier vermelde proeven werden gedaan om de invloed van verschillende faktoren op de afzonderlijke processen van de bloeiketen te onderzoeken.
2. In de lang-korte-dag plant *Bryophyllum daigremontianum* werd de LD noch de KD inductie kwalitatief beïnvloed door temperatuur, terwijl de resulterende geïnduceerde toestand en het reeds gesynthetiseerde bloeihormoon werden vernietigd door een te hoge temperatuur.
3. In de korte-dag plant *Kalanchoë blossfeldiana* werd de inductie beïnvloed door de temperatuur, waaruit blijkt dat in aparte plantesoorten verschillende processen van de bloeiketen kunnen worden beïnvloed.
4. In de LKDP *B. daigremontianum* konden geen 2 verschillende componenten van het bloeihormoon worden aangetoond.
5. De overdracht van bloeihormoon door enting wordt bij *B. daigremontianum* beïnvloed door de transportrichting, terwijl bij de KDP *Xanthium strumarium* de leeftijd van de receptor van belang is. De aanwezigheid van de bladeren van *X. strumarium* bevordert de groei van de okselscheuten sterk en beperkt daardoor de bloeireaktie, aangezien ontbladering van receptors overdracht van bloeihormoon bevordert.
6. Vroege lijnen van *K. blossfeldiana* en *X. strumarium* hebben minder bloeihormoon nodig voor een bloei-reactie dan late lijnen. De eersten hebben een lagere drempelwaarde voor het bloeihormoon.
7. De tegenstelling tussen de goede donoreigenschappen van *B. daigremontianum* voor *K. blossfeldiana* en de omgekeerde combinatie wijst op een lager bloeihormoon-niveau in *K. blossfeldiana* dan in *B. daigremontianum*.
8. Ook in *X. strumarium* konden verschillende bloeihormoon-niveaux, met verschillende bloeistadia en verschillende donorcapaciteiten, worden onderscheiden.
9. Uit 5, 6, 7 en 8 blijkt dat vele faktoren de bloei van een receptor, geënt op een donor, kunnen beïnvloeden. Daarom is het de vraag of planten, waarvan geen bloeihormoon door enting kan worden aangetoond, geen bloeihormoon hebben, terwijl negatieve resultaten bij enting van verschillende soorten niet toegeschreven behoeven te worden aan verschillende bloeihor-

monen. Nieuwe, succesvolle combinaties tussen entpartners met verschillen in daglengtebehoefte wijzen op een meer algemeen bloeihormoon.

10. De werking van het bloeihormoon is niet beperkt tot bloei-initiatie. In *K. blossfeldiana* is bovendien de aanwezigheid van voldoende bloeihormoon nodig voor bloemontwikkeling, terwijl in *K. blossfeldiana* en in *X. strumarium* het aantal bloemen afhangt van het bloeihormoon-niveau. In *B. daigremontianum* is de aanwezigheid van bloeihormoon een voorwaarde voor voortgezette bloei.
11. De term 'niet-gelokaliseerde synthese van bloeihormoon' werd ingevoerd voor het verschijnsel dat receptors van enkele soorten, die in bloei gebracht zijn door enting, in een volgende enting donor kunnen zijn. Het is waarschijnlijk dat de verschillen tussen *B. daigremontianum* en *K. blossfeldiana*, betreffende gelokaliseerde synthese van het bloeihormoon, veroorzaakt worden door inwendige milieuverschillen tussen deze planten in plaats van door verschillende bloeihormonen. Ook de bloeioverdracht tussen *Perilla crispa* en *Silene armeria* wijst op deze veronderstelling.
12. Aangezien verschillen in bloei-reacties verklaard kunnen worden door verschillen in bloeihormoon-niveaux en/of door verschillen in inwendige milieu's van de planten waarin het bloeihormoon gaat werken, blijft het bestaan van één algemeen bloeihormoon mogelijk.

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Errata:

p. 27, line 5 from below: For "Rünger" read: "Penner".
p. 76, line 18 from above: . . . . . induction and realization, the latter subdivided in synthesis, transport and . . . .
p. 78, lines 9 and 11 from above: For "Rünger" read "Penner".
p. 79, line 12 from above: "geotrophism" read: "geotropism".