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FLOWER FORMATION AS STUDIED BY GRAFTING

MET EEN SAMENVATTING

ONDERZOEKINGEN OVER BLOEMKNOPVORMING MET BEHULP VAN ENTEN

by

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CONTENTS

CHAPTER I

GENERAL

1. INTRODUCTION

Flowering is such a striking event in the ontogenesis of most Angiosperms that it has since long attracted attention. After germination seedlings always begin to form roots, stems and leaves until they have reached a certain size. Then floral primordia are initiated and flowering can take place. Nowadays it is common knowledge that flowering of many plants can be affected by various external factors. The plant's genetical constitution, however, determines which factors can influence its development.

The process of flowering is not only of theoretical interest but also of extreme importance for agricultural and horticultural practice as it is the preparatory stage to reproduction. In floriculture flowers themselves are the terminal product. In all crops which are grown for their fruits or seeds, flowering is a prerequisite. On the other hand products of market gardening as lettuce, spinach, beets *etc.* have only commercial value if they are in the vegetative state. Therefore, practice has since long tried to alter at will the onset of flowering and the numbers of flowers formed. It will be clear, however, that such attempts hardly met with any success until more fundamental knowledge of the

flowering-process came available. This did not occur until studies were initiated on the external factors and subsequent internal changes which govern the transition from a vegetatively growing plant to a flowering one. Nowadays this field of investigation is known as the physiology of flowering. In view of the many more or less extensive reviews on this subject (3, 10, 13, 25, 43, 47, 48, 49, 52, 73, 74, 82, 95, 96, 105, 106, 111, 135, 148, 149, 152) a brief historical outline will suffice.

SACHS (124, 1865[1]); 125, 1887; 126, 1892) may be considered as the founder of the modern physiology of flowering. From his well-known experiments with partially darkened *Tropaeolum* plants and with *Begonia* leaf-cuttings he concluded that leaves in the light produce "flower-forming substances" in very small quantities which direct the assimilates to the formation of flowers. SACHS' ideas were opposed by LOEW (85, 1905) and FISCHER (37, 1905) who stated that a certain concentration of sugars within the plant is the only prerequisite for flowering.

KLEBS (67, 1913; 68, 1918) clearly demonstrated that flowering in *Sempervivum funkii* is largely dependent on external factors as *e.g.* light, humidity and mineral nutrition. He put forward the theory that a high ratio carbohydrates/mineral nutrients determines the onset of flowering. KRAUS and KRAYBILL (70, 1918) elaborated this idea to the carbohydrate/nitrogen ratio. This theory has played a dominating rôle in horticultural literature for a long period.

Further development of the research on flowering has been greatly influenced by the attention paid to two external factors which can determine the time of flowering, *viz.* low temperature and daylength.

Low temperature. – This line of research started with GASSNER's discovery (42, 1918) that exposure of germinating seeds of winter cereals to low temperature resulted in normal flowering although sowing took place in summer. A number of biennials flowered only if hibernated at low temperature and not if kept in a warm greenhouse.

The action of low temperature has a typical after-effect, *i.e.* the effect is not manifested until plants are transferred to a normal temperature. It has been called *vernalization* (153, 1933). The site of perception of low temperature is not separated from the place where the effect manifests itself: both are localized in the growing apex. During low-temperature treatment a certain amount of energy substrate and oxygen are necessary (see 43, 152; 1948).

For biennial *Hyoscyamus niger* MELCHERS (92, 1936) showed that the low-temperature requirement can be overcome by grafting a vernalized growing-point near to a non-vernalized one. The same result was obtained after grafting with an annual strain of *Hyoscyamus niger*, tobacco and *Petunia* (93, 1937), indicating that a stimulus passes the graft union and induces flowering in non-vernalized *Hyoscyamus*. With the short-day plant Maryland Mammoth tobacco flower formation in the biennial stock occurred irrespective of whether the donors were kept in short day or long day. So, when this tobacco variety was not capable of flowering itself, it could still supply a stimulus which caused flowering in the unvernalized stocks. Therefore, MELCHERS (94, 1939) concluded that the transmissible stimulus in biennials (produced by a low-temperature treatment) is different from *florigen* (see below). To stress their separate natures, he called the former *vernalin*.

[1]) First number refers to literature references on p. 82–88, second number indicates year of publication.

Daylength. – GARNER and ALLARD (40, 1920) demonstrated that daylength is a factor of first importance with respect to flowering. They suggested the term *photoperiodism* to designate the response of plants to the daily photoperiod. Three groups of plants were distinguished: *viz.* long-day plants, short-day plants and day-neutral plants.

Many experiments were undertaken to elucidate which organs perceive the daylength. KNOTT (69, 1934) was the first who produced clear-cut evidence that in the long-day plant spinach the daylength is perceived by the green leaves. He suggested that some substance or stimulus is produced in the leaves and transported to the growing-points. Soon afterwards similar findings were reported with other plants: CAJLACHJAN (14, 1936) and MOSHKOV (99, 100; 1936) for *Chrysanthemum*, PSAREV (115, 1936) for soybean, LJUBIMENKO and BUSLOVA (83, 1937) for *Perilla* and many others with various plants afterwards. MOSHKOV (99, 1936) stressed that the youngest fully expanded leaves are most sensitive to photoperiodic induction.

Reports on a successful transmission of the photoperiodic stimulus across a graft union to a vegetative plant came almost simultaneously from different authors with various plants: CAJLACHJAN (15, 1936) with *Perilla* and *Helianthus*, KUYPER and WIERSUM (71, 1936) with soybean and MOSHKOV (101, 1937) with tobacco.

Probably influenced by the discovery of a *growth hormone* by WENT in 1928, CAJLACHJAN (14, 1936) postulated that a *flower hormone* regulates the processes of development just as the growth hormone does in the growth processes. He christened this hypothetical substance *florigen* (15, 1936). It would have the same nature in different plants. Afterwards CHOLODNY (25, 1939) proposed the term *anthesin* and VAN DE SANDE BAKHUYZEN (3, 1947) following WENT's nomenclature (150, 1938) of organ-forming substances introduced the term *anthocaline*.

Of course many investigators have tried to isolate an active principle which can induce flowering in vegetative plants, but all attempts met with no success or yielded irreproducible results. This led to a reappraisal of the previously obtained results and culminated in the *flower-inhibition* hypothesis. After preliminary remarks by LONA (89, 1948), GREGORY (43, 1948) and RESENDE (116, 1949), this idea was clearly defined by VON DENFFER (30, 1950) in his well-known paper: Flower hormone or flower inhibition? He arrived at the conclusion that a plant is always capable of flowering but when it does not do so some factor inhibits this capacity. *E.g.* in photoperiodically sensitive plants flowering would be inhibited in the non-adequate daylength, but after transfer to the inductive daylength the inhibition is removed. VON DENFFER (30, 1950; 31, 1954) further suggested that the flower-inhibiting factor is identical with *auxin*. WELLENSIEK *et al.* (148, 1954) also concluded that photoperiodism removes an inhibition. They put forward the hypothesis that flower formation is determined by a certain balance between products of photosynthesis and auxin.

The flower-inhibiting effect of externally applied auxin in high concentrations has been demonstrated in many short-day plants (*cf.* 128, 1955), but appropriate timing of application (128, 1955) as well as low doses (157, 1956) can also result in *promotion* of floral initiation. Until now there is no evidence that a causality exists between changes in the native auxin level and flowering (10, 1953; 82, 1955), but it is possible that auxin is only active in combination with a receptor complex which cannot be measured in the usual assays.

2. GRAFTING AND FLOWERING

The grafting of plants has been employed for a wide diversity of practical as well as theoretical purposes (*cf.* 122). One of the newest uses of grafting involves the transmission of the flower-inducing stimulus from a flowering plant (donor) to a non-flowering one (receptor). The first successful transmissions were reported in 1936 with different plants (15, 71, 92); see p. 3 and 4. LANG (73) has tabulated all positive results obtained in experiments of that type up to 1952. Since then some new cases have been reported.

Within the same species. – *Pharbitis nil* (60), and *Kalanchoë* (24). Only quantitative effects in flowering response have been reported with grafts of late *Pisum* varieties (56, 112).

Between different species. – From *Sedum* to *Kalanchoë* (24); from *Erigeron annuus*, *Rudbeckia bicolor*, and *Centaurea cyanus* to *Xanthium canadense* (109); from *Kalanchoë* to *Bryophyllum* and *Cotyledon* (119, 120).

Non-flowering varieties of *Ipomoea batatas* have been induced to flower by grafting onto certain *Ipomoea* species of the non-storage root type (65, 72, 163). Negative results were obtained after grafting onto a flowering variety of *I. batatas*, but STINO and HASSAN (141) did obtain positive results in such grafts.

3. SCOPE OF THE PRESENT INVESTIGATIONS

Data in literature bearing upon the flower-hormone hypothesis have been obtained almost exclusively with plants in which floral initiation is controlled by low temperature and/or daylength. In the case of photoperiodism such evidence came from two closely related types of experiments:

1) If only part of a plant is induced, flowering can also occur in the part exposed to the non-inductive daylength.

2) Grafting an induced plant (donor) to a non-induced plant (receptor) can result in flower formation in the latter.

The results obtained in these experiments indeed do indicate that there is a transmission of the photoperiodic effect to the receptor, but in the absence of identification it seems premature to assume that it is a single substance which is similar throughout the Vegetable Kingdom.

According to literature successful transmission of the floral stimulus from donor to receptor is possible only provided the receptor is defoliated. Supporters of the flower-hormone hypothesis (*cf.* 73) have explained this as a *translocation phenomenon*. The floral stimulus would be translocated together with the stream of assimilates in the phloem. However, transmission to the receptor would be possible only if a food deficit is created by defoliating the receptors. This explanation implicates that the inhibiting effect of leaves on the receptor is a non-specific one.

On the contrary, promotors of the flower-inhibition hypothesis (30, 148) state that defoliation of the receptors removes an inhibition whereas assimilates are furnished by the leaves present on the uninhibited donor. Flowering does not take place on a defoliated receptor which has been grafted to a non-induced "donor" because the leaves supply a flower-inhibiting factor. So, according to this explanation a *specific flower-inhibiting influence* would originate from non-induced leaves.

From section 1 it appeared that grafting has contributed considerably to the present knowledge of the flowering-process. However, the controversy described

above illustrates that the results obtained in grafting-experiments are open to different interpretations. Moreover, it must be clear to one who is familiar with the many varied applications of grafting in horticulture that all possibilities of this technique have not yet been explored in studies on flowering with herbaceous plants. Therefore, it seems logical that this type of experiments was reassumed by a horticulturist who is interested in the physiology of flowering.

The present investigations have been undertaken to study the physiology of flowering with the aid of grafting. In order to obtain a fairly complete picture of the contribution to our knowledge of flowering which can be obtained by means of the grafting-technique, the investigations included a variety of problems. Among these problems are: variation in sensitivity to photoperiodic induction of leaves of different ages and locations; the way in which the induced state is retained after transference to the non-inductive daylength; photoperiodic treatment of leaves without buds and/or roots; the possible similarity of the floral stimulus in related species; characteristics of the translocation of the floral stimulus; the nature of the flower-inhibiting effect of leaves on the receptor.

In the course of the investigations it became evident that *Perilla* is an extremely suitable plant for grafting-experiments so that this plant has been studied most extensively. But other species have been investigated as well to obtain an idea how far controversial viewpoints might have their origin in results obtained with different experimental plants.

CHAPTER II

MATERIAL AND METHODS

1. PLANT MATERIAL AND GROWTH CONDITIONS

The experiments were carried out with the following plants:

Perilla crispa (THUNB.) TANAKA.
Xanthium pensylvanicum WALLR.
A commercial strain of *Kalanchoë blossfeldiana* v. POELLN.
Nicotiana tabacum L. cv. 'Maryland Mammoth'
} Short-day plants.

Sedum ellacombianum PRAEG.
Sedum spectabile BOR.
Nicotiana sylvestris SPEGAZ et COMES.
} Long-day plants.

Bryophyllum daigremontianum (R. HAMET et PERR.) BERG., a long-short-day plant.

Nicotiana tabacum L. cv. 'Delcrest', a day-neutral plant.

Seeds of *Perilla*, *Kalanchoë*, *Nicotiana sylvestris* and Delcrest tobacco were obtained from commercial sources. *Xanthium* seeds were generously supplied by Dr. A. LANG, Los Angeles, seeds of Maryland Mammoth tobacco by Dr. H. L. HYLAND, Beltsville.

Sedum ellacombianum and *Bryophyllum* were propagated vegetatively by means of cuttings and leaf plantlets respectively. Plants of *Sedum spectabile* were dug from the garden early in spring when they just began to sprout.

All plants were cultivated in greenhouses which were heated during winter. Temperature varied between 15° and 25°C, but on extremely hot days it occasionally rose above 30°C. Plants were grown individually in pots with fertile soil and dug in benches with peat. Only tobacco plants to be used for experiments under long-day conditions were firstly grown in pots and later planted in a greenhouse.

Detached leaves of *Perilla* and *Xanthium* were grown on nutrient solution in black-painted bottles of 50 cc. Nutrient solution was different from that used in previous experiments (159). Very satisfactory results were obtained with diluted KNOP's nutrient solution to which some boric acid was added; 1.0 g $Ca(NO_3)_2$, 0.25 g $MgSO_4$, 0.25 g KH_2PO_4, and 0.031 g H_3BO_3 were dissolved in 5 l water to which some drops of a $FeCl_3$-solution were added.

In some experiments detached *Perilla* leaves were rooted in a mixture of two parts of sand and one part of peat. After formation of roots at the base of the petiole, leaves were further grown in pots.

Required daylength conditions for plants to be used as receptor were applied immediately after emergence of the seedlings. Plants to be used as donor were grown in a non-inductive daylength until they had reached the required size. Then they were transferred to a daylength suitable to flowering.

Graft-combinations of donor and receptor and their controls were always grown under non-inducing daylength for the receptor, *i.e.* long day for short-day plants and short day for long-day plants.

Equipment for short-day treatment consisted of a bench in the greenhouse which was covered with black cloth from 4.30 p.m. until 8.30 a.m., so that plants received 8 hours of natural daylight and 16 hours of darkness.

Long-day conditions for *Perilla* consisted of the natural daylength supplemented during the whole night with light from 60 Watt incandescent lamps. For other species natural daylength was supplemented with light from incandescent lamps to a total length of at least 16 hours. Day-light extension was controlled automatically by means of electric clocks. Experiments with tobacco under long-day conditions were carried out in natural long days prevailing during summer.

Experiments with different light intensities were performed in an equipment devised and described by DE ZEEUW (156). At one end of the installation (from which daylight was excluded) 9 fluorescent tubes (type warmtint) and two high pressure mercury lamps were mounted vertically. Decreasing light intensities were obtained by placing plants at increasing distances from the light source. Light intensities were measured with a spherical radiation meter (147) and expressed in μ W/cm^2 Ø sphere.

2. GRAFTING-TECHNIQUE

Throughout the investigations cleft-grafting was used. Special variations of this technique will be described in the respective experiments.

In general grafting was carried out as follows: tops of plants to be used as stocks were removed and the wedge-shaped scions were inserted into the cleft stems and bound tightly with two pieces of wetted raffia. Care was taken that grafting was performed in young, growing internodes which had not yet become woody. Preparation of the scions as well as cleaving the stocks were done with new razor blades. Before grafting all scions were dipped in water. A high air-humidity around the scion was maintained with the aid of polyethylene bags (0.05 mm thick) which were fixed around the stem below the graft union with a metal ring. Thanks to this method grafted plants could be kept in the greenhouse without taking special precautions. Only on sunny days they had to be shaded in order to prevent the temperature in the bags from rising too high. When a good graft union had been established (mostly within 10 to 12 days) the bags were removed.

In the first experiments *Perilla* and *Xanthium* scions in the polyethylene bags were often severely attacked by grey mould. Therefore in later experiments they were soaked in a $\frac{1}{2}$% aqueous solution of tetramethylthiuram disulphide (T.M.T.D.) during some minutes before grafting. This pre-treatment yielded very satisfactory results and especially in *Perilla* all scions remained free of grey mould so that almost no failures occurred.

The presence of assimilating leaves on the scions turned out to have a beneficial effect on the quick establishment of a good graft union. Therefore, even when the scions had to be defoliated, grafting was still carried out with some leaves on them. Scions were not defoliated before a good graft union had been established.

For *Perilla* a method for grafting single leaves has been described already in a preliminary communication (158). This method can be considered as a kind of biological assay which

permits to test rather quickly and accurately whether *Perilla* leaves are induced or not. Generally 30 cm² leaf area was cut out from the base of a blade along a plastic quadrangle. The prepared leaves were soaked in a solution of T.M.T.D. for some minutes. The plants to be used as stocks were completely defoliated except the second pair (counted from the base). Grafting was mostly performed in the 4th internode but in some experiments in the 3rd or 5th, depending on the age of the plants. At the moment of grafting the internode should not be woody; the best stage has been reached when its length is 2 to 3 cm. The wedge-shaped petiole of the prepared leaf was inserted into the cleft internode and bound tightly with raffia. The leaf was kept in a small polyethylene bag for 8 to 10 days. See photo 1 at the end. The secondary buds at the 4th node which had the size of a pin's head at the moment of grafting were allowed to develop as receptor shoots under the influence of the donor leaf. Unfolding leaves on these shoots were removed regularly until appearance of flower buds. With the exception of the two receptor buds all shoots and buds on the stocks were removed.

3. ABBREVIATIONS AND SYMBOLS

For shortness' sake the following abbreviations and symbols will be used throughout:

LD: long day.
SD: short day.
LDP: long-day plant.
SDP: short-day plant.
DNP: day-neutral plant.
LSDP: long-short-day plant.
/ : Graft symbol, to be read as "on": Thus, *Sedum/Kalanchoë* means: "*Sedum* scion on *Kalanchoë* as stock."
+: Leaves present on stock, interstock or scion.
– : Leaves removed from stock, interstock or scion.

Grafts within the same species, *e.g.* within *Perilla*, between induced (donor) and non-induced partner (receptor) will be indicated by SD and LD respectively, although the whole combination was kept under LD conditions since the date of grafting. Thus, for *Perilla* "SD+/LD–" means: "Scion with leaves as donor on defoliated stock as receptor; whole combination under long-day conditions."

The term *floral stimulus* will be used to designate the unknown factor(s) which lead(s) to flowering after photoperiodic induction.

4. OBSERVATIONS

As it was the first aim to see whether receptors could be induced to flower by grafting to donors, the number of flowering receptors out of the total number of successful grafts is given for all experiments. Such statements, however, hardly permit any quantitative analysis of the results obtained. To overcome this objection the following data have been recorded:

1) Mean number of days (rounded off to the nearest whole number) from grafting until appearance of macroscopically visible flower buds and in some experiments also until opening of first flower.

2) For *Sedum ellacombianum* and Delcrest tobacco the number of leaves on the scion to the terminal inflorescence.

3) Stem length of scions of Maryland Mammoth tobacco as in this plant floral initiation and development are accompanied by rapid stem elongation.

When an experiment was discontinued, growing-points from receptor plants which did not show flower buds, were dissected under a binocular (20 × magnification) and examined on the presence of floral primordia.

CHAPTER III

EXPERIMENTS WITH *PERILLA CRISPA*

1. SUITABILITY OF DIFFERENT PAIRS OF LEAVES TO FUNCTION AS DONOR

1.1. *Introduction*

It was taken for granted that the sensitivity of leaves to photoperiodic induction increases until full expansion and then gradually decreases (48, p. 64; 73, p. 283) until it was demonstrated by KHUDAIRI and HAMNER (66) that in *Xanthium* the half-expanded leaves are most sensitive; at the same time these leaves are the most rapidly expanding ones (23, 128). Besides the variation in sensitivity of leaves of different ages, the age (or size) of the plant has a pronounced effect on the reaction to the inductive treatment. Shortly after germination plants are completely insensitive or at least much less sensitive than at a later stage of ontogenesis (*e.g.*, 11, 48, 54, 59, 90, 102, 106, 111). MOSHKOV (102) experimenting with *Perilla nankinensis* (probably identical with our *Perilla crispa*) found that during the first 20 days after emergence seedlings were completely insensitive to SD treatment. WELLENSIEK (unpublished data) subjected a series of *Perilla* plants of different ages (12, 19, 26, ... 89 days old since the date of sowing) to SD and observed that in the youngest plants it lasted 51 days until flower buds appeared. This figure decreased with increasing age of the treated plants until it reached a constant value of approximately 24 days in plants which were at least 75 days old when they were transferred to SD.

The suggestion made by BORTHWICK and PARKER (11), and HAMNER (48) that the sensitivity to photoperiodic treatment is related to total leaf area seems untenable as has been pointed out already by LANG (73, p. 283) because "it takes the same number of cycles to induce an intact plant and a plant defoliated to one leaf".

The following three suggestions can be made to explain the insensitivity of young plants to daylength:

1) The primarily formed leaves are insensitive or at least less sensitive than later developed ones, that is to say they produce no or only small amounts of floral stimulus.

2) Translocation of the floral stimulus from leaves into growing-points is hindered in young plants.

3) Growing-points of young plants are not able to respond by floral initiation to supply of the floral stimulus.

In order to make a choice between these three suggestions the following experiments were designed.

1.2. *Variation in sensitivity of differently located pairs of leaves*

In *Perilla* the arrangement of the leaves is decussate. The two leaves on one node, which are morphologically identical, were found to respond to any given photoperiodic treatment in the same way. Therefore, they will be considered as a unity of two identical leaves. The pair of leaves appearing after the cotyledons will be designated as the 1st pair of leaves, the next one as the 2nd, *etc.* Hence the pairs of leaves are numbered successively from the base upwards. Fully expanded leaves of the first 5 pairs of leaves of one and the same plant are shown in fig. 1.

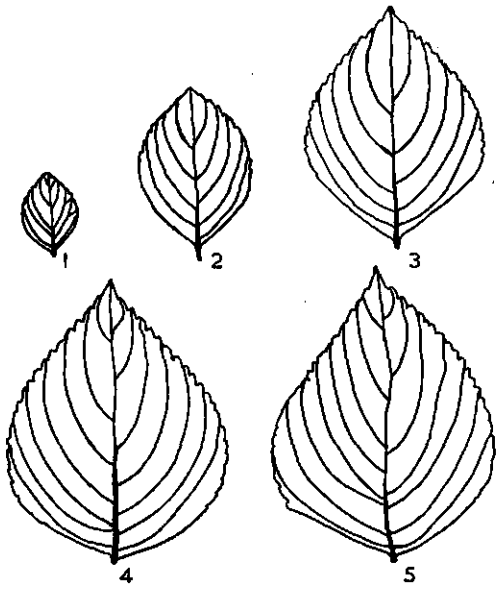

FIG. 1.
Leaves from first 5 successive pairs of leaves on the same plant; each pair represented by only one specimen. Figures refer to node number (counted from the base) from which leaves originate. × 1/4.

The presented leaves had an area of 8, 33, 75, 110, and 115 cm², respectively. Besides the area, other characteristics of the blades also change. The first two pairs are characterized by an oval blade, only little anthocyanin and an almost smooth margin. The 4th pair and higher located ones contain so much anthocyanin that the colour is purple-brown; the shape of the blade is ovate whereas the margin is smooth at the base and for the rest serrate. The 3rd pair of leaves forms a clear transitional stage between the 2nd and 4th pair.

Experiment 1. – Plants with the 3rd pairs of leaves just fully expanded were subjected to 3 weeks of SD treatment. During this treatment blades of the 5th pairs of leaves, which had an initial length of 2 to 4 cm, expanded to a length of 15 to 18 cm, so that after 3 weeks of SD treatment two fundamentally different types of leaves were available: the 3rd pairs which were already fully expanded *before* SD treatment and the 5th pairs which fully expanded *during* induction. After 21 SD leaves from both pairs were grafted onto stocks in LD. Leaf area of donor leaves was varied as indicated in table 1. Area of intact leaves was determined after terminating the experiment by drawing the outline of each leaf on paper and subsequently measuring the area with a planimeter.

From the results in table 1 it is clear that only leaves belonging to the 5th pair could induce LD stocks to flower. A leaf area of 10 cm² seems to be below the optimum as two stocks remained vegetative, but with 30 cm² the optimal flowering response seems to be reached in view of the number of days to flower buds. Moreover, flower buds and flowers developed better with an area of 30 cm² than with intact leaves. All 6 × 8 stocks grafted with completely comparable LD leaves remained vegetative.

From this experiment it seems as if only those leaves have been induced that fully expanded *during* the inductive period. Supposing this to be the correct explanation, the 3rd pair of leaves would reach the induced state provided it would expand during SD. This suggestion was investigated in the following experiment.

TABLE 1. *Experiment 1.* Flowering response of groups of 8 LD stocks after grafting with different areas from 3rd or 5th pair of leaves. Donor leaves received 21 SD. Data 88 days after grafting

Pair of leaves	Area of donor leaves (cm^2)	Number of stocks		Mean number of days to flower buds
		Generative	Vegetative	
3rd	10	0	8	–
3rd	30	0	8	–
3rd	$61 \pm 3^1)$	0	8	–
5th	10	6	2	62
5th	30	8	0	26
5th	$139 \pm 2^1)$	8	0	34

[1]) Standard error of mean.

Experiment 2. – For this experiment 3 homogeneous groups of plants were selected from 3 different sowings and transferred to SD. In order to obtain more quantitative data concerning the development of leaves during the SD treatment all blades were measured along the midrib at the beginning and at the end of the treatment. Results of these measurements are shown in table 2. As two opposite leaves of one pair of leaves are practically identical, only one leaf of each pair is represented in the diagrams. (The leaves, the arrangement of which is actually decussate, are represented here as being all in one plane). After receiving 24 SD the degree of induction of each pair of leaves, starting with the 2nd one, was tested by grafting 30 cm^2 leaf area onto LD stocks. The results obtained 50 days after grafting are presented in table 2 (see p. 12). They indicate that the 2nd pairs of leaves from all 3 groups did not induce LD stocks to flower (with the exception of one leaf in group I). For group I there was little difference between the pairs 4, 5 and 6, which fully expanded, or nearly so, during SD treatment. The same tendency is found in group II.

For group IIIa results were completely negative, although the 2nd and 3rd pairs of leaves expanded fully during SD treatment. After 24 SD none of the *donor* plants of group IIIa showed flower buds. In a comparable group (IIIb) which received 46 SD this occurred after 32 days. Leaves from this group were grafted and as can be seen from the results in table 2 nearly all leaves tested did induce LD stocks to flower.

It must be concluded that lower located leaves, *in casu* the 2nd and 3rd pair, need more SD to reach a state in which they can induce LD stocks than higher located ones.

With plants older than those of group I in table 2 it was established experimentally that the 4th and 5th pairs of leaves, fully expanded at the beginning of a treatment with 24 SD, could induce flowering in almost 100 % of LD stocks onto which they were grafted. So, it follows that the capacity of leaves to function as donor for LD stocks (which is thought to reflect the degree of induction) after receiving a given number of inductive cycles, is primarily determined by their position on the plant whereas the physiological age of leaves at the beginning of SD treatment is a factor of minor importance. This conclusion is further substantiated in the following experiment.

Experiment 3. – Two homogeneous groups of plants (I and II), comparable with groups I and III in table 2 respectively, were exposed to SD. Macroscopical

TABLE 2. *Experiment 2.* Diagrams showing growth of blades during SD treatment and degree of induction of various pairs of leaves as tested by grafting onto vegetative stocks. Lengths of blades are indicated in cm; each value represents a mean of 10 measurements. Numbers refer to position of leaves on plants. Data on flowering response of stocks after 50 days

Group and date of sowing	Length of blades in cm at 22/6/56, the beginning of SD treatment	Length of blades in cm at 16/7/56, after 24 SD for groups I, II and IIIa; at 7/8/56, after 46 SD for group IIIb	Flower buds on donor after . days	Number of stocks		Mean number of days to flower buds on stocks
				Generative	Vegetative	
I, 18/4/56	7 0.0 6 1.0 5 4.0 4 8.5 3 10.5 2 8.5	7 7.5 6 14.0 5 15.0 4 13.0 3 11.0 2 9.0	19	–	–	–
				10	0	22
				9	0	21
				10	0	22
				8	2	36
				1	9	45
II, 2/5/56	6 0.0 5 1.5 4 5.5 3 9.5 2 8.5	6 10.0 5 15.5 4 15.0 3 12.0 2 8.5	20	9	1	29
				10	0	28
				9	1	24
				8	2	36
				0	10	–
IIIa, 24/5/56	4 0.0 3 1.0 2 4.0	4 8.5 3 12.0 2 9.0		0	10	–
				0	10	–
				0	10	–
IIIb, 24/5/56		6 9.5 5 14.5	32	–	–	–
				–	–	–
	4 0.0 3 0.5 2 3.5	4 14.5 3 12.0 2 9.0		10	0	24
				9	1	25
				9	1	27

flower buds appeared after 20 and 30 days, respectively. Grafting of 2nd and 3rd pairs of leaves onto LD stocks was carried out after 24 and 48 SD. Moreover, leaves from plants of group II were also grafted after 31 SD, when flower buds appeared.

The results presented in table 3 indicate that leaves, fully expanded at the beginning of SD treatment and originating from the 2nd pair of leaves, could induce vegetative stocks to flower if they were exposed to 48 SD. Leaves of group II showed an increasing potency for inducing LD stocks with increasing duration of SD treatment. The results obtained with leaves from plants of

TABLE 3. *Experiment 3.* Flowering response of LD stocks after grafting with 2nd or 3rd pair of leaves. Donor leaves received different numbers of SD and originated from two different groups of plants

Group I: 3rd pair of leaves fully expanded at beginning of SD treatment.

Group II: 2nd pair of leaves less than half-expanded at beginning of SD treatment. Data 98 days after grafting

Group	Pair of leaves	Number of SD	Number of grafts	Number of stocks		Mean number of days to flower buds
				Generative	Vegetative	
I	2nd	24	10	0	10	–
I	2nd	48	8	7	1	23
I	3rd	24	10	3	7	68
I	3rd	48	8	8	0	23
II	2nd	24	10	0	10	–
II	2nd	31	10	8	2	26
II	2nd	48	9	9	0	23
II	3rd	24	10	1	9	72
II	3rd	31	10	6	4	26
II	3rd	48	10	10	0	20

group II are in good agreement with those presented in table 2 for the groups IIIa and IIIb.

A repetition of this experiment yielded the same results: leaves originating from the 2nd pair of leaves, which were fully expanded at the beginning of SD treatment, induced all stocks to flower after receiving 37 SD.

From this and the previous experiment it must be concluded that *all fully expanded leaves are sensitive to SD treatment. The great quantitative differences in sensitivity which exist between various pairs of leaves are caused by different positions on the plant and not by differences in physiological age.*

It might further be suggested that sensitivity in higher located leaves is favourably influenced by lower located mature ones. That this is not true follows from the following observation: sensitivity of the 6th pair of leaves, expanding during photoperiodic treatment, was not altered when lower located leaves had been removed.

Experiment 4. – The difference in sensitivity to daylength of different pairs of leaves described above was further demonstrated as follows: two groups of plants, sown with a 4-week interval, were grown in LD until in the older group of plants the 5th pairs of leaves had just got fully expanded. At the same time this was also true in the younger group of plants for the 2nd pairs. Leaves reduced to an area of 30 cm^2 from these two pairs of leaves were grafted in the usual way onto LD stocks above the 4th node, so that plants were "synthesized" that were similar except that the leaves originated from differently located pairs of leaves whereas their age was nevertheless the same. The experimental treatment was started 9 days after grafting. With the aid of small light-proof bags of black paper SD treatment was applied to the "donor" leaves which had thus far received only LD. In order to assure complete darkness the bags were closed at the base with two paper-clips. See photo 2. This treatment was continued for 4 weeks. Flowering response was traced on the secondary shoots at the 4th node of the stocks. The data shown in table 4 were obtained after 63 days. The

number of generative stocks as well as the number of days to flower buds clearly indicate that the 5th pair of leaves was much more sensitive to the SD treatment than the 2nd pair. This result confirms the conclusion reached in experiment 3.

TABLE 4. *Experiment 4.* Flowering response of LD stocks after grafting with 30 cm² from 2nd or 5th pair of leaves. SD treatment of donor leaves started 9 days *after* grafting. Data 63 days after beginning of SD treatment

Pair of leaves	Number of SD	Number of grafts	Number of stocks		Mean number of days to flower buds
			Generative	Vegetative	
2nd	0	6	0	6	–
2nd	28	9	3	6	51
5th	0	6	0	6	–
5th	28	9	8	1	30

Experiment 5. – This experiment was designed to obtain quantitative data on the difference in sensitivity to SD treatment of the 2nd and 5th pairs of leaves with the same technique as used in the previous experiment.

Selection of leaves and SD treatment were exactly the same as in experiment 4 except that this time "donor" leaves had an area of 25 cm². Duration of SD treatment was varied as indicated in table 5.

TABLE 5. *Experiment 5.* Flowering response of LD stocks after grafting with 25 cm² from 2nd or 5th pair of leaves. SD treatment of donor leaves started 10 days *after* grafting. Data 120 days after beginning of SD treatment. 10 plants per treatment

Pair of leaves	Number of SD	Number of stocks		Mean number of days to flower buds	Mean number of days to opening of first flower
		Generative	Vegetative		
2nd	0	0	10	–	–
2nd	14	0	10	–	–
2nd	21	2	8	78 ± 32.0	–
2nd	28	10	0	43 ± 1.0	84 ± 1.6
2nd	35	10	0	38 ± 0.8	95 ± 6.2
5th	0	0	10	–	–
5th	14	10	0	31 ± 3.0	94 ± 7.8
5th	21	10	0	23 ± 1.3	65 ± 4.9
5th	28	10	0	22 ± 0.7	48 ± 4.2
5th	35	10	0	22 ± 0.7	41 ± 1.4

The results obtained show once more the difference in sensitivity to SD treatment between the two pairs of leaves tested. The quantitative differences are clearly demonstrated in fig. 2 and 3.

Considering firstly a 100% flowering response of the stocks and secondly the number of days to flower buds and first open flowers it follows from table 5 that a 2-week SD treatment of the 5th pair of leaves resulted in about the same effect as a treatment during 4 weeks of the 2nd pair of leaves. Thus, the ratio of relative sensitivity of both pairs of leaves tested can be expressed in a "sensitivity-coefficient" which in the present case has a value of around 2.

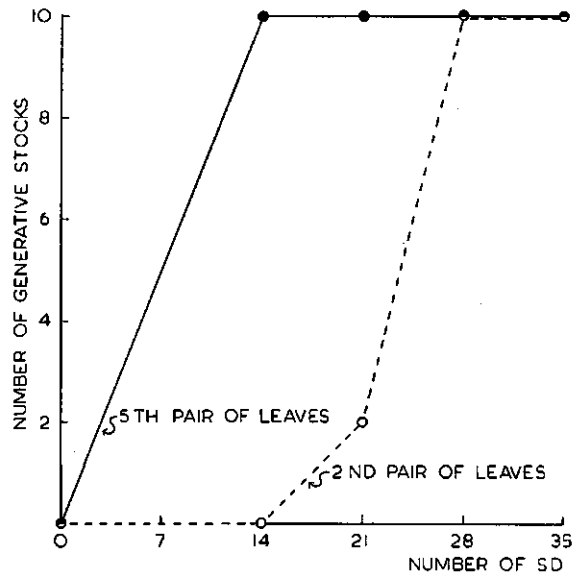

FIG. 2.
Experiment 5. Flowering response of LD stocks as influenced by original position of donor leaves. SD treatment applied to donor leaves after grafting. Per treatment 10 plants.

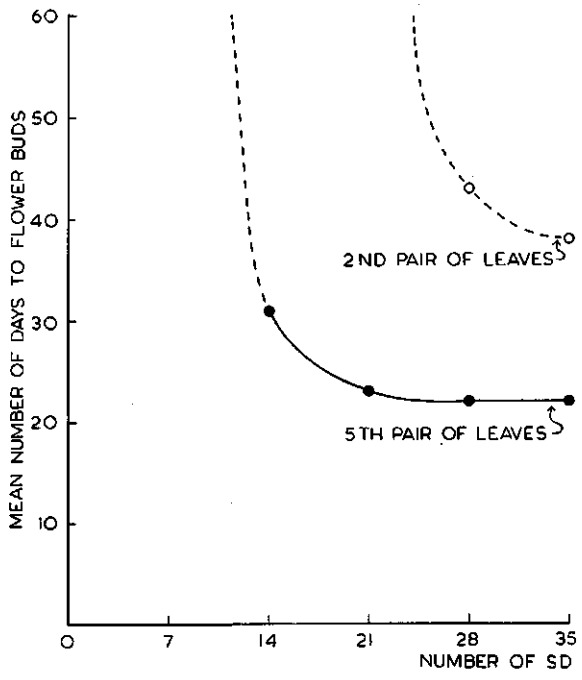

FIG. 3.
Experiment 5. Number of days to flower buds visible on LD stocks as influenced by original position of donor leaves. Compare fig. 2.

The different results with the 2nd pair of leaves obtained in tables 4 and 5 can be ascribed to different climatical conditions. Experiment 4 was carried out in autumn, experiment 5 in summer during a very warm period.

1.3. *Discussion*

For *Xanthium* KHUDAIRI and HAMNER (66, already cited on p. 9) concluded that the sensitivity of a leaf to photoperiodic treatment is determined by its physiological age: half-expanded leaves were always most sensitive irrespective of their position on the stem. From our experiments it follows that this does not hold true for *Perilla:* in this plant the physiological age of a leaf is a factor of minor importance for its sensitivity. It is, however, the leaf's position on the plant which determines its sensitivity to the inductive treatment. For, leaves located on the 2nd node needed about twice as many SD as those located on the 5th node to reach a state in which they could induce vegetative stocks to flower, even when they were of the same physiological age. Although no tests were carried out with the 1st pair of leaves, it seems probable that this is the least sensitive one. This would include that the plant's sensitivity to SD treatment increases with each new pair of leaves appearing until the maximum sensitivity has been reached in about the 5th pair or even higher located ones. According to WELLENSIEK's data (mentioned on p. 9) sensitivity really increased with increasing age and size until the plants were approximately 75 days old. It must be stressed that this increase in photoperiodic sensitivity is accompanied by a gradual morphological change of the leaf blades as can be seen in fig. 1 (p. 10). If it is assumed that all parts of a blade are equally sensitive to SD treatment, the increase of leaf area in successive pairs of leaves does not explain the differences in sensitivity as we always tested 30 cm^2. (That this assumption is warranted follows from data in literature (19, 53, 87) on photoperiodic treatment of basal or apical halves of leaves).

The difference in number of days to flower buds for groups I and IIIb in table 2 on p. 12 (19 *versus* 32) can be explained as well with the difference in sensitivity of the leaves being expanding at the beginning of SD treatment. It thus appears that the suggestion made *sub* 1 on p. 9 is, at least in *Perilla*, the major cause of the low sensitivity of young plants to photoperiodic treatment. Data in literature seem to support this conclusion. BEHRENS (5) experimenting with the LDP *Sempervivum alpinum* found that young rosettes sent out from a parent plant never flowered until in their fourth year of growth when grown as single plants, but attached to a flowering parent plant they could flower already in the second year of growth. She concluded that leaves of rosettes in the second year are insensitive to daylength (produce no "flowering hormone") whereas the growing-points can already respond to a supply of "flower hormone" by a parent plant. She defined the "juvenile stage" (which lasts 3 years in this species) as the period during which the leaves of detached rosettes do not produce "flower hormone." A similar phenomenon has been described for young runner plants of *Fragaria vesca* L. var. *semperflorens* DUCH. (134). If these plants were still attached to induced plants flowering took place at a stage in which they were insensitive to photoperiodic treatment when detached from the parent plants. Again this indicates that in young plants growing-points can react to a supplied floral stimulus, but the leaves seem to be unable to produce it.

The results presented in the preceding section give rise to make some remarks about the minimum number of leaves formed in photoperiodically reacting plants before floral primordia can be initiated. VON DENFFER (28) suggested this number to be identical with the number of leaf primordia already present in the embryo. ZIERIACKS (162) presented evidence that a definite leaf area – the

critical leaf area – must be present before plants can react by floral initiation to the inductive daylength. Therefore she extended VON DENFFER's supposition as follows: the minimum leaf number represents the number of leaf primordia present in the embryo plus those initiated before the critical leaf area has been reached. A similar explanation was given by PARKER and BORTHWICK (111). In all plants investigated by ZIERIACKS the critical leaf area was reached already as soon as the cotyledons and/or primary leaves had expanded so that continuous removal of further appearing leaves did not prevent flowering under inductive daylength conditions.

It is probable that the critical leaf area in *Perilla* is reached already in the cotyledons, and surely in the 1st pair of leaves (156, p. 21). Suppose that a certain threshold value of floral stimulus must be reached before growing-points can be induced to initiate flower buds. Such a threshold value will be reached much more rapidly in old than in young plants. Consequently more leaf initials will be differentiated in the latter than in the former. In fact we could establish that *Perilla* plants subjected to SD treatment from the very beginning of emergence formed 6 pairs of leaves before the terminal inflorescence. When the cotyledons were fully expanded only 2 pairs of leaf initials could be detected. In old plants only 1 pair of leaves was differentiated in addition to those already present at the beginning of SD treatment. Thus, it is probable that the difference in sensitivity between differently located leaves finds its expression in the 3 extra pairs formed in young plants.

JENNINGS and ZUCK (64) found that in *Xanthium* the cotyledons were completely insensitive to SD treatment whereas foliage leaves with an area even smaller than the cotyledons, could induce flowering. Possibly this finding combined with our results obtained in *Perilla* offers an explanation for the data presented by HOLDSWORTH (59) for *Eupatorium adenophorum*. In this plant more than 30 leaves were formed before the inflorescence. Only 2 pairs of leaf initials were present in the seedlings. In old plants about 10 leaves were initiated in addition to those already present at the beginning of SD treatment. So, the sum of these two was still about 20 short of the total number formed. It seems quite well possible that in this plant the first leaves are completely insensitive to daylength. Therefore, it would seem of interest to know how a young plant would react to a supply of floral stimulus, *e.g.* by grafting a seedling onto a flowering stock. If such a treatment would result in a very rapid flowering this would indicate that the leaves are not able to produce the floral stimulus and there would be no further need for accepting the minimum leaf number as a criterion for an obligatory vegetative growth preceding the condition known as "ripeness to flower" as HOLDSWORTH does.

2. FACTORS INFLUENCING THE FLOWERING RESPONSE OF VEGETATIVE STOCKS

As it appeared in the preceding section great differences in the capacity to induce vegetative stocks to flower exist between differently located pairs of leaves. In order to prevent these differences from interfering with other factors under investigation, all following experiments were carried out with 4th or higher located pairs of leaves. For each separate experiment donor leaves were selected from equal pairs of leaves (same position) on a homogeneous group of plants to minimize variation in reaction.

2.1. *Number of SD applied to donor leaves*

Experiment 6. – Groups of 5 plants, coming from one sowing, were transferred to SD with intervals of 1 week. This was continued until 6 different groups had received SD treatments of 0, 1, 2, 3, 4, or 5 weeks. Plants were then moved back to LD and at the same time donor leaves (area 30 cm²) from the 6th pairs of leaves were grafted onto stocks in LD.

Plants reacted to SD treatments of various duration as follows: when exposed to 0 or 7 SD they remained vegetative, but with 14 SD a very low flowering response occurred; flower buds did not appear until 35 days after beginning of SD treatment and plants reverted to vegetative growth very quickly. After receiving 21, 28 or 35 SD all plants showed flower buds 3 weeks after beginning of SD treatment, but 21 SD gave a suboptimal response. Plants which received 28 or 35 SD showed about the same flowering behaviour, the latter being slightly superior to the former.

The response of vegetative stocks to donor leaves was correlated with the flowering behaviour of plants from which the donor leaves were derived. See table 6.

TABLE 6. *Experiment 6.* Flowering response of LD stocks after grafting with 30 cm² from 6th pair of leaves. Donor leaves received different numbers of SD. 10 plants per treatment. Data 71 days after grafting

Number of SD applied to donor leaves	Number of stocks		Mean number of days to flower buds	Number of flowering stocks
	Generative	Vegetative		
0	0	10	–	0
7	0	10	–	0
14	10	0	38	0
21	10	0	25	10
28	10	0	21	10
35	10	0	20	10

All donor leaves subjected to 14 SD or more evoked floral initiation in LD stocks. However, great differences occurred which can be deduced from the number of days to appearance of flower buds and the groups with flowering stocks after 71 days. When donor leaves had received 28 or 35 SD, stocks flowered very profusely, but with 21 SD flowering was less profuse whereas with 14 SD only flower buds appeared which had not yet attained the stage of opening after 71 days.

From this experiment it can be concluded that an optimal flowering response on stocks in LD will be obtained if donor leaves have been in SD for about one month or more. As the present experiment was carried out during summer when plants exhibit an optimal response to SD treatment, in later experiments to give an optimal response, donor leaves were used which had received at least 35 SD. The flowering response obtained on a LD stock after grafting with an optimally induced leaf in summer is shown in photo 3.

2.2. *Area of donor leaves*

In exp. 1 (p. 10) it was found that donor leaves with an area of 10 cm² could induce vegetative stocks to flower. As these leaves received only 21 SD whereas about 35 SD are necessary for an optimal response it seemed obvious that even smaller leaf areas would be able to induce stocks.

Experiment 7. – Areas varying from 2 to 30 cm² were cut out at the base of blades originating from plants which had received 46 SD, and were grafted onto LD stocks. The results obtained are given in table 7.

TABLE 7. *Experiment 7.* Flowering response of LD stocks after grafting with different areas from 5th pair of leaves. Donor leaves received 46 SD. Data 60 days after grafting

Leaf area of donor in cm²	Number of grafts	Number of stocks		Number of flowering stocks
		Generative	Vegetative	
2	10	6	4 [1])	5
5	10	10	0	9
10	9	9	0	9
20	10	10	0	10
30	10	10	0	10

[1]) Senescent leaves.

Decreasing leaf areas up to and including 5 cm² induced all LD stocks to flower. Even with 2 cm² 6 out of 10 stocks became generative, the 4 vegetative ones being grafted with rather senescent leaves. Thus, it follows that the minimal leaf area to give a response is not yet reached with 2 cm². Still smaller areas had to be tried.

Experiment 8. – Leaf area was further decreased to the lowest possible limit: 0 cm², which means a petiole with 5 cm midrib without any leaf parenchyma. From the results in table 8 it can be seen that even with a midrib as donor 2 stocks out of 10 initiated flower buds.

TABLE 8. *Experiment 8.* Flowering response of LD stocks after grafting with different leaf areas from 5th or 6th pair of leaves. Donor leaves received 47 SD. Data 40 days after grafting

Leaf area of donor in cm²	Number of grafts	Number of stocks		Mean number of days to flower buds
		Generative	Vegetative	
0 [1])	10	2	8 [2])	22
1	10	8	2 [2])	25
2	10	9	1 [2])	22
30	6	6	0	19

[1]) Petiole + 5 cm midrib.
[2]) Donor leaves died within 14 days after grafting.

Unfortunately most grafts with midribs did not take as was established in several separate experiments. However, if a graft with a midrib from an induced leaf was successful, the stock concerned was always induced; this was never the case if the midrib did not "take". So, it must be concluded that the poor results obtained with midribs as donor were due rather to failure of the grafts than to inability of the midribs to induce stocks. With 1 cm² leaf area results were more successful as can be seen in table 8.

The data presented above indicate that a leaf area of 30 cm² – used in most experiments – is far above the minimum area necessary for inducing a flowering response in a vegetative stock. Probably it represents about the optimum as intact leaves gave no better results.

2.3. *Light intensity*

Experiment 9. – Leaves were grafted onto stocks and immediately after grafting the plants were transferred to an installation with artificial light as described on p. 7. Plants were placed at 4 different distances from the unilateral, continuous illumination so that they were exposed to 4 different light intensities as stated in table 9.

TABLE 9. *Experiment 9.* Flowering response of LD stocks as influenced by various light intensities after grafting with 30 cm² from 5th pair of leaves. Donor leaves received 27 SD. Data 50 days after grafting

Light intensity in μW/cm² ø sphere			Donor leaves	Number of stocks		Mean number of days to flower buds	Number of flowering stocks
Fluorescent tubes	Mercury lamps	Total		Generative	Vegetative		
1800	1000	2800	SD	13	0	23	10
			LD	0	12	–	–
1000	600	1600	SD	12	0	23	5
			LD	0	13	–	–
650	350	1000	SD	13	0	26	0
			LD	0	12	–	–
400	200	600	SD	10	2	35	0
			LD	0	13	–	–

From the results presented it appears that flowering response decreased with a decrease in light intensity. Receptor shoots at the highest intensity unfolded rapidly and had purple-brown leaves. On the contrary shoots at the lowest intensity grew slowly and were green in colour, probably owing to a low rate of photosynthesis. It seems plausible that the difference in number of days to appearance of flower buds on plants, exposed to highest and lowest light intensity, was mainly due to differences in rates of growth of receptor shoots. No flower buds had opened on plants illuminated with less than 1600 μW/cm² ø sphere when the experiment was discontinued. Again this can be explained with different rates of photosynthesis as in another experiment no flowering occurred when plants were moved from high to low light intensity after appearance of flower buds.

In experiments in the greenhouse the same experience has been obtained as in the present experiment with artificial illumination. When low light intensity prevailed during winter receptor shoots grew slowly and appearance of flower buds was delayed.

The method used in the present experiment has the disadvantage that the influence of light intensity on donor leaf and receptor stock cannot be studied separately. A change in light intensity on the donor leaf is necessarily accompanied by the same change on the receptor shoots. It seems justifiable, however, to conclude that light intensity influences the response of stocks to donor leaves only indirectly by differences in photosynthesis and subsequent different rates of growth of receptor shoots.

2.4. *Darkening of donor leaves*

The disadvantage of the method described in the previous experiment to study the influence of light intensity can be overcome by exposing donor leaf

and stock to different light intensities. Preliminary experiments yielded the somewhat surprising result that good graft unions could be established when donor leaves were darkened completely immediately after grafting. This offers the possibility to vary light intensity on the leaves from normal daylight to total darkness.

Experiment 10. – Leaves with an area of 30 cm² which had received 0, 24 or 38 SD were grafted onto LD stocks. Each group was further subdivided as follows: one half was treated normally, *i.e.* received LD (daylight and supplementary illumination) whereas in the other half donor leaves were darkened continuously with a light-proof envelope, in the way as described on p. 13, directly after grafting. Black envelopes were put over the polyethylene bags. See photo 2. Receptor shoots received normal LD treatment. After 3 weeks paper bags and donor leaves were removed. Nearly all leaves were in a good condition and had formed a graft union. Apparently donor leaves contained sufficient reserve material to remain alive and for the formation of a graft union. For the latter a certain amount of building material supplied by the donor leaf is necessary as appeared in exp. 8 (p. 19) in grafts with midribs.

The results of two separate experiments yielding similar results have been united in table 10.

TABLE 10. *Experiment 10.* Influence of continuous darkening of donor leaves on flowering response of LD stocks. 20 plants per treatment. Data 60 days after treatment

Number of SD applied to donor leaves	Donor leaves in light or darkness	Number of stocks		Mean number of days to flower buds
		Generative	Vegetative	
0	Light	0	20	–
0	Darkness	0	20	–
24	Light	20	0	25
24	Darkness	14	6	28
38	Light	20	0	20
38	Darkness	19	1	22

From this table it follows that donor leaves treated with 38 SD and darkened immediately after grafting induced flowering in almost 100 % of the stocks. However, appearance of flower buds was somewhat retarded, and flowering was less prolific than on stocks with donor leaves in light. When SD treatment of donor leaves had been suboptimal (*in casu* 24 SD) the difference in flowering response to leaves in darkness and those in light was greater than with optimally induced ones. All stocks grafted with LD leaves remained vegetative.

This experiment shows that light is a factor of minor importance for induced leaves to function as donor (at least if they are in an optimally induced state) in the case receptor shoots are in strong light. We can reach the conclusion that the functioning of donor leaves does not directly depend upon the process of photosynthesis because continuous darkness does not nullify their action. Anticipating the general discussion we conclude that certain products, formed during SD treatment, remain stored in the leaves without losing their activity and induce flowering in vegetative stocks even when donor leaves are darkened.

3. THE TISSUE UNION BETWEEN DONOR AND RECEPTOR

3.1 *Minimal duration of contact period between donor and receptor*

In preliminary experiments on the minimal duration of the contact period between donor and receptor necessary to evoke a flowering response in the latter, variable results were obtained. Finally it looked as if the results varied with the degree of induction of the donor, which would also be expected in view of the results obtained in exp. 6 (p. 18). The next experiment was designed to determine the exact relationship.

Experiment 11. – Completely comparable leaves (5th pair, area 30 cm^2) from plants of one sowing which had received 0, 26 or 40 SD were grafted onto vegetative stocks above the 4th node. At various times after grafting donor leaves were removed from groups of 12 plants. This was done every 2 days for leaves which had received 40 SD, starting 4 days after grafting. Groups of leaves with a suboptimal induction (26 SD) were removed with 3-day intervals in order to cover a wider space of time than was planned with optimally induced leaves. Stems were pinched immediately above the 4th node with the aid of a pair of tweezers. It should be stressed that petioles of donor leaves were taken away completely. Unfolding leaves on receptor branches were retained.

The results obtained have been plotted in fig. 4.

For obtaining flowering on all stocks, the leaves had to remain on the receptors for at least 10 days when they had been induced for 40 days. The shift from 0 to 100% flowering took place within 4 days. However, when leaves had re-

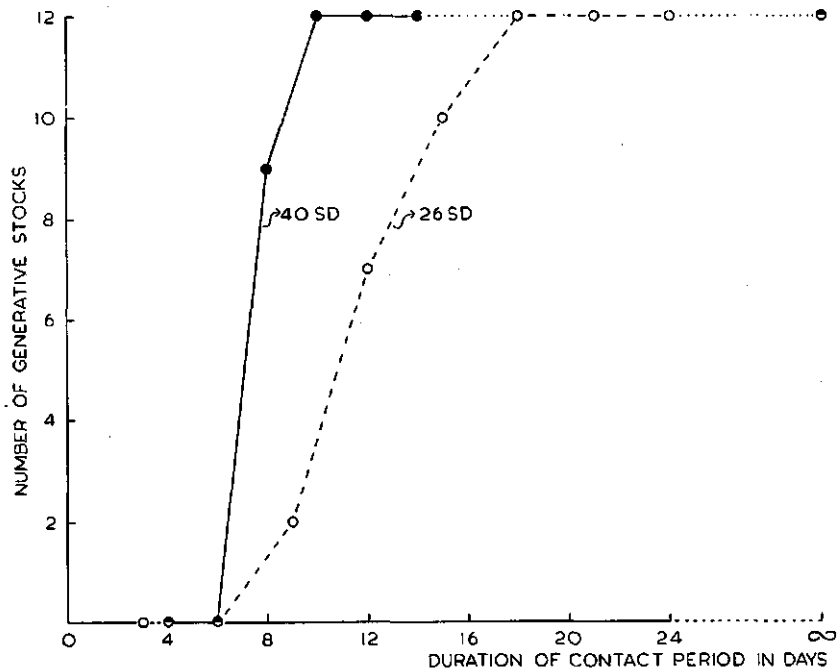

FIG. 4. *Experiment 11.* Number of generative stocks as influenced by duration of contact period between donor and receptor. Donor leaves received 26 or 40 SD prior to grafting. Per treatment 12 plants.

ceived only 26 SD a gradual increase in flowering response from 0 to 100% continued over a period of 12 days. Maximal response was not attained until graft contact had lasted 18 days or more. In this connection it is worth while to mention that removal of polyethylene bags 6 days after grafting did not cause wilting of donor leaves in bright sunlight.

The stocks onto which LD leaves had been grafted and subsequently removed, remained vegetative.

The two curves in fig. 4 clearly demonstrate that the contact period necessary to induce all stocks was shorter when the period during which donor leaves had been induced was longer. Unfortunately these curves give no impression of the *amount* of flowering on receptors. It was clear at first sight that in this respect great differences existed between various groups of plants. When, after 51 days, the experiment was discontinued, the numbers of macroscopically visible flower buds, flowers and fruits per group were counted in order to be able to express differences quantitatively. This method gives at least an idea about the number of axillary buds which had become reproductive. It does not account, however, for different rates of development of buds into open flowers and subsequent fruits. *E.g.* when stocks had been in contact with donor leaves, induced for 40 days, flowering and fruiting were more profuse with increasing duration of contact period. When leaves had received 26 SD, the first two or three nodes on receptor shoots remained vegetative and even 51 days after grafting only a few flower buds had opened. The counting yielded results which are shown graphically in fig. 5.

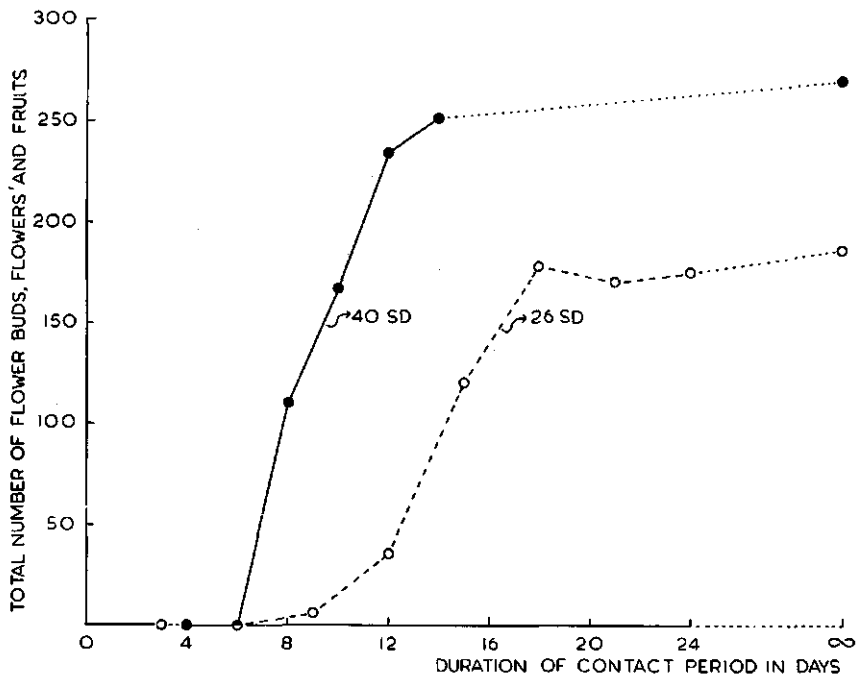

FIG. 5. *Experiment 11.* Number of macroscopically visible flower buds, flowers and fruits per group of 12 stocks as influenced by the number of SD applied to donor leaves, and duration of contact period between donor and receptor. Data 51 days after grafting.

It is clear that two typical saturation-curves are obtained. The level of saturation is determined by the degree of induction of the donor leaves, the level being highest with optimally induced donors.

Various experiments on the minimal duration of contact period between donor and receptor have been carried out. Results varied slightly with prevailing external conditions but flowering never occurred when leaves remained less than 6 days on stocks. Fig. 6 gives data of an experiment in which leaves were removed with 1-day intervals.

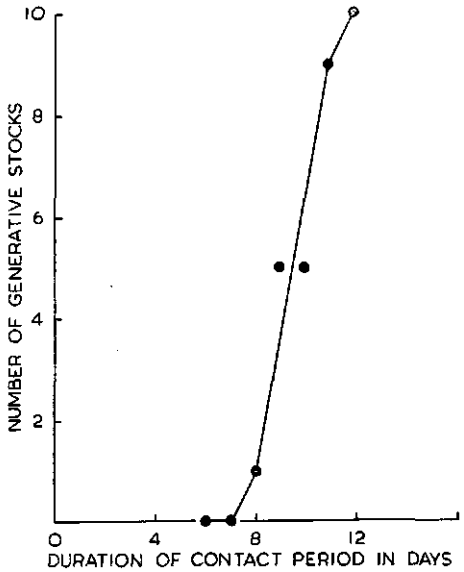

FIG. 6.
Number of generative stocks as influenced by duration of contact period between donor and receptor. Donor leaves received 35 SD. Per treatment 10 plants.

The resulting curve shows the same trend as the left one in fig. 4, although in comparison with the latter it is shifted slightly to the right. Exp. 11 (p. 22) was carried out during a period with bright weather and high temperature whereas the results shown in fig. 6 were obtained when cloudy weather and lower temperature prevailed. It was a general impression, gained in all experiments of this type, that high temperature (25°–30 °C) and light of high intensity could shorten, to some degree, the minimal duration of graft contact to obtain flowering. However, as mentioned already before, the contact period had always to be at least 6 days in order to get any response. Such a response was then obtained only when plants were exposed to uninterrupted light of high intensity at rather high temperature and when donor leaves were optimally induced.

Of course the question arises which tissues of donor and receptor should form a functional connection for obtaining flowering on stocks. From literature it is apparent that the floral stimulus probably moves in the phloem (*cf.* 73). If this would be valid in the present case it follows that the minimal period of contact, necessary for inducing a stock to flower, must coincide or exceed the time required for establishing a phloem continuity between donor leaf and stock.

3.2. *Anatomical observations*

Anatomical studies of graft unions at various stages of development have been made. It appeared that a union between the petiole of a donor leaf and the stem of a stock was established in the same way as described in literature for a union between two stems as graft partners (6, 26, 140).

The following *résumé* of the origin of a union between stem and petiole is especially valid for the *lower* part of a petiole which has been inserted into a cleft stem.

Shortly after grafting, callus parenchyma was differentiated on both sides of the isolating layer, although in general more actively on the side of the stock than on that of the scion. On the fourth day callus tissue was still completely meristematic. In the following days differentiation into new tissues occurred rapidly. Among the new elements observed xylem vessels were easily distinguishable. These apparently differentiated from callus parenchyma as they had the same shape and size as surrounding cells. Xylem elements united to vascular strands and connected scion and stock through local openings in the isolating layer. Sometimes such a connection could be observed already after 8 days.

As phloem tissue in *Perilla* consists of small elements it was very difficult to identify phloem continuities from scion to stock. Strands of cells, rich in plasm, which parallelled the xylem strands in the region where phloem should be present, most probably consisted of phloem elements.

CRAFTS (26) assumed that in tobacco xylem elements and sieve tubes differentiated simultaneously so that the finding of any mature xylem vessel was a good indication that both tissues had become functional. If this would hold true for *Perilla* functional phloem would have arisen by the time that xylem does so, that is to say approximately 8 days after grafting. This value fairly well agrees with the minimal duration of contact period necessary for a flowering response. It will be clear, however, that this general statement is inadequate evidence to assume that a phloem continuity is necessary for transmission of the floral stimulus. For that purpose one should investigate a great number of graft unions and find a correlation between establishment of phloem contact and flowering response. Besides that this would be laborious it would not yield very satisfactory results. The ideal cases described above in which a vascular continuity between stock and scion could be detected at a young stage, were rare. As pointed out already by DE STIGTER (140) this is due to the fact that connecting tissues mostly make bendings in all three dimensions so that one hardly ever finds the whole required region in one and the same section.

3.3. *Translocation of labelled sucrose via the graft union*

Besides the anatomical approach there is still another method to determine when a functional phloem continuity has been established, *viz.* by timing when translocation in the phloem from donor leaf to stock is possible. Theoretically this sounds quite simply, but for the experimental approach one should have the disposal of a substance, by preference natively occurring in the phloem, which is readily translocated and moreover easily detectable in the stock. This latter condition is fulfilled by dyes which, however, do not naturally occur in the phloem, and further by labelled organic compounds.

In the experiments to be described in this section totally C^{14}-labelled sucrose was employed to determine when a functional phloem connection between

donor leaf and stock was established. Gross autoradiograms were used to show the distribution of labelled carbon in the treated plants. For basic principles of autoradiography we can refer to a recent review by DUGGER (32).

The design of translocation experiments was as follows: a homogeneous group of stocks was grafted with optimally induced leaves. Beginning 4 or 5 days after grafting donor leaves were removed from groups of stocks with 24-hour intervals to establish the minimal duration of grafting contact necessary for flowering on stocks under prevailing conditions (Flower test). At the same time translocation experiments with labelled sucrose were carried out (Translocation test). As plants used in the latter test had to be killed, transmission of the floral stimulus and translocation of labelled sucrose could not be studied in one and the same plant. In order to overcome this objection two plants each were grafted with a partner of the same pair of leaves and were considered to be equivalent: one plant was used in the flower test, the other in the translocation test.

Translocation tests were always carried out in a room with constant illumination and controlled temperature. Illumination was obtained from 20 Watt fluorescent tubes (type TL 33); intensity at the level of the donor leaves was approximately 3000 $\mu W/cm^2$ Ø sphere. Temperature was kept constant at 25 °C.

Labelled sucrose was applied in a droplet to the donor leaf inside a lanolin ring 3 to 4 cm from the base along the midrib. In general the droplet was dry within 60 minutes. At the end of a 4-hour period of treatment the pieces of raffia around the graft union were cut so that the two longitudinal halves of the stem could be pulled loose from the inserted petiole. In this way donor leaf and stock were separated carefully to be sure that all labelled carbon detectable in the stock had passed the graft union *during* the 4-hour period of treatment. The top of the stem was cut into sections of 2 cm, the lower part was discarded. Plant material was dried in a plant press between some layers of filter-paper. After being dried plant parts were mounted in the original position with the aid of small pieces of sellotape on a sheet of drawing-paper, placed on "Gevaert Osray" X-ray film, size 30 × 40 cm, and kept under pressure between plates of glass in light-proof packing-envelopes. Exposure time was always 4 weeks. As a control untreated plants were likewise placed on films to be sure that the plant material itself did not cause formation of an image without presence of labelled carbon (*cf.* 32, pp. 361 and 370).

X-ray films were developed at 20 °C for 5 minutes, rinsed with water and fixed during 10 to 15 minutes afterwards.

Translocation of labelled sucrose from donor leaf to stock was assumed to have taken place when receptor shoots had caused an image on the film. In general young leaves on receptor shoots gave most intensive images. Stems mostly gave no or only vague images, probably due to low amount of labelled carbon present and/or absorption of the weak β-radiation in the stem tissue. (Even sellotape which was used for mounting the plants on paper, was found to absorb the radiation completely; see interruptions in image of stem in photo 4). Results of a typical experiment are reported below.

Experiment 12. – Donor leaves with an area of 30 cm^2 which had received 40 SD were grafted onto a group of stocks above the 3rd node. Immediately after grafting plants were moved into the room at 25 °C under continuous illumination. Beginning on the 5th day after grafting flower- and translocation tests were started and repeated with 24-hour intervals. Every day 2 pairs of leaves were tested. Results are given for each pair of leaves separately in table 11 (see p. 27).

The first flowering response was obtained when grafting contact had been uninterrupted for at least 6 days. This coincided with duration of contact necessary for translocation of a detectable amount of labelled sucrose to receptor shoots. Photo 4 shows an autoradiogram which was obtained from a plant treated on the 9th day after grafting.

One pair of leaves looking very senescent and treated 10 days after grafting neither gave a flowering response nor was labelled sucrose translocated, thus providing experimental evidence for a suggestion made previously, *viz.* that senescent leaves "have reached a state in which they are physiologically very

TABLE 11. *Experiment 12.* Minimal duration of grafting contact between donor leaves and receptor stocks necessary for translocation of labelled sucrose and transmission of the floral stimulus. Donor leaves received 40 SD prior to grafting

Duration of contact between donor and receptor in days	Presence (+) or absence (–) of labelled carbon in receptor shoots after a 4-hour period of treatment	Flowering response of stocks induced by corresponding donor leaves
5	–	–
5	–	–
6	+	+
6	–	–
7	+	+
7	+	+
8	+	+
8	+	+
9	+	+
9	+	+
10	– [1])	– [1])
10	+	+

[1]) Senescent pair of leaves.

inactive and during which little or no organic substances including the floral stimulus, are supplied any longer to the stock" (158).

In this and other experiments grafted donor leaves did not show symptoms of wilting in bright light when polyethylene bags were removed 1 or 2 days *before* labelled sucrose could be translocated, probably indicating that a xylem continuity between donor leaves and stocks had established by that time. Moreover, labelled sucrose moved downwards *via* the graft union, so that it seems warranted to conclude that translocation of tracer during the 4-hour period of treatment took place *primarily* in the phloem.

Similar results as those presented in table 11 have been obtained in several separate experiments. Most often first translocation of labelled sucrose and first transmission of floral stimulus (as measured by flowering response on stocks) occurred simultaneously. *Flowering was never obtained when duration of contact between donor and receptor had been shorter than necessary for translocation of labelled sucrose.* The close correlation found in these experiments between minimal duration of grafting contact, necessary for a flowering response and for first translocation of sucrose, strongly supports the view that the floral stimulus cannot pass the graft union until a functional phloem continuity between donor leaf and stock has been established.

3.4. *Discussion*

From the preceding sections it is obvious that grafting contact between donor and receptor should be uninterrupted for at least 6 days in order to obtain flowering on vegetative stocks. Apparently tissue connection is at first insufficient for transmission of the floral stimulus. The translocation experiments with labelled sucrose provide evidence that the initial period of approximately 6 days is the time required for the establishment of a functional phloem con-

tinuity. So, when we determine the minimal duration of grafting contact necessary for obtaining a flowering response, we really determine the time required for the establishment of a phloem connection. The results obtained with optimally induced leaves demonstrate that the shift from 0 to 100% flowering receptors took place within 4 and 5 days respectively in fig. 4 and 6 (pp. 22 and 24). This very short period means that there was only little variation among the individuals concerning time required for establishing a phloem union. During grafting of the plants no special attention was paid to good or poor approximation of phloem of stock to phloem of petiole, so that this may have varied from poor to good. Nevertheless stocks always reacted very uniformly so that we can conclude that the initial approximation of phloem had little or no influence on the period necessary for the establishment of a functional phloem connection. HAYWARD and WENT (57) experimenting with peas reached a similar conclusion. Growth factors (caulocaline) coming from the roots could pass a graft union only if a vascular continuity had been established. The establishment of such a continuity, however, was not affected by the initial approximation of the vascular bundles of stock and scion.

As noticed already on p. 24 high temperature could shorten, to some degree the minimal duration of graft contact to obtain flowering. This indicates that the rate of establishing a phloem connection increased with increasing temperature. A similar finding was reported by ESCHRICH (36) for regeneration of sieve tubes between the ends of a severed bundle in *Impatiens* and *Coleus*.

As soon as translocation *via* the newly established phloem connection has started, the floral stimulus will reach the receptor shoots and exert its morphogenetic effect. From fig. 5. (p. 23) it appears that the number of flower buds formed on these shoots increased with increasing duration of grafting contact until a maximal value was attained. This phenomenon can easily be interpreted: if the donor leaves have been removed shortly after the beginning of phloem translocation, only a small amount of stimulus will reach the receptor shoots with the consequence that only one or two nodes on a receptor shoot will become reproductive. With longer duration of contact more stimulus will be translocated to the shoots and induce more profuse flowering. Thus, there seems to be a quantitative relation between amount of stimulus received by the shoots and number of subsequently produced flower buds.

In the preceding it was concluded that flowering on vegetative stocks can be induced as soon as a phloem continuity has become established. This holds true only if optimally induced donor leaves are involved. The results obtained in exp. 11 (p. 22) indicate clearly that for obtaining the same flowering response a longer contact period was necessary with suboptimally induced leaves than with optimally induced ones. As there was no indication that duration of SD treatment affected the rate of establishment of tissue connection afterwards, it seems that the *amount* of stimulus supplied by the donor leaves became the limiting factor for the induction of vegetative stocks. It was established experimentally with leaves which had received only 21 SD that under favourable conditions grafting contact had to be uninterrupted for more than 10 days for obtaining any flowering response. In view of this result and those obtained in exp. 6 (p. 18) it seems justified to predict that in general under equal conditions and with comparable leaves *minimal duration of grafting contact necessary for flowering on all stocks will decrease with increasing number of SD applied to donor leaves to an ultimate value of approximately 6 days.*

From literature it appears that there are no exceptions to the rule that a tissue connection is necessary for transmissio nof the floral stimulus from donor to receptor (*cf.* 39; 96, p. 157). The minimal duration of contact necessary for inducing receptors to flower has been investigated in several plants.

MOSHKOV (103) found that in *Perilla* non-induced scions had to remain on induced stocks for more than 10 days to obtain flowering. He stated that tissue union occurred after 9 to 12 days and concluded that such a union was necessary before the floral stimulus could be transferred.

HEINZE *et al.* (58), experimenting with soybeans, obtained flowering on 7 out of a total of 15 receptor plants when splice-grafted donor leaves were removed after 4 days. A shorter period of contact, however, did not result in floral initiation. These authors did not record peculiarities about the graft union but in view of the good approximation of vascular bundles obtained with splice-grafting it seems possible that a phloem connection had been established after 4 days.

In *Xanthium* WITHROW and WITHROW (155) observed flowering on 20% of receptor plants when direct contact with donors lasted 8 days. Such a period was sufficient for the establishment of a tissue union. These authors thought it most likely that the floral stimulus was translocated in the phloem.

In biennial *Hyoscyamus niger* MELCHERS (94) ascertained that the grafting contact between vernalized and non-vernalized specimens had to last for at least 5 days for the induction of floral primordia on the receptors. In a recent communication AACH and MELCHERS (1) quote unpublished experiments in which this period could be shortened to 3 days. This seems too short a time for the establishment of a phloem union in tap root tissue, so that in this case probably a union between living parenchyma cells will suffice for transmission of the effect evoked by low temperature in *Hyoscyamus*.

Although not directly related to the present subject it seems worth while to mention briefly some other "factors" or "stimuli" of which the nature is completely unknown up to now. They all seem to have in common that they move in living tissue, most probably in the phloem. Therefore they cannot pass a graft union until a tissue union has been established. *E.g.* WENT's calines (57, 150, 151), a swelling factor in peas (150), a tuber-forming stimulus in *Helianthus* (51) and potatoes (44), a flower-inhibiting factor in peas (112) and the natural gibberellin factor in peas (84).

Only the cambial stimulus and the stimulus which inhibits the growth of axillary buds can cross a protoplasmic discontinuity (137, 138). It is probable that auxin is involved in these processes.

In this connection BENNETT's (6) experiments on transmission of a "known" principle *viz.* curly-top virus (which is strictly limited to the phloem) should also be mentioned. Infected tobacco scions were grafted onto healthy stocks and removed with 24-hour intervals for 15 days. No virus could pass the graft union until a phloem continuity had differentiated. The data presented by BENNETT are plotted in fig. 7 (p. 30), together with results from our fig. 4 and 6.

It will be clear that the curve for virus-infected tobacco stocks is similar, also concerning time, to those for generative *Perilla* stocks.

Even more instructive were results when the same experiment was repeated (7) with a mixture of two viruses, *viz.* a parenchyma-inhabiting virus and curly-top virus. The former, moving from cell to cell, already passed the graft union on

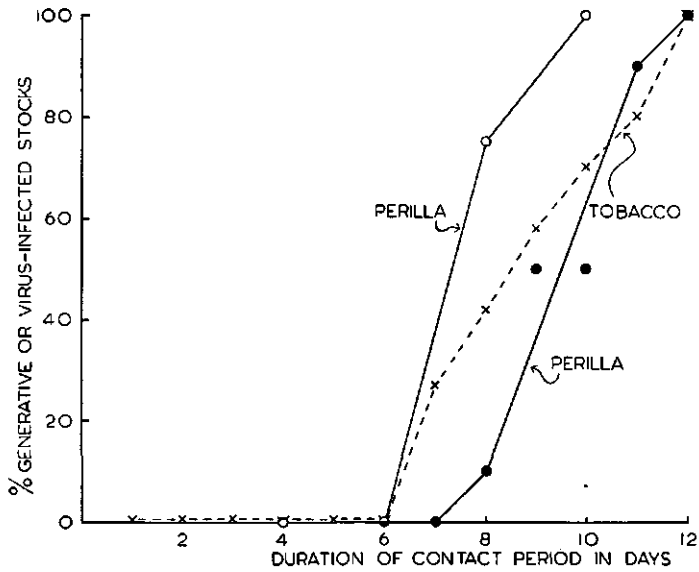

FIG. 7. Percentage generative *Perilla* stocks and virus-infected tobacco stocks as influenced by duration of contact period between donor and receptor, and virus-infected scions and healthy stocks respectively.
Data for *Perilla* are taken from fig. 4 and 6 (pp. 22 and 24). Data for tobacco after BENNETT (6, p. 680).

the 2nd day after grafting. The latter, needing a bridge of phloem, did not begin to pass the graft union until the 5th day.

4. THE MECHANISM OF PHOTOPERIODIC INDUCTION

4.1. *Photoperiodic induction as an irreversible phenomenon*

In a preliminary communication (158) it was concluded that in *Perilla* the effect of photoperiodic induction is completely irreversible. The experimental results which led us to this conclusion have been confirmed and extended. New evidence, even more convincing than previously published results, is reported in the following experiments.

Experiment 13. – *Perilla* leaves (5th pair, area 30 cm^2) induced during 40 days were grafted onto a group of vegetative stocks. Every 14 days these leaves were regrafted onto a new group of stocks in the way as described before (158). Table 12 shows the results.

TABLE 12. *Experiment 13.* Effect of induced leaves grafted successively onto 5 different groups of LD stocks. Donor leaves received 40 SD prior to 1st grafting

Number of grafting	Days in LD after 1st grafting	Number of leaves grafted	Number of stocks		Mean number of days to flower buds
			Generative	Vegetative	
1st	0	12	12	0	18
2nd	14	12	11	1	20
3rd	28	12	12	0	19
4th	42	12	11	1	20
5th	56	12	12	0	19

It appears that leaves continued to induce flowering in each new group of stocks onto which they were grafted. There was no indication that the flowering response had diminished in the 5th group in comparison with that in the 1st group.

Experiment 14. – In this experiment donor leaves previously exposed to 28 SD were grafted onto 7 groups of LD stocks in succession. The first 6 graftings were carried out with intervals of 15 days, the 7th grafting, however, 22 days after the preceding one. The results shown in table 13 once more indicate that the effect of photoperiodic induction did not get exhausted but remained transmissible to vegetative stocks.

TABLE 13. *Experiment 14.* Effect of induced leaves grafted successively onto 7 different groups of LD stocks. Donor leaves received 28 SD prior to 1st grafting

Number of grafting	Days in LD after 1st grafting	Number of leaves grafted	Number of stocks		Mean number of days to flower buds
			Generative	Vegetative	
1st	0	15	14	1	27
2nd	15	15	11	4	27
3rd	30	15	15	[1]0	26
4th	45	15	15	0	20
5th	60	15	14	1	21
6th	75	15	14	1	20
7th	97	14	10	4 [1])	22

[1]) Donor leaves died within 20 days after grafting.

Donor leaves did not lose their induced state although more than three months elapsed since they received the last inductive cycle. After the 7th grafting leaf blades became yellowish between the veins and several died afterwards. No attempts were made to regraft surviving leaves onto a next group of stocks. Differences in numbers of days until appearance of flower buds shown in table 13 cannot be considered as significant as conditions in the greenhouse varied in the course of the experiment.

From table 13 it appears that in some groups flowering was not obtained on all stocks. In view of the results obtained in exp. 11 (p. 22) it seems probable that the period of contact was too short for transmission of sufficient floral stimulus in some plants as donor leaves had received only 28 SD. In experiment 13 the leaves were induced optimally and although they were removed with 14-day intervals almost 100 % flowering was observed in all groups. On the other hand it was noticed in these and other experiments that leaves tended to show symptoms of senescence earlier the longer they had been induced. It thus appears that the number of stocks which can be induced to flower by one and the same donor leaf is determined by two factors which are – to a certain extent – complementary to another, *viz.:*

Firstly, by the period during which the leaf can be kept in a good condition; this period decreases with increasing duration of SD treatment.

Secondly, by the minimal duration of contact between leaf and stock necessary for transmission of the floral stimulus; this period also decreases with increasing duration of SD treatment.

It should be pointed out that in both experiments non-induced leaves were regrafted onto several groups of LD stocks successively at the same time as induced leaves. These control stocks never initiated flower buds.

Besides regrafting leaves consecutively onto several groups of stocks as described above there are other methods for demonstrating the irreversible state in induced leaves. *E.g.* one can expose plants to SD and move them back to LD afterwards. In course of time these plants can be tested on their ability to induce flowering in vegetative stocks. Previously it has been reported (158) that the effect of photoperiodic induction reached during 21 SD was not altered by a LD after-treatment of 4 weeks. Later experiments, not to be reported in detail, have confirmed and extended this result: leaves on induced plants retained the induced state as long as they remained in a good condition. A modification of this type of experiments is reported below.

Experiment 15. – A number of plants was subjected to 28 SD whereas comparable plants remained in LD. At the end of this treatment leaves on the 6th node were detached from all plants by excising the petiole about 5 mm from the base. One leaf of each pair was subjected to SD, the other to LD, so that the experiment included 4 different treatments of donor leaves as indicated in table 14. Detached leaves were grown on nutrient solution. All produced roots very abundantly except those which received SD both on the plants and after they were detached (Table 14, first treatment). Presence or absence of induced state was demonstrated by grafting leaves with an area of 30 cm^2 onto vegetative stocks. The results compiled in table 14 show that all leaves which received SD, either on the plants or as detached leaves, induced LD stocks to flower.

TABLE 14. *Experiment 15.* Effect of photoperiodic treatment applied to detached leaves which received 28 SD or LD before they were detached. Leaves were grown on diluted KNOP's nutrient solution + boric acid

Photoperiodic treatment of donor leaves		Root production of detached leaves	Number of stocks		Mean number of days to flower buds
On the plant	Detached		Generative	Vegetative	
28 SD	35 SD	Few	12	0	22
28 SD	35 LD	Many	11	1 [1])	20
LD	35 SD	Many	12	0	21
LD	35 LD	Many	0	12	–

[1]) Senescent leaf.

Repetitions of this experiment gave similar results, so that it can be concluded that leaves, detached from plants after they have been induced, retain the induced state under LD conditions afterwards just as leaves do which remain attached to plants.

It will be noticed that root production on the petioles in no way reflected whether leaves were in the induced state or not.

Results in table 14 also provide evidence that detached leaves without axillary buds can be induced. See also p. 37.

From exp. 6 (p. 18) and 11 (p. 22) it followed that flowering response on stocks in LD was more abundant with increasing duration of SD treatment applied to donor leaves. Thus, it looks as if in *Perilla* leaves a gradual transition can exist from non-induced state to a condition which can induce a maximal flowering response. It seems of interest to know whether a certain "degree" of induction remains existent or that, once set going, it tends to reach its maximal value. The results of the next two experiments present evidence for the former alternative.

Experiment 16. – Three comparable groups of plants received 0, 24, or 38 SD. The treatments ended for all groups at the same date. Comparable leaves with an area of 30 cm^2 from all three groups were grafted onto vegetative stocks and regrafted onto other stocks 34 days after the 1st grafting. The results presented in table 15 once again show that flower buds were visible earliest and developed most rapidly when leaves had been optimally induced.

TABLE 15. *Experiment 16.* Degree of photoperiodic induction attained in leaves which received different numbers of SD, as tested at the end of SD treatment and after 34 days in LD. Data 50 days after grafting

Number of SD applied to donor leaves	1st or 2nd grafting	Number of stocks		Mean number of days to flower buds	Number of flowering stocks
		Generative	Vegetative		
0	1st	0	10	–	–
0	2nd	0	10	–	–
24	1st	10	0	26	0
24	2nd	10	0	27	0
38	1st	10	0	21	10
38	2nd	10	0	22	7

The 2nd grafting yielded similar results as the 1st one except that stocks reacted more slowly owing to lower temperature and lower-intensity light. The same results have been obtained in several separate experiments.

Experiment 17. – Donor leaves used in this experiment originated from the same groups of plants as those tested in exp. 6 (p. 18). After plants had received 0, 1, 2, 3, 4, or 5 weeks of SD they were moved back to LD. After having been for 4 weeks in LD the "degree" of induction was tested by grafting the 7th pair of leaves. The results obtained are presented in table 16.

TABLE 16. *Experiment 17.* Effect of LD after-treatment on degree of induced state in leaves which were exposed to different numbers of SD. LD after-treatment lasted 28 days. Data 58 days after grafting. Compare table 6 on page 18

Donor leaves received	Number of successful grafts	Number of stocks		Mean number of days to flower buds	Number of flowering stocks
		Generative	Vegetative		
0 SD	10	0	10	–	0
7 SD	10	0	10	–	0
14 SD	10	7	3	51	0
21 SD	10	10	0	26	2
28 SD	9	9	0	23	8
35 SD	5	5	0	21	5

After comparison of tables 6 (p. 18) and 16 it is obvious that almost similar results were obtained with leaves tested at the end of SD treatment and with those exposed to 28 LD since the last inductive cycle. Again the slower reaction of stocks in this experiment compared with that in exp. 6 (p. 18) can be ascribed to lower temperature and lower-intensity light.

From this and the preceding experiment it can be concluded that *various "degrees" of the induced state are retained in LD to the same extent in which they were present at the end of SD treatment.*

4.2. *Photoperiodic induction as a localized phenomenon*

Data presented in the preceding section provided clear-cut evidence that the ability of induced leaves to produce the floral stimulus is fully retained under LD conditions. Two interesting problems, however, remained undiscussed until now, *viz.*:

1) Is the ability to produce the floral stimulus (the induced state) transmissible to non-induced leaves in LD?

2) Is it possible to translocate the floral stimulus to LD leaves so that the latter can be induced *indirectly* (*i.e. not* by SD treatment) to function as donor?

Preliminary observations (158) indicated that both these questions should be replied in the negative. Data reported below support this conclusion.

Experiment 18. – A group of stocks was induced to flower by grafting them with leaves which had received 35 SD. At various times after grafting, as indicated in table 17, indirectly induced shoots were tested on their ability to induce LD stocks to flower. Shoots grafted after 22 or 30 days flowered normally but finally reverted to vegetative growth just as ungrafted shoots did.

To be sure that the shoots to be tested – if induced – could produce the floral stimulus, a number of stocks with flowering shoots was subjected to 28 SD when 50 days had elapsed since grafting.

From the results presented in table 17 it follows that indirectly induced shoots which flowered abundantly, could never function as donor for vegetative stocks. However, if these indirectly induced shoots were exposed to 28 SD and then grafted, they did induce flowering in all stocks.

TABLE 17. *Experiment 18.* Effect of indirectly (= *via* grafting) induced shoots of *Perilla* after grafting onto vegetative stocks. Photoperiodic treatment of donor shoots (first column) indicates number of days elapsed since stocks from which shoots originated, were grafted with induced leaves

Photoperiodic treatment of donor shoots	Number of shoots grafted	Number of stocks	
		Generative	Vegetative
22 LD	9	0	9
30 LD	9	0	9
50 LD [1])	12	0	12
78 LD [1])	6	0	6
50 LD + 28 SD [1]) . . .	12	12	0

[1]) Flowers and axillary buds removed from donor shoots, only 2 pairs of leaves left.

All similar experiments with shoots as well as with single leaves always yielded negative results.

If it is taken for granted that the floral stimulus is translocated in the phloem (*cf.* section 3, p. 22) with the assimilates, it might be possible to direct this stream to leaves depleted of carbohydrates.

Experiment 19. – Leaves induced during 48 days with an area of 60 cm^2 were grafted onto vegetative stocks above the 4th node. Leaves on the 2nd and 4th node were retained whereas all axillary buds were removed. After 6 days leaves on the 4th node were reduced to an area of 30 cm^2. At the same time one leaf of each 4th pair was darkened with a bag of black cloth which transmitted around 5 % daylight. Fig. 8 gives a diagram of one experimental plant.

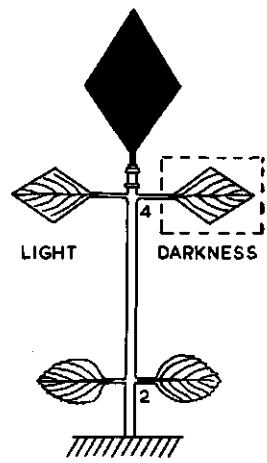

FIG. 8. *Experiment 19.* Diagram of *Perilla* plant grafted with an induced leaf. One LD leaf on the 4th node darkened. White: non-induced. Black: induced during 48 SD.

After 19 days (so 25 days after grafting) leaves from the 4th pair were grafted onto vegetative stocks, and donor leaves were grafted onto a second group of stocks. Leaves darkened during 19 days had become green in colour, but were still in a good condition so that they could be grafted with 100 % success. The results obtained can be summarized very briefly: previously SD treated leaves induced stocks to flower, but stocks grafted with leaves from the 4th pair, either kept in light or darkness, never showed any flowering response. The same negetative result was obtained in several separate experiments. Of course one can wonder whether translocation from donor leaves to darkened leaves had really taken place. This was investigated with the aid of labelled sucrose. The method used was the same as described in section 3.3 (p. 25), but period of translocation also included 8 hours. Autoradiograms revealed that no labelled carbon had ever been translocated to leaves on the 4th node, whether these were in light or in darkness. This somewhat surprising result is in full agreement with data presented by ARONOFF (2; *cf.* also 27, p. 220). This author exposed soybean leaves to $C^{14}O_2$ and found that labelled products were translocated from the leaves very rapidly. However, translocation to adjacent, mature leaves, either in light or darkness, did not occur.

4.3. *Discussion of two preceding sections*

Experimental data presented in the preceding two sections have been diagrammed in fig. 9 (p. 36).

For the sake of clearness two phenomena should be distinguished:

1) The *induced state* (*i.e.* the ability to produce the floral stimulus), gradually built up under the influence of SD, which is irreversible and strictly localized.

2) The *floral stimulus* which is transmissible from induced leaves to growing-points where it exerts its morphogenetic effect.

Exp. 18 (p. 34) and 19 (p. 34) demonstrate that in plants "synthesized" by grafting *the induced and non-induced (or vegetative) state coexist side by side in one and the same plant without influencing each other: the SD treated leaves retain the induced state, the LD treated ones the vegetative state.* A similar phenomenon was established by LONA (87) when he applied SD treatment to only one leaf

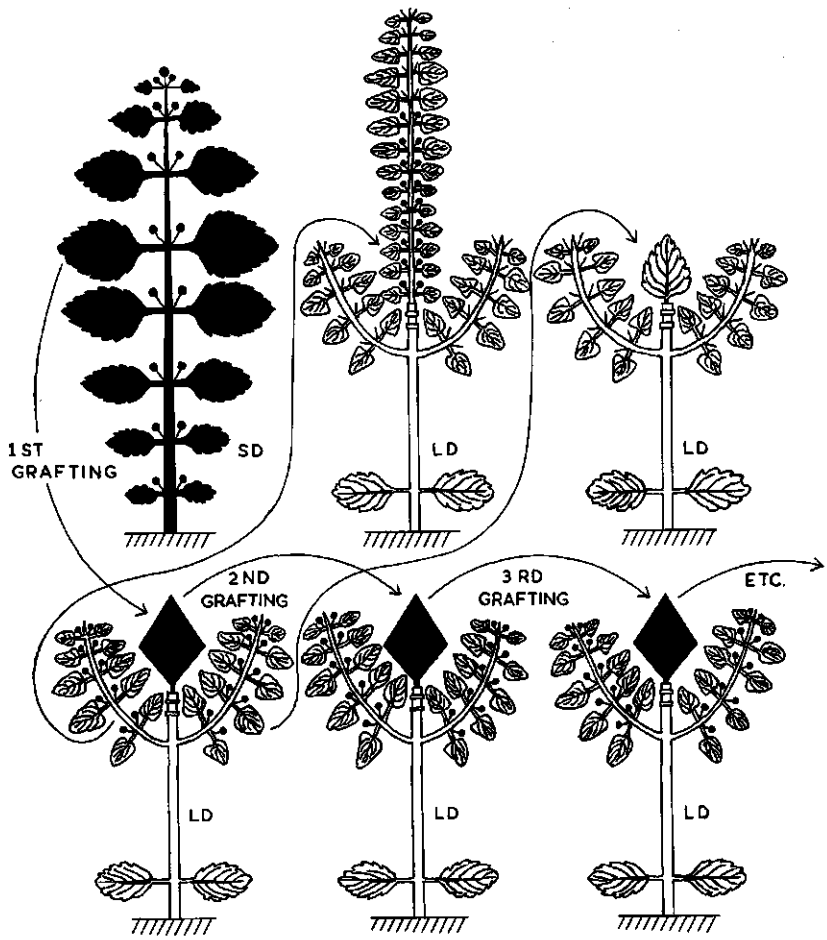

FIG. 9. Diagram of grafting and regrafting one and the same donor leaf onto several LD stocks in succession; all stocks are induced to flower. An indirectly induced shoot does *not* function as donor.
Leaves in black are in induced state, those in white in the vegetative state.

| = vegetative axillary bud.
ƚ = generative axillary bud.

on a *Perilla* plant in LD. He introduced the term *functional chimera* to designate this phenomenon, but we prefer the term *physiological chimera* (used incidentally by LONA (86) in an earlier publication) because both types of leaves exhibit a different physiological behaviour (In fig. 8 and 9 induced and non-induced leaves have been indicated in black and white respectively to underline the difference in physiological behaviour). CAJLACHJAN (19) and subsequently LONA (87) defoliated *Perilla* plants except for one leaf. Various combinations of LD and SD treatment were applied to the basal or apical half of the blade. Flowering occurred if the lower half was in SD and the apical half in LD. However, if the apical half was in SD and the basal half in LD, flowering was

strongly retarded. LONA (87) grafted such a basal, LD treated half onto a LD stock and observed that no flowering occurred. This clearly shows that the *induced state* had remained localized in the apical half and that no or insufficient stimulus for inducing a vegetative stock to flower had been translocated to the basal half. From our exp. 18 (p. 34) it follows that the very leaves expanding in LD under the influence of a donor leaf neither acquired the induced state nor accumulated floral stimulus. Apparently every new cell coming into being in LD gets the vegetative state which can only be changed into the induced state under the *direct* influence of SD. See further discussion on p. 51.

4.4. *Photoperiodic induction as a non-correlative phenomenon*

In the foregoing it appeared that the effect of photoperiodic induction is irreversible and strictly localized. There is no doubt that the induced state comes into being in the blade under the influence of SD. The possibility remains, however, that other organs besides the leaf are concerned with the origin of the induced state. LONA (91), BOCCHI *et al.* (9) and the present author (159) presented evidence that in *Perilla* no actively growing buds are necessary for photoperiodic induction. The same has been noticed incidentally in exp. 15 (p. 32). More evidence is presented in the following experiment.

Experiment 20. – The 7th pair of leaves, just fully expanded, on a homogeneous group of plants, was treated as follows: one leaf of each pair was detached by cutting through the petiole whereas the remaining leaves were detached together with pieces of stem which were about 5 cm in length. In the latter group all axillary buds were removed carefully by excavating the axils with a razor blade. After formation of roots in a mixture of sand and peat, leaves were potted and exposed to SD treatments of different duration as indicated in table 18. After terminating this treatment (for all groups at the same date) leaves were grafted onto LD stocks. The results are given in table 18.

TABLE 18. *Experiment 20.* Effect of photoperiodic treatments of different duration applied to leaves on disbudded cuttings or to detached leaves. Cuttings and leaves were rooted in a mixture of sand and peat, and afterwards grown in pots. Data after 48 days

Number of SD applied	Leaves on cuttings			Detached leaves		
	Number of stocks		Mean number of days to flower buds	Number of stocks		Mean number of days to flower buds
	Generative	Vegetative		Generative	Vegetative	
0	0	5	–	0	5	–
14	0	6	–	0	6	–
21	2	4	42	3	3	33
28	6	0	25	5	1	25
35	5	0	21	6	0	21

It follows once more that no actively growing buds were necessary for photoperiodic induction and the same can be concluded with regard to pieces of stem attached to leaves. For obtaining any flowering response at least 21 SD were necessary whereas in exp. 6 (p. 18) with leaves attached to plants 14 SD were sufficient for a 100 % response. However, photoperiodic treatment in the present experiment was performed under less favourable conditions than in exp. 6. Although no experiments were done with comparable leaves under equal conditions the general impression was gained that photoperiodic induction in

detached leaves takes place at a somewhat slower rate than in those attached to plants.

From this experiment it is evident that detached and afterwards rooted leaves can be induced, but the possibility remains that *roots* play a rôle in the process of photoperiodic induction. In order to investigate this it is necessary comparing leaves with roots and those deprived of all roots. In *Perilla* roots are formed especially at the bases of stems and petioles so that small pieces of stem or petiole could be removed regularly. As the inductive treatment, however, had to be continued for at least 4 weeks it was an extremely difficult task keeping petioles or stems completely free of root primordia. Although results obtained did not indicate any effect of roots in the process of photoperiodic induction, a better method, described in the next experiment, was designed which enabled us to grow leaves absolutely free of any roots for at least one month.

Experiment 21. – Old plants grown in LD were defoliated to one pair of leaves which had just got fully expanded. All axillary buds were removed carefully. Stems were cut off at soil-level so that cuttings were obtained with one pair of leaves, and stems of 40 to 50 cm in length. Cuttings were placed in pots which contained a small amount of nutrient solution so that only the utmost ends of the stems (about 1 cm in length) were wetted. In groups of control cuttings roots were only formed at the wetted bases. In the experimental groups bases about 2 cm in length were removed every other day. In this way cuttings remained free of roots during the whole treatment of 31 days. Results obtained after grafting onto LD stocks are shown in table 19.

TABLE 19. *Experiment 21.* Flowering response of LD stocks after grafting with 30 cm^3 from leaves which were induced on disbudded cuttings. Cuttings were grown with or without roots on diluted KNOP's nutrient solution + boric acid

Number of SD applied to cuttings	Cutting + or – roots	Number of grafts	Number of stocks		Mean number of days to flower buds
			Generative	Vegetative	
0	+	12	0	12	–
0	–	11	0	11	–
31	+	12	12	0	21
31	–	10	10	0	21

It will be clear from this table that presence or absence of roots on cuttings was of no importance for reaching the induced state. Repetitions of this experiment always gave similar results.

From this and the previous experiment it is obvious to conclude that *the process of photoperiodic induction in Perilla does not depend upon any correlative phenomenon.* The only condition to be satisfied for reaching the induced state is that a leaf blade, whether connected with other organs or not, is exposed to the adequate daylength.

4.5. *Photoperiodic induction as an indestructible phenomenon*

In order to get any idea about the nature of the induced state brought about by SD, various attempts were made to destroy it.

Light intensity. – From experiments described in section 4.1 it appeared that daylight supplemented with light from incandescent lamps did not nullify

the effect of SD treatment. Exactly the same result was obtained when induced leaves were grafted onto vegetative stocks and exposed to continuous high- or low-intensity light in the equipment described on p. 7.

Auxin. – In view of the flower-inhibiting influence of auxins in many SDP (*cf.* 128) some experiments were done with the potassium salt of naphthalene acetic acid (NAA). Soaking induced leaves in 10^{-5}, 10^{-4}, or 10^{-3} M solutions of NAA during 2 hours prior to grafting turned out to have no influence on flowering response of stocks. Another experiment was designed in such a way that NAA was applied regularly *during* transmission of the floral stimulus from leaves to stocks. This is described in the next experiment.

Experiment 22. – Induced leaves were grafted onto vegetative stocks and sprayed daily with 0, 10^{-7}, 10^{-5}, or 10^{-3} M solutions of NAA during the first 25 days after grafting. Graft unions were established normally except in the group sprayed with a 10^{-3} M solution. Polyethylene bags on stocks of this group were not removed for good until 20 days after grafting, but still all donor leaves wilted and subsequently died. Moreover, receptor buds on these stocks did not develop due to re-establishment of apical dominance by applied auxin, so that this group had to be discarded. Axillary buds on stocks sprayed with 10^{-5} M solution of NAA developed somewhat more slowly than those on stocks sprayed with a concentration of 0 or 10^{-7} M. Table 20 gives the results.

TABLE 20. *Experiment 22.* Effect of daily application of naphthalene acetic acid (NAA) to donor leaves on flowering response of stocks. Donor leaves were exposed to 40 SD

Concentration of applied NAA, molar	Number of stocks		Mean number of days to flower buds
	Generative	Vegetative	
0	10	0	25
10^{-7}	10	0	25
10^{-5}	10	0	29
10^{-3}	– [1])	–	–

[1]) No successful grafts.

A concentration of 10^{-5} M solution of NAA caused delayed appearance of flower buds for 4 days but this must be ascribed to the somewhat slower rate of development of receptor shoots, just as was observed for shoots developing under the influence of low-intensity light in exp. 9 (p. 20). So, the flower-inhibiting effect of auxin was only indirect and not due to partial nullification of the induced state. This conclusion is in sharp contrast to results obtained by SALISBURY and BONNER (128, 129, 130) in *Xanthium*. They showed that flowering was inhibited only if auxin was applied to leaves before translocation of the floral stimulus to receptor buds was complete. It was suggested that auxin brought about a destruction of the floral stimulus in the leaves. In *Perilla* translocation of the floral stimulus *via* a graft union does not occur in measurable amounts during the first week after grafting (compare section 3, p. 22). Although in the above described experiment spraying was done daily since grafting, so before any translocation of the floral stimulus from leaves could have taken place, no direct effect on flowering response was observed. This indicates that the auxin concentrations tested did affect neither the induced state nor production and transmission of the floral stimulus.

High temperature. – Some experiments were performed to test the effect of a short period of high temperature before induced leaves were grafted. A representative experiment is described below.

Experiment 23. – Cuttings with one pair of leaves were prepared from induced plants and set in pots with pre-warmed water in thermostats which were kept at constant temperature of 42° or 45 °C. At the end of a 5-hour treatment only some leaves at 45 °C showed small regions along the margin which had been killed; for the rest there were no visible signs of damage. Treated leaves were reduced to an area of 30 cm^2 and grafted onto vegetative stocks. Table 21 shows the results obtained.

TABLE 21. *Experiment 23.* Effect of heat treatment on the induced state in *Perilla* leaves. Donor leaves were exposed to 45 SD

Heat treatment	Number of leaves grafted	Number of surviving leaves	Number of stocks		Mean number of days to flower buds
			Generative	Vegetative	
Control	10	10	10	0	18
5 hours at 42 °C	12	12	12	0	18
5 hours at 45 °C	15	10	10	0	21

It is clear that a 5-hour treatment of donor leaves at 42 °C did not affect flowering response of stocks. Leaves exposed to 45 °C behaved as follows: five died shortly after grafting, the stocks concerned remaining vegetative. Five other leaves showed signs of damage, but nevertheless all stocks became reproductive, although appearance of flower buds was somewhat retarded. The remaining 5 leaves looked normal and behaved as if they had not been exposed to 45 °C. Similar results were obtained in several experiments. It appeared that 45 °C is about the maximum temperature which *Perilla* leaves can stand. But in all experiments leaves which survived heat treatment always induced flowering in LD stocks. Apparently *the induced state in Perilla leaves is completely heat stable.* Again this finding is not in agreement with results obtained with other plants. *E.g.* SACHS (127) experimenting with the LSDP *Cestrum nocturnum* established that the effect of LD treatment was not translocated from the leaves, but remained localized quite analogous to the effect of SD treatment in *Perilla.* In contrast to the latter plant, however, SACHS found that in *Cestrum* the effect of LD was *heat labile:* a 2-hour exposure to 42 °C decreased floral initiation to 18 % of that of control plants. The final floral stimulus which in the case of *Cestrum* is only produced in SD in leaves previously exposed to LD was found to be heat labile as well.

Enzyme inhibitors. – The substances 2,4-dinitrophenol (DNP) and sodium azide (NaN_3) are known as inhibitors of certain enzymatic processes. Therefore, their effect on the induced state in *Perilla* leaves was investigated. Application of substances to induced leaves was done by putting cut stems in solutions of concentrations required. During uptake transpiration was promoted by exposing leaves to bright light. At the end of a 5-hour treatment leaves were grafted onto stocks in LD. For both substances tested concentrations of around 10^{-3} M or higher had a harmful effect so that most leaves died quickly after grafting. However, all leaves surviving a treatment with DNP or NaN_3 always induced flowering in LD stocks.

The results obtained in this section can be summarized very briefly as follows: as far as appears from the factors and substances investigated *the induced state in Perilla leaves is completely indestructible*; it can be lost only by killing the leaves.

5. Translocation of the floral stimulus

In the experiments described in the preceding sections single leaves were grafted and their effect was traced on the receptor shoots of the stocks. So, translocation of the floral stimulus out of the donor leaves *via* graft unions to shoots was a *conditio sine qua non*. However, translocation of the floral stimulus as such was not studied. This question will be dealt with in the experiments to be described in the following sections.

5.1. *Single grafts*

Experiment 24. – Leaves (area 30 cm^2) which had been subjected to 36 SD were grafted in the usual way onto vegetative stocks above the 5th node. In one group of grafted plants receptor shoots were located on the 5th node, but in other groups shoots were allowed to develop on the 3rd node, so that the floral stimulus had to move over a distance of two internodes. There was no delay in appearance of flower buds on shoots of the 3rd node as compared with those on the 5th node, indicating that the floral stimulus was translocated very rapidly through the stem. In order to study this translocation, various treatments were applied to the piece of stem situated between the donor leaf and the receptor shoots on the 3rd node, when 4 days had elapsed since grafting.

Steam treatment. – A jet of steam was applied around the stem over a length of approximately 2 cm during 1 to 2 minutes. This resulted in a marked shrivelling of the stem so that the upper part with the donor leaf had to be supported. Although donor leaves kept in polyethylene bags remained in a good condition for 3 to 4 weeks, no flower buds were observed on the receptor shoots. Apparently the floral stimulus was not translocated through the dead piece of stem. This conclusion is in agreement with similar results obtained by other investigators when petioles of induced leaves were killed with steam (39, 155) or when pieces of stem between donor leaves and receptor buds were killed by electrically heating a constantan wire looped around the stems (63).

Girdling. – As stems had not yet become very woody, girdling was performed by carefully scraping off the bark up to the xylem over a length of 2 cm immediately above the 3rd node. The results obtained clearly indicated that the floral stimulus could not pass the girdled piece of stem: 8 out of a total of 10 stocks remained vegetative, 2 showed flower buds only 1 day later than non-girdled ones. However, when these latter 2 stocks were examined afterwards, it appeared that in both a very thin strand of bark had remained undisturbed, thus keeping up a phloem connection. Evidently these thin strands of phloem were sufficient for transmission of the floral stimulus.

Removal of half a ring of bark. – Bark was removed as described above from one half of the stem just above a receptor shoot. Appearance of flower buds was recorded for each shoot separately. From the data thus obtained it appeared that flower buds on the shoots below the girdled half became visible 3 to 4 days later than those on the shoots located on the opposite side of the stem below the non-girdled half. Further development of flower buds below the

girdled half was delayed as well. From these results it must be concluded that the floral stimulus was not only translocated in lengthwise direction in the bark but also in lateral direction. These and the above reported observations fully confirm CAJLACHJAN's (16) results obtained with girdled *Perilla* plants.

A ring of 2, 3, 5-triiodobenzoic acid (TIBA). – According to NIEDERGANG-KAMIEN and SKOOG (107) TIBA applied in lanolin as a ring around the stem would stop polar transport of auxin. In the present experiment the effect of TIBA on the transmission of the floral stimulus was investigated. TIBA was applied as a 1 % lanolin paste to the stem which was wounded superficially in order to promote uptake of TIBA. Although leaves on the receptor shoots had become somewhat deformed by TIBA, appearance of flower buds was not retarded, indicating that applied TIBA did not interfere with transmission of the floral stimulus.

In grafts with single leaves as described in the previous experiments transmission of the floral stimulus could be studied only in downward direction. In order to be able to investigate *translocation both in downward and upward direction* grafts with *shoots* being either donor or receptor, had to be made. It soon became evident with these grafts that in certain combinations LD leaves on receptors exerted a marked inhibitory influence on the transmission of the floral stimulus. A representative experiment clearly demonstrating this flower-inhibiting effect is described below.

Experiment 25. – A group of plants was subjected to 28 SD whereas comparable plants remained in LD. At the end of SD treatment when the 6th pairs of leaves had become half-expanded all axillary buds and shoots were removed whereafter two graft-combinations were performed between induced and non-induced plants, *viz.* LD/SD and SD/LD. These combinations have been diagrammed in fig. 10.

From this figure it will be clear that stocks which functioned as donor retained only the 4th and 5th pairs of leaves. The scions originated from plants in LD and consisted of pieces of stem with the 5th pairs of leaves. Secondary buds in the leaf axils on the 5th node were allowed to develop as receptor branches.

In the case scions had to function as donor, shoots from induced plants with the 5th and 6th pairs of leaves were grafted onto LD stocks above the 4th node. Secondary buds on that node functioned as receptor branches.

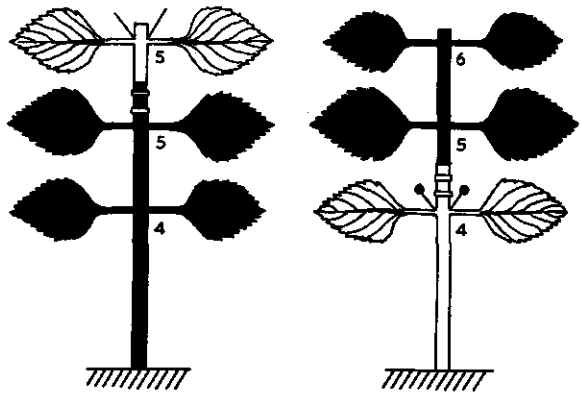

FIG. 10.
Experiment 25. Diagrams of graft-combinations LD+/SD+ (left), and SD+/LD+ (right).
Figures at nodes refer to node numbers before grafting.
White: non-induced.
Black: induced during 28 SD.

As controls similar graft-combinations were made except that "donor" shoots were derived from plants in LD.

Both cleft- and splice-grafting were applied. When 20 days had elapsed since grafting, in half the number of plants of each combination the pairs of LD leaves were removed so that 4 different groups were obtained as listed in table 22. In all groups receptor *branches* were defoliated continuously until flower buds became visible macroscopically. Table 22 gives the results.

TABLE 22. *Experiment 25.* Upward and downward movement of the floral stimulus as influenced by presence or absence of one pair of leaves on receptors. Donors received 28 SD prior to grafting. Scions were cleft- or splice-grafted. LD leaves were removed after 20 days. Per treatment 10 plants

Graft-combination	Mean number of days to flower buds	
	Cleft-grafting	Splice-grafting
LD+/SD+	99 ± 5.1	111 ± 2.9
LD−/SD+	47 ± 0.9	46 ± 1.0
SD+/LD+	32 ± 1.3	29 ± 0.5 [1])
SD+/LD−	31 ± 0.8	29 ± 0.9

[1]) Only 9 plants.

It is obvious that in all groups cleft- and splice-grafting yielded similar results.

When stocks functioned as receptor, flower buds appeared almost simultaneously in all groups indicating that the LD leaves did not interfere with transmission of the floral stimulus in downward direction. In the reciprocal combination, however, pairs of LD leaves remaining on the scions greatly retarded formation of flower buds. This will be clear if one compares the results obtained in the combinations LD+/SD+ and LD−/SD+. In the former combination it took more than twice as many days until all scions showed flower buds. It should be added that on the whole flower buds did not appear on scions in the combination LD+/SD+ until LD leaves showed clear symptoms of senescence; the longer these leaves remained in a good condition the longer flowering was postponed.

From the results obtained with LD−/SD+ and SD+/LD− it looks at first sight that there is a strong preference for translocation of the floral stimulus in downward direction. However, this statement can hardly be maintained if the following facts are taken into consideration. It is probable that in the combination LD+/SD+ leaves on the receptor inhibited transmission of the floral stimulus almost completely during the first time after grafting (compare also exp. 27, p. 44). In LD−/SD+ the inhibiting leaves were removed 20 days after grafting, so that reckoned from the day of excision it took 27 days until flower buds appeared. In SD+/LD+ leaves on the stock did not inhibit transmission of the floral stimulus, so that transport can be assumed to have begun as soon as a phloem connection was established, suppose about 10 days after grafting. So, we arrive at 21 days for downward transmission *versus* 27 days for upward movement.

All receptor branches in control grafts *viz.* in LD+/LD+, LD−/LD+, LD+/LD+, and LD+/LD− were vegetative after 129 days when the experiment was discontinued.

The results obtained in this experiment show that a pair of LD leaves can inhibit transmission of the floral stimulus only in acropetal direction. This

flower-inhibiting effect of leaves on receptor-scions was studied more quantitatively in the following two experiments.

Experiment 26. – The first two pairs of leaves as well as all axillary buds were removed from a group of plants which had been exposed to 29 SD. Shoots with the 5th pairs of leaves from plants in LD were grafted onto the induced plants above the 5th nodes. On these scions secondary buds functioned as receptor branches. After 20 days the area of the pairs of LD leaves was reduced to 0, 2 × 15, or 2 × 40 cm^2 for different groups. In another group LD leaves were fully retained. Table 23 gives the results.

TABLE 23. *Experiment 26.* Upward movement of the floral stimulus in the graft-combination LD+/SD+ as influenced by various leaf areas on the receptors. Donors received 29 SD prior to grafting. Leaf blades were reduced to the required area 20 days after grafting. Data 90 days after grafting

Leaf area on LD scions	Number of scions		Mean number of days to flower buds
	Generative	Vegetative	
0 cm^2	10	0	40 ± 0.6
2 × 15 cm^2	10	0	47 ± 1.2
2 × 40 cm^2	10	0	77 ± 3.1
2 complete leaves	0	10	>90

It is clear that a close correlation exists between leaf area present on scions and number of days until flower buds became visible. After 90 days when the experiment was discontinued all 10 scions which had retained the pair of LD leaves, were vegetative. At that time the LD leaves were still in a good condition but most leaves on stocks had abscised.

Experiment 27. – Again groups of induced and non-induced plants were grafted in the combination LD+/SD+. Donor plants had received 36 SD; LD scions were grafted above the 6th nodes of the stocks. LD leaves on scions were excised from different groups with intervals of 15 days beginning on the 15th day after grafting.

LD leaves removed from scions after 30 days were grafted onto LD stocks. None of the stocks initiated flower buds. This observation once more illustrates that the induced state remained strictly localized. Thus, plants of the graft-combination LD+/SD+ are another example of *physiological chimeras*; compare p. 36.

The results are given in table 24.

TABLE 24. *Experiment 27.* Upward movement of the floral stimulus in the graft-combination LD+/SD+ as influenced by time of removal of LD leaves on receptor. Stocks received 36 SD prior to grafting. Data 75 days after grafting

Period between grafting and removal of LD leaves on scion in days	Number of scions		Mean number of days to flower buds	
	Generative	Vegetative	Since grafting	Since removal of LD leaves
15	10	0	36 ± 1.0	21 ± 1.0
30	10	0	53 ± 0.6	23 ± 0.6
45	10	0	65 ± 0.7	20 ± 0.7
∞	5	5	70 ± 3.5 [1])	.....

[1]) Only for 5 generative scions.

It is clear that the time since removal of LD leaves until appearance of flower buds remained constant. Apparently the leaves on receptors inhibited transmission of the floral stimulus completely at least during the first 45 days after grafting. In the group with non-defoliated scions leaves on some scions began to die after about 60 days. These naturally defoliated scions promptly initiated flower buds. However, when leaves had remained in a good condition scions were vegetative after 75 days.

Stocks which functioned as donor in the preceding experiments always retained some leaves. So, it seems logical to suppose that the floral stimulus which induced LD scions to flower, was supplied by these leaves. The next experiment was designed to find out whether besides the leaves stems could as well generate the floral stimulus.

Experiment 28. – A group of plants was exposed to 36 SD. These plants were grafted with LD shoots (4th node with one pair of leaves) above the 5th node. All leaves and shoots were removed from 10 stocks immediately before grafting. For the remaining stocks this was done 9 days later. The pairs of LD leaves on scions were severed after 16 days. Moreover, the first pair of leaves on each receptor shoot was removed as soon as it had become fully expanded. The results can be stated very briefly: all scions of both groups except one which had not united well with the stock, had initiated flower buds after 31 days. There was no difference between the two groups. This result indicates that induced stocks which were defoliated before grafting, could induce LD scions to flower, *i.e.* they could supply the floral stimulus without presence of leaves. None of the control grafts (LD–/LD–) initiated flower buds.

A similar experiment was performed by MOSHKOV (104), but he obtained no flowering on LD scions when induced stocks were defoliated before grafting. However, MOSHKOV's plants received a suboptimal SD treatment (15 SD) in contrast to those in the present experiment who did get an optimal inductive treatment (36 SD). Probably this difference explains the conflicting results. MOSHKOV concluded from his results that the floral stimulus did not accumulate in stems. It seems somewhat premature to conclude the converse from our results, for it has been found by SELIM (132) and confirmed by the present author that old *Perilla* plants stripped of all leaves and shoots except the terminal bud will initiate flower buds in SD in the same time as non-defoliated plants, whereas controls in LD remain vegetative. Thus, in old plants the inductive daylength can be perceived by the stems. In view of these observations it is not necessary to assume that in the present experiment a certain amount of floral stimulus had been accumulated in the stems which, after grafting, was transmitted to the scions. It is conceivable that the stems having reached the induced state, continued to produce the floral stimulus under LD conditions just as induced leaves do (compare section 4.1, p. 30).

Two-branched plants have often been used for demonstrating the transmission of the photoperiodic stimulus from an induced donor branch to a non-induced receptor branch of the same plant; see literature cited in (133), and further (12, 53, 60, 88). A similar experiment with a slightly modified technique is described below.

Experiment 29. – Plants in LD were pinched above the 4th node so that two opposite branches developed on that node. One of these branches on each plant was grafted with an induced shoot. These shoots retained 3 pairs of leaves

and originated from plants which had been subjected to 27 SD. The non-grafted branches and the stems below the ramification were completely defoliated; secondary buds on all nodes were allowed to develop as receptor shoots. The results can be stated as follows: the buds firstly became visible on the grafted branch below the graft union and on the stem immediately below the ramification; not until several days later they appeared on the non-grafted branch and at the base of the stem. From these observations it appears that the floral stimulus moved from the induced scion to the other branch and downward to the base of the stem.

The results of the present experiment do not confirm those obtained by SELIM (133) with the same experimental plant; he failed in transmitting the photoperiodic stimulus from an induced to a non-induced branch and ascribed this to absence of direct anatomical connections between donor and receptor branches.

5.2. *Double grafts*

In the preceding section it appeared that LD leaves present on a scion as receptor strongly inhibit transmission of the floral stimulus in upward direction. The same phenomenon was observed in several experiments with double-worked plants in which the interstocks functioned as donor for both stocks and scions. A representative experiment is described below.

Experiment 30. – A homogeneous group of plants was subjected to 27 SD. At the end of SD treatment the 6th pairs of leaves had just got fully expanded. Plants of the same sowing which had remained in LD, functioned as receptor plants. They were pinched above the 6th node and the first 4 pairs of leaves were excised so that only the 5th and 6th pairs of leaves were retained. The internode between these two pairs of leaves was cut half-way transversely and a piece of stem with a pair of induced leaves was intergrafted, so that double-worked plants were obtained as shown diagrammatically in fig. 11.

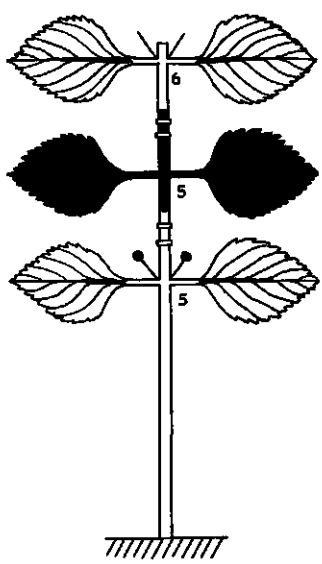

FIG. 11. *Experiment 30.* Diagram of graft-combination LD+/SD+/LD+.
Figures at nodes refer to node numbers before grafting.
White: non-induced.
Black: induced during 27 SD.

Both grafts were made at the same time. They all took for 100 %. Secondary buds on stocks and scions developed into receptor branches. After 21 days the plants were divided into 4 equal groups of 10 plants. The pairs of LD leaves were not removed, or removed from either stocks or scions, or from both, so that the experiment involved the 4 combinations which are listed in table 25.

TABLE 25. *Experiment 30.* Induced interstocks as donor for stocks and scions in double-worked plants. Donors received 27 SD prior to grafting. LD leaves were removed after 21 days. Per treatment 10 plants. Data after 101 days

Graft-combination	Number of receptors		Mean number of days to flower buds
	Generative	Vegetative	
LD+ / SD+ / LD+	3	7	97 [1])
LD+ / SD+ / LD—	10	0	53 ± 6.5
LD— / SD+ / LD+	1	9	97 [1])
LD— / SD+ / LD—	10	0	46 ± 1.9

[1]) Only for generative stocks.

These results once more illustrate the marked flower-inhibiting effect exerted by LD leaves present on scions; LD leaves on stocks, however, had no or only a slight inhibitory effect. Thus, in double-worked plants with interstocks as donor the floral stimulus is transmitted basipetally as well as acropetally. Only in the latter case LD leaves have a decided delaying effect.

5.3. *Double-stock and double-scion grafts*

Experiment 31. – Plants to be used as donor in the present experiment were subjected to 23 SD. At the end of SD treatment the following grafts were performed:

Double-stock grafts. – Two plants were put together in one big pot so that their 5th nodes were at the same level. An oblique cut was made through both stems above the 5th node in such a way that after bending plants together a wedge-shaped scion fitted in nicely. The two stems with the scion between them were bound together tightly with raffia. All three possible combinations of SD and LD stocks were made, *viz.* 2 LD, 1 LD and 1 SD, and 2 SD stocks. The scions originated from plants in LD and were allowed to develop 2 receptor branches, one above each stock. These grafts, with the results obtained, are shown in the combinations 1 to 3 in fig. 12, p. 48.

When both stocks were non-induced (combination 1) all receptor branches remained vegetative as could be expected. With both stocks induced (combination 3) flower buds appeared on both branches. In combination 2 (1 LD+

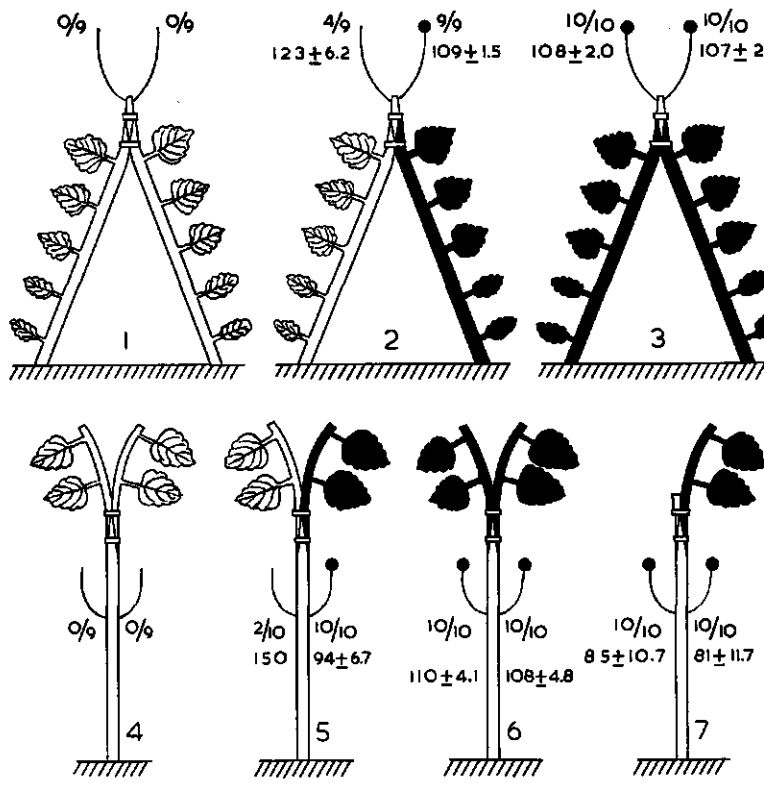

FIG. 12. *Experiment 31.* Diagrams 1 to 3 representing double-stock and 4 to 7 double-scion grafts.
White: non-induced; black: induced during 23 SD.
(In order to avoid overcrowding the figure one leaf of each pair has been omitted). Numerator of each fraction indicates total number of plants per treatment, denominator number of generative receptor branches. Figures below fractions represent mean number of days until appearance of flower buds. Data after 156 days.

1 SD) floral initiation occurred on all branches *above* the induced stock, but above the LD stock 5 out of 9 receptors were still vegetative after 156 days. Thus it seems as if transmission of the floral stimulus took place mainly unilaterally from SD stocks to the branches above these stocks.

Double-scion grafts. – In these grafts one LD stock functioned as receptor of two donor-scions. Stems of stocks were cut obliquely above both opposite leaves on the 4th node so that a wedge was obtained the point of which directed upwards. On both cut surfaces of the stock an obliquely cut donor shoot was placed. Both scions with the piece of stock between them were bound together with raffia. The three possible combinations of LD and SD scions were performed as shown in diagrams 4, 5, and 6 in fig. 12. Diagram 7 represents a stock which was grafted unilaterally with one shoot; on the other side only a piece of stem was placed on the cut surface. On each stock two branches developed as receptor, one below each scion. The results have been marked in the diagrams 4 to 7 in fig. 12. From the flowering response in combination 5,

1 LD + 1 SD scion as donor, it follows that translocation of the floral stimulus had mainly taken place unilaterally as was noticed already for the comparable double-stock graft (see above). However, from the results obtained in combination 7, with only one unilaterally grafted scion, we can learn that translocation in lateral direction was fundamentally possible; it had not even been slackened in comparison with downward transport as measured by the numbers of days until appearance of flower buds on both shoots. This throws light upon the results obtained in the combinations 2 and 5: failure of flowering on receptor branches above or below non-induced "donors" cannot be ascribed to difficulties in transmission in lateral direction. The reason why these shoots did not initiate flower buds seems to be solely that their growing-points were under the immediate control of LD leaves so that the floral stimulus could not reach them.

5.4. *Discussion*

The data presented in the previous 3 sections provide further evidence for the conclusion reached already in section 3.4 (p. 27), *viz.* that the floral stimulus is translocated in the phloem in lengthwise direction. Moreover, transmission in lateral direction has been observed after removal of half a ring of bark (p. 41), and in double-stock grafts, double-scion grafts, and unilateral grafts (diagrams 2, 5, and 7 respectively in fig. 12).

Of course one may wonder which pathway is followed by the floral stimulus after removal of half a ring of bark. In this respect ESCHRICH's (36) interesting experiments on movement of fluorescein in one severed vascular bundle in a stem of *Impatiens* offer a clear model. This investigator observed that the dye moved out of the severed bundle into the parenchyma sideways towards intact bundles. However, when about 8 days after severance of the bundle the ends were connected through regenerated sieve tubes, the dye became again restricted to the phloem elements.

With the combinations LD±/SD+, SD+/LD±, and LD±/SD+/LD± it appeared that translocation in downward direction could always take place. However, LD leaves on receptor-scions could almost completely prevent floral initiation. The interpretation of this phenomenon is clear: if LD leaves were present on the scions, phloem translocation was mainly directed basipetally, so that the receptor branches on scions were fully dominated by these leaves. If a food deficit was created on the scions by removing the LD leaves, translocation of assimilates together with the floral stimulus from stocks or interstocks in upward direction took place resulting in subsequent floral initiation.

The inhibiting effect of LD leaves on transmission of the floral stimulus from a donor-stock to a receptor-scion has been noticed already by the first experimenters on this subject (*e.g.* 16, 20, 71, 104). According to MOSHKOV (104) only fully expanded leaves of *Perilla* exert this flower-inhibiting effect; darkening these leaves had the same effect as removing them. CAJLACHJAN (cited in 23; see also 73, p. 283) found that the flower-inhibiting effect of non-induced leaves located more acropetally than the induced leaves, could be simulated by cutting off the leaf blades and applying sugar solution to the petioles.

There is still another condition which must be fulfilled in order to get a successful transmission of the floral stimulus from donor to receptor; on the former all shoots and growing-points must be removed (*e.g.* 4, 50, 104). This was remarked already by SACHS (124) as early as 1865. He stated that flower

buds on a darkened top of *Tropaeolum majus* as "Recipient" (receptor) were initiated only, if from the basal part in light all fruits, flowers and axillary shoots were removed. Probably this phenomenon can likewise be interpreted in terms of translocation and distribution of assimilates: if all growing-points and shoots are removed from the donor, transport of assimilates with the floral stimulus towards the growing receptor branches will easily take place. However, if no shoots, fruits or flowers are removed, the main stream of assimilates will be directed towards these carbohydrate-consuming sites and no or insufficient amounts of floral stimulus will reach the receptor.

6. GENERAL DISCUSSION ON *PERILLA*

In section 4.1 (p. 30) clear-cut evidence was presented that production of the floral stimulus in leaves which were exposed to SD continues unhampered when *Perilla* plants are transferred from SD to LD. This upsets CAJLACHJAN's original idea that the "florigen" is only produced in the inductive daylength and becomes exhausted in the non-inductive daylength afterwards (*cf.* also 158). In graft-combinations between induced and non-induced partners the induced and non-induced state coexist side by side which gives rise to "physiological chimeras" (p. 36). The interaction between both partners might be conceived as follows: the induced partner produces the floral stimulus which is translocated to the receptor shoots along the same pathway as the assimilates; we shall call this the reproductive stream. Likewise leaves on the receptor will produce a vegetative stream. The former can induce differentiation of floral primordia, the latter leaf primordia. The development of receptor shoots reached by both streams will be determined by the dominating partner.

On p. 28 we established that a quantitative relationship exists between the amount of stimulus received by the shoots and the number of subsequently produced flower buds. This, combined with the observation in exp. 6 (p. 18) that flowering response on LD stocks increases with increasing duration of SD treatment permits to conclude that the capacity to produce the floral stimulus will increase with increasing duration of inductive treatment until after a certain number of SD the maximal productive capacity has been reached. It appears from the results in section 1 (p. 9) that the building-up of this capacity takes place at different rates in differently located leaves: the higher the leaves are located, the more rapid this will occur which is valid at least for the first 5 pairs of leaves. This building-up can be stopped at any given moment by moving the plants back to LD, resulting in sub-optimally induced plants.

The floral stimulus can be transmitted both in acropetal and basipetal direction. If special conditions are fulfilled transmission can be demonstrated in lateral direction as well. Movement of the floral stimulus can take place only in living tissue (p. 41). The experiments with labelled sucrose (p. 26) provide direct evidence that the tissue involved is the phloem. All observations support the viewpoint that the translocation of the floral stimulus is very closely correlated to or probably inseparably connected with transport of assimilates from donor leaves to receptor shoots. This conclusion does not disagree with the observation (exp. 10, p. 21) that leaves treated with SD and continuously darkened after grafting can induce LD stocks to flower, because several investigators of phloem transport (27, p. 220; 131, 154) have established a food export from mature, darkened leaves.

Once the floral stimulus has reached the axillary buds it exerts its morphogenetic effect which is observed afterwards as differentiation of floral primordia. If shoots are no longer defoliated after appearance of flower buds, the buds in the axils of bracteal leaves on 12 to 15 nodes may become reproductive, but finally normal leaves will appear and the buds in their axils will be vegetative. Apparently the terminal growing-point does not differentiate into a floral primordium. A similar phenomenon occurs in intact plants which have been subjected to a limited number of SD. It seems probable that all cells being mature during SD treatment acquire the induced state. Only meristematic cells will retain the non-induced state, so that newly formed organs at the top of the stem will acquire the non-induced state as well. In course of time the influence exerted on the terminal growing-point by lower located leaves will diminish and instead of bracts with flower buds normal leaves with vegetative axillary buds will be differentiated. Only if SD treatment is continued for a very long period plants will not revert to vegetative growth. Most probably this is due to the fact that the terminal growing-point has died or become inactive.

Reviewing the results gathered in our experiments with *Perilla* as a whole we shall have to discuss the controversy flower hormone or flower inhibition as stated on p. 5.

Firstly, it should be noticed that in all experiments control grafts were performed with non-induced leaves or shoots as "donor". No flowering was ever observed on receptor branches of these control grafts, although they were regularly defoliated. So, defoliation of receptors was in itself not sufficient for obtaining a flowering response under LD conditions. On the other hand it appeared in exp. 25 and 30 (pp. 42 and 46) that stocks as receptor need not be defoliated in order to get such a response. Shoots incidentally arising on these stocks below the LD leaves always initiated flower buds. The same phenomenon has been observed in grafts with single leaves when shoots developed on the 1st node, *i.e.* below the pair of LD leaves located on the 2nd node. In several experiments (*e.g.* exp. 11, p. 22) shoots developing from secondary buds were not defoliated, but nevertheless they could flower abundantly. Immediately after grafting these buds are very small indeed, so that they can be fully controlled by the donor leaves. This is not the case, however, when primary buds grow out to receptor shoots. Yet they were induced to flower by donor leaves. All these observations indicate that *flowering on receptor shoots cannot simply be explained in terms of removal of flower-inhibiting leaves.* Moreover, completely defoliated plants remain vegetative in LD, but flower in SD.

If in the graft-combination LD+/SD+ non-induced leaves on the receptors should produce a flower-inhibiting factor (*cf.* p. 5) one would expect that the longer these leaves are retained, the more time will elapse from defoliation of receptors until appearance of flower buds. But it appeared in exp. 27 (p. 44) that this period remained constant. So, it can be concluded that the LD leaves prevented only the floral stimulus from reaching the receptor shoots without producing flower-inhibiting substances themselves.

Flowering on LD stocks increased with increasing duration of contact period between donor and receptor (exp. 11, p. 22), so that there can be no other explanation than that "something" was transmitted from the donor leaves to the growing-points on the receptor shoots. (In this connection BENNETT's investigations on virus transmission *via* a graft union (see fig. 7, p. 30)

furnish a clear example). Whether this "something" provides the growing-points with the factor(s) which is (are) absolutely limiting for the formation of flower buds or that it has to neutralize a flower-inhibiting effect present in the *growing-points* remains undecided. Only on grounds of plausibility we might have a preference for the former possibility.

The statement that "something" is transmitted from donor leaves to receptor shoots does not mean that we accept without further evidence the existence of one single substance with which we can induce flowering in a *Perilla* plant at will whereas the same plant will remain absolutely vegetative in the absence of the substance in question.

It is obvious that grafting-experiments cannot furnish the decisive proof for the existence of one single flower-inducing substance: transmission of the floral stimulus is always so closely correlated with the possibility of phloem transport from donor to receptor that it is quite well possible that the floral stimulus is merely a definite combination of nutrients which – in quantitative as well as qualitative respect – might be different from the combination supplied by non-induced leaves.

Therefore, for the present we can conclude no more than that SD treatment induces an alteration in the metabolism of the leaves (the induced state) which results in the production of the floral stimulus even when the leaves are subjected to LD afterwards. The results presented in section 4.5 (p. 38) demonstrate that the induced state is an integrant element of the protoplasm which cannot be lost without killing the leaves.

The findings that induced leaves of a very small size (exp. 7 and 8, p. 19) and induced, completely darkened leaves (exp. 10, p. 21) could successfully function as donors might be arguments in favour of a single substance. On the other hand it was observed that indirectly induced shoots did not function as donor (exp. 18, p. 34). When these shoots were pinched and deprived of all flower buds, newly developing secondary buds which were probably in a dormant state at pinching, always remained vegetative. This means that the floral stimulus did not exert its influence on dormant buds. Moreover, this indicates that no floral stimulus remained stored until the buds became actively growing as would be expected when only nutrients would be involved in the flower-inducing effect.

It can be concluded that the present experiments have considerably increased our knowledge of the mechanism of photoperiodism in *Perilla*, although they have not revealed the nature of the floral stimulus. The controversy flower hormone or flower inhibition has been answered so far that it is clear now that induced leaves produce a flower-inducing stimulus. Non-induced leaves can interfere with transmission of this stimulus, but it seems improbable that they produce flower-inhibiting substances themselves.

CHAPTER IV

EXPERIMENTS WITH *XANTHIUM PENSYLVANICUM*

As appeared in the preceding chapter one of the most prominent features regarding photoperiodic induction in *Perilla* is that the induced state remains strictly localized, so that shoots induced indirectly (*i.e.* by grafting in LD) do

not function as a donor. According to data in literature a quite different situation exists in *Xanthium*. LONA (86) induced one leaf on a *Xanthium* plant and established that young leaves located near the top which were continuously kept in LD, could, after grafting onto stocks in LD, induce flowering. BONNER and LIVERMAN (10, p. 292), quoting unpublished data, reported that indirectly induced shoots of *Xanthium* can function as donor. So, in this plant the floral stimulus seems to possess certain characteristics of a virus.

In view of the negative results with *Perilla* after grafting with indirectly induced shoots (exp. 18, p. 34) we have tried to confirm the above mentioned data for *Xanthium* under the same conditions as those prevailing during the experiments with *Perilla*.

Experiment 32. – A number of *Xanthium* plants was subjected to SD. After having received 14 inductive cycles the tops with 2 or 3 leaves were cut off and grafted onto vegetative stocks in LD (SD/LD). As a check non-induced shoots were grafted onto LD stocks (LD/LD). When after approximately 40 days flower buds had appeared on the stocks of the combination SD/LD the indirectly induced shoots were cut off and grafted onto a next group of LD stocks. As a control comparable vegetative shoots from the stocks in the combination LD/LD were grafted. The indirectly induced shoots caused flower formation on the stocks whereas the control shoots did not. Again the flowering shoots from the LD stocks were grafted, *etc.* In this way the floral stimulus could be transmitted *via* 4 successive grafts. Then the experiment was discontinued. There was no indication that the flowering response had diminished after the 4th grafting in comparison with the 1st one. Transmission did not always occur in 100 % of the plants tested; it was noticed that it mostly coincided with the presence of expanding leaves on the donor shoots. All control grafts remained strictly vegetative.

It has been claimed (23, 128) that actively growing buds are necessary for the *stabilization* of the floral stimulus in *Xanthium*. However, it is still uncertain whether any bud is necessary for the *production* of the floral stimulus *in rapidly expanding leaves*. The following experiment was carried out to elucidate this point.

Experiment 33. – *Xanthium* plants were completely defoliated except the half-expanded leaves. In one group all buds were removed carefully with the aid of a razor blade; in another group only the buds in the axils of the remaining leaves were retained. The plants received 10 SD whereas controls remained in LD for that period. Immediately after terminating the SD treatment the leaves were grafted with the petioles onto stocks in LD. They had become fully expanded by that time. The results of two separate experiments are joined together in table 26, p. 54.

It is clear that leaves induced either in connection with an axillary bud or without, could cause LD stocks to flower. Only a low percentage of the stocks was induced but this must be ascribed to the fact that *Xanthium* leaves often die shortly after grafting or do not take at all. It is impossible to keep grafted *Xanthium* leaves in a good condition for 2 or 3 months as can be done with *Perilla* leaves (p. 31).

Transmission of the floral stimulus from leaves, induced on disbudded plants, to vegetative stocks has been observed in several separate experiments. Thus, it can be concluded that production of the floral stimulus is possible in rapidly

TABLE 26. *Experiment 33.* Flowering response of LD *Xanthium* stocks as affected by presence or absence of buds on donor plants during an inductive period of 10 SD. Data 70 days after grafting

Treatment of leaves	Donor plants + or – buds	Flowering response of stocks		% generative stocks
		Generative	Vegetative	
LD	+	0	20	0
SD	+	10	10	50
SD	–	12	18	40

expanding leaves in the absence of buds. CARR (22) did not obtain a flowering response on *Xanthium* scions grafted onto plants which were disbudded before SD treatment. But this may be due to the fact that the leaves were *mature* when the treatment started. Moreover, he neglected to demonstrate that comparable stocks induced *with* buds could transmit the floral stimulus to the scions.

Our results further demonstrate that the floral stimulus in *Xanthium* is more stable than has been suggested by other investigators (23, 128; see also 82, pp. 186 and 187). According to our experience with *Xanthium* leaf-grafts it takes at least 10 days until a graft union has been established. It is obvious that a certain amount of floral stimulus present in the leaf blades has not lost its activity at the end of that period; otherwise it would be impossible to induce flowering in LD stocks by grafting single leaves. Another explanation may be that the SD treated leaves continue – just as induced *Perilla* leaves do – to produce the floral stimulus in LD (see below). SALISBURY (128) suggested that the floral stimulus had become dissipated 6 days after the inductive period. This is a much shorter time than follows from our results, but in our experiments the leaves received 10 SD. Leaves which had received only 1 SD did not cause flowering on LD stocks under our experimental conditions. So, besides a translocation of the floral stimulus from the leaves to the growing-points with the assimilates (*cf.* 23) it appears that *in the leaves* a certain accumulation of the effect of successive inductive cycles can take place.

It has been reported (21, 159) that detached *Xanthium* leaves cannot be induced. At first sight this does not agree with the above reported results that no buds are necessary for the production of the floral stimulus. CARR (21) used leaves "which had just attained maturity," *i.e.* leaves which are rather insensitive to SD treatment. We (159) subjected detached, half-expanded leaves to SD, but nevertheless the stocks remained vegetative after grafting with these leaves. However, it is very difficult to keep detached *Xanthium* leaves in a good condition for a long enough period; after detachment they expand hardly any further and the chlorophyll content decreases. It is probable that the change in metabolism (*cf.* 121), rather than the absence of buds, is the cause that no detectable amounts of floral stimulus are produced.

In fact we could establish in new experiments with a small number of detached leaves that they could induce flowering in LD stocks. These leaves were selected as the best ones from a much larger group; they had formed several roots on the petioles at the end of the treatment with 21 SD. This observation indicates that *production* of the floral stimulus is principally possible in detached *Xanthium* leaves. For further investigations regarding this point it may be useful to make

use of the finding (121) that kinetin is capable of extending the life-span of detached *Xanthium* leaves.

SALISBURY's statement (128) that "auxin can replace the requirement for active buds in the induction of *Xanthium*" is somewhat misleading. His results show only that the axillary buds resumed growth earlier after the application of auxin. According to the same author, LINCOLN has shown "that very young leaves can replace the requirement for actively growing buds". This is in full agreement with LONA's observations (86, already cited on p. 53) that very young leaves in LD can be induced by a SD treated leaf to *produce* the floral stimulus. So, if no buds are active as in LINCOLN's experiments, the very young leaves will acquire the induced state. These leaves in their turn will induce the buds to flower when the latter begin to grow actively.

From the above discussed results it can be concluded that *in Xanthium, unlike Perilla, the induced state is maintained in actively growing buds* (young leaves and growing-points). It is clear that the ability to produce the floral stimulus is not localized in the once induced leaves, but becomes operative in all young leaves expanding in LD afterwards.

It is still uncertain whether induced *Xanthium* leaves after full expansion retain (as *Perilla*) or lose their induced state upon transference to LD, although there are indications (66, 86, 128) which seem to support the latter alternative.

CHAPTER V

EXPERIMENTS WITH *CRASSULACEAE*

1. EXPERIMENTS WITH *KALANCHOË*

According to CARR and MELCHERS (24) the SDP *Kalanchoë blossfeldiana* can be induced to flower in LD by grafting with previously induced shoots. As the photoperiodic behaviour of this species is well-known owing to the extensive investigations by HARDER and coworkers (see *e.g.* 52), it was used in the present investigations.

Experiment 34. – In order to have an idea about the minimal size of induced scions required for obtaining a flowering response of defoliated *Kalanchoë* stocks, grafts were performed with the donors listed in table 27, p. 56. Donor-scions originated from plants which had received 42 SD. They were grafted onto defoliated stocks above the 4th or 5th node. Two continuously defoliated shoots on the stocks below the graft union functioned as receptors.

Young and old leaves were taken from the same donor plants, but the former were located 2 nodes higher than the latter. Single leaves were either cut off through the petioles or they were grafted attached to a piece of stem.

It appears from the results in table 27 that single leaves grafted with the petioles could not induce flowering in all stocks. It is remarkable that the percentage of generative stocks increased when pieces of stem were attached to the leaves. The number of flower buds on receptor shoots showed a great variation: on some stocks normal inflorescences with more than 300 flower buds developed; on other shoots, however, only some buds were initiated and maximal phyllody occurred (*cf.* 52).

With 1 pair of leaves as donor all stocks were induced, but with 3 pairs of

TABLE 27. *Experiment 34.* Effect of varying sizes of donor-scions on the flowering response of *Kalanchoë* receptor-stocks. Donors received 42 SD prior to grafting. Old leaf indicates: leaf expanded at beginning of SD treatment. Young leaf indicates: leaf less than half-expanded at beginning of SD treatment. Data 140 days after grafting

Donor	Number of stocks		Mean number of days to flower buds	Mean number of flower buds + flowers
	Generative	Vegetative		
1 old leaf . . .	9	10	57 ± 3.0	156 ± 32.8
1 young leaf . .	13	6	53 ± 2.8	138 ± 27.3
1 old leaf [1]) . .	18	1	53 ± 2.3	134 ± 22.9
1 young leaf [1]) .	15	4	47 ± 1.6	151 ± 34.9
1 pair of leaves .	8	0	45 ± 2.5	196 ± 32.1
3 pairs of leaves	10	0	42 ± 1.4	311 ± 63.4

[1]) + piece of stem.

leaves on the donors the stocks flowered more abundantly, indicating that the response increased with increasing leaf area on the donors.

Experiment 35. – This experiment was carried out to demonstrate that the induced state is retained in *Kalanchoë* plants when they are moved back to LD after being subjected to a SD treatment.

Two kinds of donors were used, *viz.* plants which had received 42 SD, and comparable plants which had received 21 SD, followed by 21 LD. Reciprocal grafts between donors and receptors were performed as listed in table 28. Donor-stocks and -scions were deprived of all axillary shoots; they retained 3 pairs of leaves. Receptor-scions were grafted with 1 pair of leaves; these leaves were excised after 18 days. From the results shown in table 28 it is clear that all receptors except one were induced to flower by the donors.

TABLE 28. *Experiment 35.* Effect of LD after-treatment on the induced state in *Kalanchoë*. The pairs of LD leaves on scions in the first and second combination were removed after 18 days. Data 140 days after grafting

Graft-combination	Donor received prior to grafting	Number of stocks		Mean number of days to flower buds	Mean number of flower buds + flowers
		Generative	Vegetative		
LD—/SD+	42 SD	9	0	54 ± 2.9	364 ± 82.2
LD—/SD+	21 SD, 21 LD	10	0	57 ± 2.2	248 ± 36.8
SD+/LD—	42 SD	10	0	42 ± 1.4	311 ± 63.4
SD+/LD—	21 SD, 21 LD	8	1	56 ± 1.8	149 ± 32.4

The response to donors that received 42 SD prior to grafting was somewhat superior to that evoked by donors which were exposed to 21 SD and after that to 21 LD, but this may be due to the difference in numbers of SD the donors had received. At least the results obtained in this experiment indicate that the effect of photoperiodic induction was not nullified in subsequent LD but remained transmissible to receptor-shoots.

Transmission in basipetal as well as in acropetal direction was demonstrated with *Perilla* in exp. 25 (p. 42). A similar experiment was carried out with *Kalanchoë*; it is described below.

Experiment 36. – A group of plants was subjected to 38 SD whereas comparable plants remained in LD. At the end of SD treatment all axillary shoots were removed from the donor plants whereafter two graft-combinations were performed between induced and non-induced plants, *viz.* LD/SD and SD/LD. In the first combination grafting was carried out above the 5th node; the stocks retained all leaves. The LD scions consisted of pieces of stem with 1 pair of leaves. Buds in the axils of these leaves were allowed to develop as the receptor shoots. In LD–/SD+ the pairs of LD leaves were removed after 23 days.

Donor-scions were grafted with 5 pairs of leaves; the receptor-stocks retained all leaves (SD+/LD+) or were defoliated immediately before grafting (SD+/LD–). Shoots on the nodes directly below the graft union functioned as receptors. The results are presented in table 29.

TABLE 29. *Experiment 36.* Upward and downward movement of the floral stimulus in *Kalanchoë* as influenced by presence or absence of LD leaves on receptors. Donors received 38 SD prior to grafting. The pairs of LD leaves in the second combination were removed after 23 days. Data after 132 days

Graft-combination	Number of receptors		Mean number of days to flower buds	Mean number of flower buds + flowers
	Generative	Vegetative		
LD+/SD+	13	0	44 ± 0.9	130 ± 35.2
LD–/SD+	9	1	36 ± 0.7	265 ± 19.5
SD+/LD+	11	0	73 ± 2.6	87 ± 23.7
SD+/LD–	11	0	50 ± 2.8	96 ± 15.9

It appears from these results that LD leaves present on receptors delayed appearance of flower buds but did not completely prevent it. Receptor shoots in the combination LD–/SD+ flowered abundantly (see photo 5), but when 1 pair of LD leaves was retained (LD+/SD+), the inflorescences showed phyllody (photo 6). The same behaviour of receptor shoots was observed in the reciprocal combinations.

From the results in table 29 it should not be concluded that there is a preference for transmission of the floral stimulus in acropetal direction because the receptor shoots on the scions grew much more vigorously than those on the stocks so that the combinations LD/SD and SD/LD are not completely comparable.

It can be concluded from the results presented in this experiment that *transmission of the floral stimulus in Kalanchoë can occur via a graft union both in acropetal and basipetal direction. Transmission is retarded, but not completely inhibited by LD leaves on the receptor.*

2. EXPERIMENTS WITH *SEDUM*

Both *S. ellacombianum* and *S. spectabile* are known as qualitative LDP (29, 41). The differences in habit observed between plants growing in SD and LD have been described already in a preceding communication (161).

Several attempts have been made with both species to induce flowering in

non-induced shoots by grafting them onto previously induced specimens, but with as little success as reported by TINCKER (145) for *S. spectabile*. Our results are summarized below.

Plants of both *Sedum* species were grown in LD until flower buds were clearly visible. Then the stems were excised below the terminal inflorescences and vegetative shoots from SD were cleft-grafted onto these stems. On stocks of *S. ellacombianum* approximately 15 leaves remained present, on stocks of *S. spectabile* only 8 to 10. Scions retained 1 or 2 expanded leaves. The plants were grown in SD after grafting. All scions, either defoliated or not, remained vegetative; they did not even show a change towards LD habit which always precedes floral initiation. Scions of *S. ellacombianum* continued developing new leaves, but those of *S. spectabile* stopped growth after having developed 3 to 5 new leaves. The previously induced stocks always reverted to vegetative growth: all axillary shoots remained vegetative and exhibited a SD habit. So, there seems to be no photoperiodic after-effect when *Sedum* plants are transferred from LD to SD. In fact this could be demonstrated with intact *S. ellacombianum* plants which were moved back to SD before they had initiated flower buds. These plants remained vegetative and newly appearing leaves showed SD habit. This absence of a photoperiodic after-effect explains the negative results obtained after grafting. It remains to be determined whether a flower-inducing effect can be transmitted in plants which are only locally subjected to the inductive day-length, *e.g.* by continuing after grafting the application of LD treatment to the stocks. The results obtained by MEYER (97) with *S. kamtschaticum* give an indication that this might result in flowering: she observed that the habit-changing effect was transmitted from LD leaves towards shoots in their axils which received SD, but she did not report on flowering responses.

3. EXPERIMENTS WITH *BRYOPHYLLUM*

B. daigremontianum is a LSDP (117, 118) which means that plants of this species will not flower if kept continuously in LD or SD; flower formation will only occur after transfer from LD to SD. According to our own experience plants raised from leaf plantlets will react by floral initiation to the shift from LD to SD only if they have developed at least 10 pairs of leaves.

In several separate experiments we could establish that *B. daigremontianum* is a very suitable plant for demonstrating the transmission of the floral stimulus from induced to non-induced specimens. Observations made in these experiments are reported below.

Experiment 37. – Plants were grown in LD or SD until they had developed at least 15 pairs of leaves. At that time a number of plants was transferred from LD to SD. One half of these plants remained there for at least 20 days until flower buds began to differentiate. Then they were moved back to LD and grafted with tops from plants that had always been in LD. At the same time the other half of induced plants, remaining in SD, was grafted with tops from plants that had always been subjected to SD. As control grafts LD–/LD+ and SD–/SD+ were performed for both groups respectively.

Results were always clear-cut: flower buds appeared on all receptor-scions both in LD and SD within a period of 30 to 40 days after grafting. Ungrafted plants kept in LD or SD, and scions on all control grafts remained vegetative. Flowering on induced scions was as abundant as on ungrafted plants which had

been induced by moving the plants from LD to SD. Presence of a pair of leaves on receptor-scions did not inhibit or delay the appearance of flower buds on scions.

These results indicate that vegetative plants of Bryophyllum daigremontianum either grown continuously in LD or SD can be induced to flower by grafting them onto previously induced plants.

4. *KALANCHOË* AS DONOR FOR *SEDUM*

In a preliminary communication (161) we reported that *Kalanchoë* can induce both *S. ellacombianum* and *S. spectabile* to flower in SD. Incidental observations made it probable that leaves must be present on the *Kalanchoë* stocks in order to get a transmission of the floral stimulus to *Sedum* scions. A detailed investigation regarding this point is described in the next experiment.

Experiment 38. – *Kalanchoë* plants were transferred from LD to SD. After having received 42 SD they were grafted with *S. spectabile* shoots which retained only 1 or 2 leaves. These shoots originated from plants that were dug up from the garden early in spring and grown in SD afterwards. By the time they were grafted, growth had stopped completely.

Defoliation of groups of *Kalanchoë* stocks was performed with 12-day intervals beginning at the day of grafting. Scions were defoliated until flower buds appeared with the restriction that defoliation was stopped as soon as the stocks were deprived of their leaves. The results are presented in table 30.

TABLE 30. *Experiment 38.* Flowering response of *Sedum spectabile* receptor-scions in SD after grafting onto *Kalanchoë* as affected by defoliation of donor-stocks at different times after grafting. *Kalanchoë* stocks were transferred to SD 42 days prior to grafting

Stocks defoliated after ... days	Number of plants	Number of *Sedum* scions		Mean number of days to	
		Generative	Vegetative	flower buds	opening of first flower
0	12	1	11	41	76
12	11	10	1	41 ± 0.0	86 ± 4.3
24	10	8	2	42 ± 0.9	85 ± 3.0
36	11	10	1	42 ± 1.3	85 ± 1.4
48	10	10	0	44 ± 1.7	89 ± 3.0
∞	22	22	0	44 ± 0.9	89 ± 1.4

From these results it will be clear that defoliating the stocks immediately before grafting resulted in a very weak flowering response: only 1 out of a total of 12 plants initiated flower buds; the remaining 11 scions formed some new leaves after grafting, but growth stopped afterwards. They all remained in the rosette stage; see photo 7. However, when the leaves on the *Kalanchoë* donor-stocks were retained for at least 12 days after grafting almost all scions were induced to flower. Further development of flower buds was not influenced by removal of leaves from the stocks as follows from the numbers of days until opening of first flowers.

The results obtained in this experiment show that the *leaves* on the *Kalanchoë* stocks are the organs which cause *Sedum* scions to initiate flower buds in SD. In order to get a flowering response these leaves need to be present only during the period immediately after grafting.

We also found (161) that flower buds on *Sedum* scions appeared earlier when *Kalanchoë* stocks had received a SD treatment before grafting than without such a pre-treatment. Data of a more extensive series carried out afterwards are given below.

Experiment 39. – This experiment was performed simultaneously with the previous one. *Sedum* scions originated from the same source. They were defoliated until flower buds appeared. *Kalanchoë* plants to function as donor-stocks had received 0, 14, 28, or 42 SD prior to grafting. The results are given in table 31.

TABLE 31. *Experiment 39.* Flowering response of *Sedum spectabile* receptor-scions in SD after grafting onto *Kalanchoë* stocks as affected by number of SD applied to donor-stocks prior to grafting

Stocks transferred to SD . . . days prior to grafting	Number of plants	Number of *Sedum* scions		Mean number of days to	
		Generative	Vegetative	flower buds	opening of first flower
0	11	11	0	61 ± 2.5	107 ± 3.4
14	9	9	0	49 ± 1.7	92 ± 2.4
28	10	10	0	45 ± 1.4	87 ± 2.0
42	22	22	0	44 ± 0.9	89 ± 1.4

It is clear that flower buds on *Sedum* scions appeared earlier with increasing duration of SD pre-treatment applied to *Kalanchoë* stocks. Transferring the plants to SD 14 days before grafting was performed, accelerated appearance of flower buds 12 days, so that the effect was almost equal to the duration of the pre-treatment. A further increase in duration of the pre-treatment did not significantly hasten flower formation.

These results are plausible if the following facts are taken into consideration. For inducing optimal flowering in *Kalanchoë* approximately 20 SD suffice. If it is assumed that the floral stimuli in *Kalanchoë* and *Sedum* are identical as will be demonstrated in exp. 41 (p. 61), scions of the latter will respond most rapidly with flower formation if they are grafted onto optimally induced stocks of the former. Probably the establishment of a graft union takes at least 7 days. Therefore, in grafts performed with stocks which had received 14 SD before grafting, transmission of the floral stimulus could not begin until 21 days after the beginning of SD treatment, when in the meantime the donors had reached the optimally induced state.

Combining the results of the present experiment with those obtained in the preceding one, it becomes very probable that for inducing *Sedum* scions to flower, leaves on donor-stocks can be removed earlier when they have received a SD pre-treatment than without such a treatment. In fact this could be ascertained in the next experiment in which scions of *S. ellacombianum* functioned as receptor.

Experiment 40. – Vegetative scions of *S. ellacombianum* with 1 or 2 expanded leaves were grafted onto *Kalanchoë*. The stocks had been grown in LD until then or they had received 39 SD prior to grafting. One half of the donors of each group was defoliated, the other half retained the leaves continuously, so that the experiment included 4 different treatments of donor-stocks. *Sedum* scions were not defoliated.

The whole procedure was repeated 11 days later when the induced plants had received 50 SD. As the two experiments yielded exactly the same results, data obtained in comparable groups have been joined together in table 32.

TABLE 32. *Experiment 40.* Flowering response of *Sedum ellacombianum* receptor-scions in SD after grafting onto *Kalanchoë* as affected by number of SD applied to donor-stocks prior to grafting and defoliation of stocks at date of grafting

Number of SD applied to *Kalanchoë* stocks prior to grafting	Stocks + or − leaves	Number of *Sedum* scions		Mean number of days to		Mean number of leaves to inflorescence
		Generative	Vegetative	flower buds	opening of first flower	
0	+	14	0	46 ± 1.6	71 ± 1.8	23.2 ± 1.3
0	−	0	20	−	−	−
39 or 50	+	17	0	40 ± 1.6	67 ± 2.1	20.8 ± 0.9
39 or 50	−	6	14	53 ± 11.8	79 ± 8.5	27.3 ± 1.9

Sedum scions grafted onto non-defoliated *Kalanchoë* stocks showed a gradual change from leaves with a SD habit towards leaves with a LD habit. All scions formed flower buds. Subsequent flowering was very prolific, 10 to 25 flowers being present per inflorescence.

Defoliated stocks which received 0 SD before grafting did not induce *Sedum* scions to flower. These scions were richly branched; they developed many new leaves which all retained their SD habit.

However, when previously induced stocks were defoliated at the date of grafting, 6 out of a total of 20 *Sedum* scions became generative. Only 1 or 2 flower buds appeared per scion; they developed to normal flowers afterwards. Appearance of flower buds on these scions was not preceded by such a clear change in habit as was observed when stocks retained their leaves.

Thus, just as in exp. 38 (p. 59) it must be concluded that the leaves on the *Kalanchoë* stocks are the organs which produce the floral stimulus. However, defoliated stocks which had previously received SD treatment could – to some extent – also function as donor. This cannot be ascribed solely to production of the floral stimulus by the stems, as stocks which had not received a SD treatment before removal of the leaves, did not evoke a flowering response on *Sedum* scions. Therefore, there can be no other interpretation than that a certain amount of floral stimulus, produced before defoliation, had been translocated to the stems and remained stored there without losing its activity until it could induce flower formation in *Sedum* scions.

The fact that *Sedum* scions can easily be induced to flower in SD by grafting onto induced *Kalanchoë* stocks is not yet a conclusive proof for the identity of the floral stimuli in both plants (*cf.* 73, pp. 269 and 271). This conclusion would be warranted only when *Kalanchoë* "donors" would be able to induce floral initiation in *Sedum* receptors if the former were induced themselves and not if they were non-induced. The results presented in table 31 (p. 60) give an indication that this will really occur as in this experiment a SD treatment prior to grafting resulted in earlier flowering than without such a pre-treatment. Direct evidence for the similarity of the floral stimuli in *Kalanchoë* and *Sedum* is presented in the next experiment.

Experiment 41. – *Kalanchoë* plants raised from cuttings were grown in LD until they had expanded approximately 5 pairs of leaves. Then they were

pinched and two shoots were allowed to develop on each plant in the axils of the highest located pairs of leaves. After 3 months one branch on each plant was completely defoliated. These defoliated branches were trained through small holes (diameter approximately 5 mm) punched in the side-walls of a box – 3 m long, 0.75 m wide, 0.5 m high – which had been constructed with board. The holes were further filled up with wadding. The non-defoliated branches with the main stems remained outside the box in LD. The defoliated branches inside the box were grafted with vegetative *S. spectabile* or *S. ellacombianum* scions. The former retained 3, the latter 1 or 2 leaves. The scions received 8 hours of daylight, the box being covered by a lid from 4.30 p.m. to 8.30 a.m. So, in this case the stocks were *non-induced*. As a check two-branched *Kalanchoë* plants with *Sedum* scions were inside the box so that these stocks were *induced*. The following observations were made:

S. spectabile: the results are presented in table 33.

TABLE 33. *Experiment 41.* Flowering response of *Sedum spectabile* receptor-scions in SD after grafting onto *Kalanchoë* as affected by induced (SD) or non-induced (LD) donor-stocks

Kalanchoë donor-stocks exposed to	Number of *Sedum* scions		Mean number of days to	
	Generative	Vegetative	flower buds	opening of first flower
SD	3	0	58.0 ± 0.0	100 ± 1.5
LD	0	10	–	–

Scions grafted onto induced stocks responded normally with flower formation. Scions on non-induced stocks, however, formed only 2 to 4 new leaves without any stem elongation. They were vegetative when the experiment was discontinued after 80 days.

S. ellacombianum: one half of the scions on induced as well as on non-induced stocks was defoliated; the other scions retained all leaves. The results are given in table 34.

TABLE 34. *Experiment 41.* Flowering response of *Sedum ellacombianum* receptor-scions in SD after grafting onto *Kalanchoë* as affected by induced (SD) or non-induced (LD) donor-stocks. Data after 80 days

Kalanchoë donor-stocks exposed to	Scions + or – leaves	Number of *Sedum* scions		Mean number of days to		Mean number of leaves formed after 80 days
		Generative	Vegetative	flower buds	opening of first flower	
SD	+	10	0	39 ± 1.2	62 ± 1.3	24.9 ± 0.9[1]
SD	–	10	0	41 ± 1.0	63 ± 1.0	24.3 ± 1.1[1]
LD	+	0	10	–	–	22.3 ± 1.2
LD	–	0	10	–	–	22.6 ± 1.6

[1]) Counted from graft union to inflorescence.

All scions grafted onto *Kalanchoë* stocks in SD formed flower buds and flowered afterwards. There was no difference in flowering response between the non-defoliated group and the group which was defoliated until flower buds were visible. A clear change from leaves with SD habit towards leaves with LD habit always preceded flower formation; see photo 9. Ungrafted *Sedum* plants

which had been transferred from SD to LD at the beginning of the experiment flowered at the same time as shoots grafted onto induced *Kalanchoë* stocks. The flowering obtained after grafting onto *Kalanchoë* was just as prolific as on the ungrafted *Sedum* plants in LD. Ungrafted *Sedum* plants kept *within* the box remained vegetative throughout.

The newly formed leaves on *Sedum* scions grafted onto non-induced *Kalanchoë* stocks always exhibited SD habit and the internodes remained very short so that the scions looked rosulate. See photo 10. All 20 scions were vegetative 80 days after grafting when the experiment was discontinued.

In another group of 10 non-induced *Kalanchoë* stocks SD treatment was started 30 days after grafting by darkening the *Kalanchoë* branches *outside* the box from 4.30 p.m. to 8.30 a.m. Non-defoliated *Sedum* scions which had already developed several leaves at the beginning of the SD treatment remained vegetative. Defoliated scions, however, were induced to flower, thus demonstrating that the bending of the defoliated branches through the small holes in the side-walls did not influence transmission of the floral stimulus.

It should be added that several *Kalanchoë* stocks initiated one or a few flower buds although they were continuously kept in LD. However, this had no effect on the *Sedum* receptors. Flowering of *Kalanchoë* plants in LD was observed only in this group of old plants raised from cuttings. According to verbal communications made to the author by Dr. R. BÜNSOW, Göttingen, Prof. Dr. G. MELCHERS, Tübingen, and Dr. W. W. SCHWABE, Rothamsted, this occurs regularly.

From the data presented in this experiment it is obvious that *Sedum scions in SD can be induced to flower only if the Kalanchoë donor-stocks are induced themselves, indicating that the floral stimuli are identical in both plants.*

The findings of the present experiment also bear upon the "metaplasin" hypothesis: although *Sedum* scions were grafted onto non-induced *Kalanchoë* stocks with LD habit they retained their SD habit. Therefore, these results fully support the conclusion reached in a preliminary communication (161), *viz.* "that in *Sedum ellacombianum* long-day habit and flowering are caused by one and the same stimulus."

5. *SEDUM* AS DONOR FOR *KALANCHOË*

CARR and MELCHERS (24) reported that *Kalanchoë blossfeldiana* can be induced to flower in LD after grafting with *Sedum kamtschaticum*. Similar results obtained after grafting with *S. ellacombianum* and *S. spectabile* are described in the next experiment.

Experiment 42. – *Kalanchoë* stocks grown in LD were grafted above the 4th node with *Sedum* scions. On each stock two branches functioned as receptors; they were regularly defoliated until flower buds were visible.

Scions of *S. ellacombianum* originated from plants in LD; they retained 4 expanded leaves. *S. spectabile* scions were taken from plants in SD which had stopped growth; they retained 3 to 4 leaves. As a control non-induced *Kalanchoë* shoots were grafted onto non-induced stocks which were treated in a similar way as those grafted with *Sedum*. The results are given in table 35, p. 64.

It will be clear that both *S. ellacombianum* and *S. spectabile* induced flowering in all *Kalanchoë* stocks onto which they had been grafted. However, this occurred much more rapidly with the former than with the latter donor. Probably this was due to the fact that *S. ellacombianum* had been induced already prior to grafting which resulted in appearance of flower buds on the scions shortly after

TABLE 35. *Experiment 42.* Flowering response of *Kalanchoë* receptor-stocks in LD after grafting with *Sedum* as donor. Defoliation of stocks was performed 21 days after grafting. Data after 230 days

Donor-scions and control	Number of *Kalanchoë* stocks		Mean number of days to flower buds
	Generative	Vegetative	
S. ellacombianum	24	0	57 ± 3.7
S. spectabile	24	0	115 ± 6.7
Kalanchoë in LD	0	12	–

growth was resumed. In half the scions the inflorescences were removed, but this had no effect on the flowering response of the stocks concerned.

On the other hand *S. spectabile* scions which had previously been in SD, formed nothing but leaves during more than 2 months after grafting. Then inflorescences appeared, subsequently followed by formation of flower buds on the *Kalanchoë* receptors. Apparently the floral stimulus was not transmitted to the receptors, at least not in sufficient quantities, before the donors were able to produce flower buds themselves. Moreover, axillary buds on the 4th nodes of several *Kalanchoë* stocks failed to develop as receptor branches. Finally, however, all these stocks produced a pair of shoots from the root collar as often occurs in *Kalanchoë* (*cf.* 52, 53). On these shoots only flower buds were present, indicating that the floral stimulus was supplied in excess when the buds became active.

All receptor branches of control grafts were strictly vegetative when the experiment was discontinued after 230 days.

Results of the reciprocal graft-combination *viz. Kalanchoë/Sedum* in LD are given in the following experiment.

Experiment 43. – In this experiment induced *S. spectabile* stocks functioned as donor for *Kalanchoë* scions in LD. Donors were raised from cuttings in LD until flower buds were visible. The tops bearing the inflorescences were excised, so that 4 to 6 nodes with leaves were retained. Vegetative *Kalanchoë* scions with one pair of fully expanded leaves functioned as receptors. This pair of LD leaves was removed in half the plants as soon as the scions had taken; all younger leaves were retained. The results presented in table 36 show that the *Kalanchoë* scions responded very rapidly with flower formation.

TABLE 36. *Experiment 43.* Flowering response of *Kalanchoë* receptor-scions in LD after grafting onto *Sedum spectabile* as donor. Groups I and II were grafted at different dates. One pair of expanded leaves remained present on scions or was removed after 19 and 17 days in groups I and II respectively

Group	Number of plants	Pair of LD leaves retained (+) or removed (–)	Mean number of days to flower buds
I	10	+	35 ± 1.6
I	10	–	33 ± 1.4
II	6	+	31 ± 0.9
II	6	–	31 ± 1.2

Removal of LD leaves did not significantly influence appearance of flower buds; only one plant having retained the pair of LD leaves, showed phyllody.

Flowering on *Kalanchoë* scions was as prolific as on comparable ungrafted plants that were induced to flower by SD treatment.

In a pilot experiment the *Sedum* stocks were defoliated immediately before grafting, with the result that all *Kalanchoë* scions remained vegetative. With leaves present on the donor-stocks, however, all receptors flowered. See photo 8. Again this shows that the *Sedum* leaves are the organs which produce the floral stimulus.

The results presented in this section have clearly demonstrated that *Kalanchoë can easily be induced to flower in LD by grafting with induced Sedum donors. Transmission of the floral stimulus is possible in basipetal as well as in acropetal direction.*

6. *KALANCHOË* AND *SEDUM* AS DONORS FOR *BRYOPHYLLUM*

RESENDE (119, 120) reported that the SDP *Kalanchoë velutina* can induce *Bryophyllum daigremontianum* to flower in LD. He described 4 grafts with induced *Kalanchoë* onto *Bryophyllum* with the result that only 1 of the receptor-stocks showed "a completely vegetative inflorescence" (119, p. 71), the other stocks remaining vegetative. Moreover, he reported that *Bryophyllum* receptors "in SD conditions did not receive the floral stage from the same flowering donor" (120, p. 294).

Although our experiments on grafts with *Kalanchoë* and *Sedum* as donors for *Bryophyllum* were rather preliminary, we shall summarize the observations made as they extend the above quoted statements of RESENDE.

Kalanchoë as donor for *Bryophyllum* in SD. – *Bryophyllum* plants continuously grown in SD were grafted with *Kalanchoë* shoots from LD. The scions retained 3 pairs of leaves. Out of a total of 10 grafts only 3 *Bryophyllum* stocks developed axillary shoots. All 3 these stocks showed flower buds after 7 months; on 2 of them flower buds opened.

In the reciprocal graft-combination *Bryophyllum*—/*Kalanchoë*+ in SD only 2 out of a total of 10 scions showed some flower buds 5 months after grafting; the buds did not develop to open flowers. Growth of the *Bryophyllum* scions on *Kalanchoë* stocks was rather poor so that it is possible that the weak flowering response was due to a poor graft union.

All receptor shoots in control grafts in SD remained vegetative, so that it is to be concluded that the flowering *Bryophyllum* receptors were really induced by the *Kalanchoë* donors. However, the response was very weak and absolutely inferior to the flowering response exhibited by *Bryophyllum* transferred from LD to SD.

Kalanchoë as donor for *Bryophyllum* in LD. – One branch on each plant of a group of two-branched *Bryophyllum* plants in LD was grafted with a *Kalanchoë* shoot. This shoot had received 39 SD or had been kept continuously in LD. The ungrafted *Bryophyllum* branches were defoliated and functioned as receptors. After 100 days the following observations were made: 2 out of 10 *Bryophyllum* receptors grafted with induced donors had formed flower buds. After grafting with vegetative *Kalanchoë* donors 1 out of 8 receptors had become generative. All comparable ungrafted *Bryophyllum* plants were strictly vegetative. It is clear that these graft-combinations need further investigation. If the response to non-induced *Kalanchoë* donors would be reproducible, this would indicate that the floral stimuli are not identical in *Kalanchoë* and *Bryophyllum*.

Sedum spectabile as donor for *Bryophyllum* in LD. – *S. spectabile* stocks comparable to those used in exp. 43 (p. 64) were grafted with *Bryophyllum* tops from plants in LD. After 6 months all 16 *Bryophyllum* receptors were vegetative. It must be added that the *Bryophyllum* scions on the *Sedum* stocks showed very poor growth; most scions formed roots above the graft union which is an indication that no functional phloem continuities had been established (*cf.* 58). Therefore it is probable that the negative result was due to absence of a phloem connection between donor and receptor and not primarily to unidentity of the floral stimuli in *Sedum* and *Bryophyllum*.

CHAPTER VI

EXPERIMENTS WITH *NICOTIANA*

The following abbreviations will be used throughout this chapter:
N.s.: *Nicotiana sylvestris* (LDP)
M.M.: *Nicotiana tabacum* cv. 'Maryland Mammoth' (SDP).
Dc.: *Nicotiana tabacum* cv. 'Delcrest' (DNP).

1. MARYLAND MAMMOTH AS RECEPTOR

The flowering response of M.M. receptors in LD was studied both in single and double grafts.

1.1. *Single grafts*

The results obtained with single grafts can be summarized as follows (*cf.* 160):

1) The LDP N.s. turned out to be a very good donor for M.M. in LD; both stocks and scions of M.M. were induced indicating that the floral stimulus was transmitted basi- as well as acropetally. M.M. scions flowered abundantly about 50 days after grafting, resulting in a high seed-yield. Presence of several leaves on stocks in the combination N.s+/M.M.+ did not inhibit or delay the formation of flower buds on the receptors. In the reciprocal combination M.M./N.s.+ scions were grafted without any mature leaves. Removal of subsequently expanding leaves (M.M.–/N.s.+) did not hasten appearance of flower buds. All control grafts (M.M.+/M.M.– and M.M.–/M.M.+) remained vegetative. Thus, it can be concluded that flowering of M.M. receptors is not due to removal of flower-inhibiting M.M. leaves, but is brought about by a specific flower-inducing effect which is transmitted from N.s. donors to M.M. receptors.

2) All grafts performed with the DNP Dc. as donor for M.M. in LD, being completely comparable with and grown under identical conditions as the combinations *sub* 1, always yielded negative results: all shoots on M.M. receptors remained vegetative.

Some of the peculiarities regarding the flowering response of M.M. scions grafted onto N.s. stocks (M.M.–/N.s.+) are reported below.

Experiment 44. – N.s. plants in LD were grafted with vegetative M.M. shoots after the donors showed flower buds. The N.s. stocks retained 8 to 10 fully expanded leaves. These leaves were removed from different groups of donors with 5-day intervals beginning at the day of grafting. When the stocks were defoliated immediately before grafting all M.M. scions remained vegetative. Removal of leaves from the stocks 5 days after grafting resulted in flowering in 25 % of the stocks. A flowering response of 100 % was not attained until leaves remained present for 10 to 15 days after grafting. These results indicate that the leaves present on the N.s. stocks are the organs which induce M.M. scions to initiate flower buds in LD. A similar conclusion was reached already for the graft-combination *Sedum/Kalanchoë* in SD (exp. 38 and 40, pp. 59 and 60).

Experiment 45. – The leaf area on N.s. stocks in LD was reduced to 0, 1, 2, 3, 4, 6, or 8 leaves immediately before grafting with vegetative M.M. scions was performed. As a check, comparable M.M. scions were grafted onto M.M. stocks in LD. The number of leaves on these stocks was varied as well. The data obtained have been plotted in fig. 13.

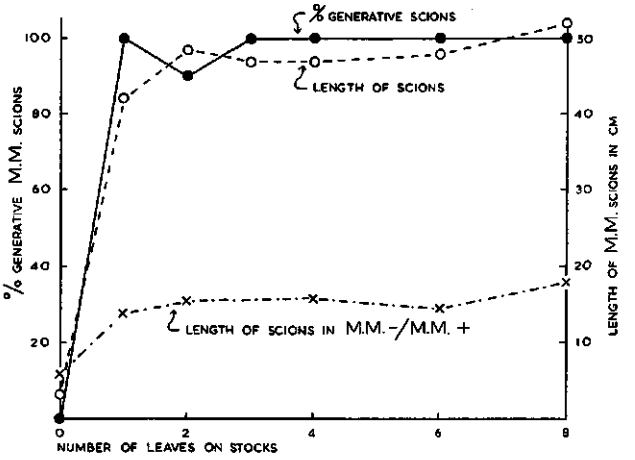

FIG. 13. *Experiment 45.* Flowering response and stem elongation of M.M. scions in the graft-combination M.M. – /N.s. + as influenced by the number of leaves present on the stocks (upper two curves). For the graft-combination M.M. –/M.M. + only data for lengths of scions. Per treatment 10 plants. Data 50 days after grafting.

It is clear that removal of all leaves from the donor-stocks did not result in flowering just as was observed in the preceding experiment. However, with one leaf per stock all M.M. scions were induced to flower. As one leaf had an area of approximately 2000 cm^2 it seems probable that even a part of a leaf might induce M.M. scions to initiate flower buds.

Appearance of flower buds on M.M. scions was always followed by a rapid stem elongation. Measurements of scions in the graft-combinations M.M.–/N.s.+ and M.M.–/M.M.+ have been plotted in fig. 13. Comparing the two curves it becomes clear that generative scions on N.s. stocks were about three times as long as vegetative ones on M.M. stocks with the same numbers of leaves. So, the length reached by the M.M. scions was determined by the type of stock and not by the numbers of leaves on the stocks.

1.2. *Double grafts*

From the results mentioned in the preceding section it is clear that a qualitative difference exists between the LDP N.s. on the one hand and DNP Dc. on the other hand, in relation to their ability to function as donors for M.M. receptors. In a preliminary communication (160) experimental evidence was presented which permitted the conclusion that this difference is not due to the establishment of a poor graft union in grafts with Dc. as donor. It was shown that M.M. scions in the graft-combination M.M.–/Dc.–/N.s.+ responded very rapidly with flower formation, thus demonstrating that the floral stimulus supplied by the N.s. donors can pass both graft unions and the Dc. interstock very easily. However, when some leaves remained present on the interstock (M.M.–/Dc.+/N.s.+) formation of flower buds on the receptor-scions was greatly delayed. This flower-inhibiting influence of leaves on the interstocks was studied more quantitatively in the following experiment.

Experiment 46. – The graft-combination M.M.–/Dc.+/N.s.+ and the controls M.M.–/M.M.+, M.M.–/Dc.+ and M.M.–/N.s.+ were performed in just

the same way and under similar conditions as described previously (160). The period between grafting of Dc. interstocks and M.M. scions was 35 days in the present experiment. The mean lengths of Dc. interstocks varied between 50 and 70 cm in various groups of plants.

The effect of leaves on the Dc. interstocks was studied in two different ways:

Firstly, by varying the *number of leaves* on the interstocks. This number was reduced to 0, 2, 4, or 6 leaves in 4 different treatments immediately after grafting.

Secondly, by varying the *time of defoliation* of the Dc. interstocks. For that purpose the interstocks retained 6 leaves. They were removed from the interstocks in different groups respectively 0, 15, 30, or 45 days after grafting. In one group the leaves were retained continuously.

When 65 days had elapsed since the M.M. scions had been grafted, the experiment was discontinued. The data obtained with *single* grafts have been compiled in table 37.

TABLE 37. *Experiment 46.* Flowering response of M.M. scions grafted in LD onto different stocks. Data 65 days after grafting

Graft-combination	Flowering response of scions		Mean number of days to flower buds	Length of scions in cm
	Generative	Vegetative		
M.M.–/M.M.+	0	12	–	58 ± 3.7
M.M.–/Dc.+	1 [1])	10	–	46 ± 4.6
M.M.–/N.s.+	12	0	24 ± 1.3	163 ± 5.2

[1]) Beginning of floral initiation microscopically visible after 65 days.

Again these results demonstrate the difference between Dc. and N.s.; although both flower in LD, only the latter donor can induce M.M. scions to flower.

In the double-worked plants several M.M. scions were still vegetative or had only macroscopically visible flower buds after 65 days. In order to be able to present the numbers of days until appearance of flower buds for each group of scions, the following assumptions were made:

Firstly, when flower buds were microscopically visible, the number of days until appearance of flower buds was assumed to be 70.

Secondly, when scions were still vegetative after 65 days flower buds were assumed to have appeared after 80 days.

Variation of the number of leaves on Dc. interstocks. – The results are given in fig. 14. The M.M. scions responded most rapidly with flower formation when all leaves were removed from the interstocks, thus confirming previous results (160). There is a tendency that flower buds appeared later with increasing number of leaves on the interstocks, but the differences obtained with 2, 4, or 6 leaves are not significant.

The time of defoliation of Dc. interstocks. – The results are plotted in fig. 15. It is clear that appearance of flower buds on the M.M. scions was not delayed when leaves remained present on the interstocks for 15 days as compared with defoliation immediately after grafting. This indicates that transmission of the floral stimulus from the N.s. stocks to the M.M. scions did not begin until about the 15th day after grafting. When the leaves were kept on the interstocks

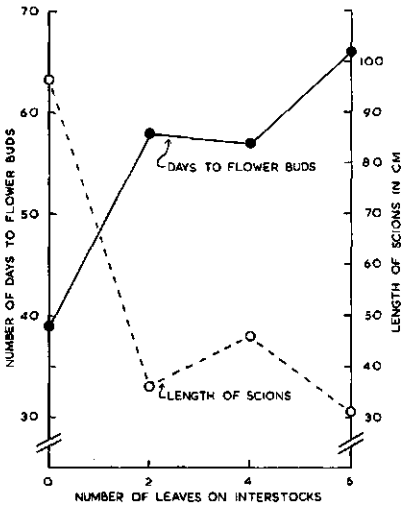

FIG. 14.
Experiment 46. Flowering response and stem elongation of M.M. scions in the double graft-combination M.M.–/Dc. + /N.s. + as influenced by the number of leaves present on the interstocks. Per treatment 10 plants. Data 65 days after grafting of scions.

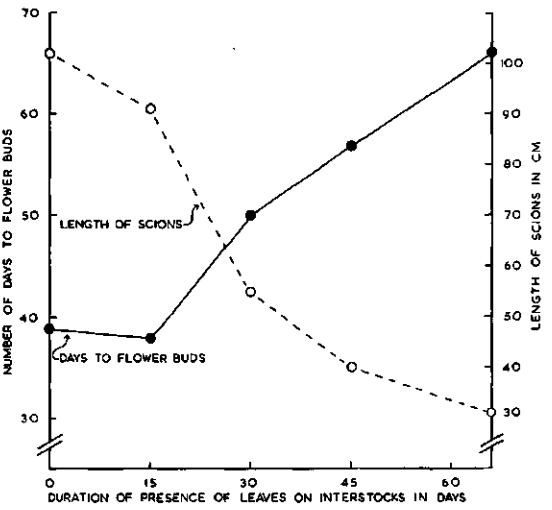

FIG. 15.
Experiment 46. Flowering response and stem elongation of M.M. scions in the double graft-combination M.M. – /Dc. + /N.s. + as influenced by time of defoliating interstocks. Per interstock 6 leaves. Data 65 days after grafting of scions.

for more than 15 days after the scions had been grafted, appearance of flower buds was retarded with increasing duration of presence of leaves. However, the increase in number of days to flower buds was not equal to the period during which the leaves remained present. It seems that the flower-inhibiting influence of leaves decreases when they become older, especially when they begin to show symptoms of senescence.

The results presented in this experiment clearly demonstrate that Dc. cannot induce M.M. scions to flower. However, a piece of Dc. stem between N.s. donor and M.M. receptor conducts the floral stimulus very easily; this means that the interstock is merely a living tube between donor and receptor. It is obvious that leaves on the interstock can considerably interfere with transmission of the floral stimulus in upward direction. But in the reciprocal combinations N.s.+/ Dc.±/M.M.– presence or absence of leaves on the interstocks does not affect the flowering response of the M.M. receptor shoots (160). These results are best explained by assuming that the floral stimulus is translocated in the phloem with the assimilates.

For a discussion of data in literature on grafts with M.M. as receptor bearing upon our results we can refer to (160). Suffice it to repeat the conclusion "that it seems improbable that the floral stimuli in Dc. and M.M. are identical".

2. *NICOTIANA SYLVESTRIS* AS RECEPTOR

Experiment 47. – In this experiment it was tried to induce flower formation in the LDP N.s. under SD conditions by grafting it onto the SDP M.M. and the

DNP Dc. For that purpose M.M. and Dc. were grown in SD in big pots until flower buds were clearly visible. After having excised the tops, the plants were grafted with vegetative N.s. scions. The stocks retained 8 to 10 leaves. The N.s. scions originated from plants which had been grown in SD and had remained in the rosette stage until then. They were defoliated except 1 or 2 leaflets; the thickened roots were cut wedge-shaped before grafting. Three scions of each group retained all leaves after grafting. This resulted in the formation of rosettes on the tops of the stocks without initiation of flower buds. See photo 11. From the remaining scions the young leaves were removed regularly until flower buds were visible. The results compiled in table 38 show that nearly all these scions had formed flower buds when the experiment had to be discontinued.

TABLE 38. *Experiment 47.* Flowering response of N.s. scions grafted in SD onto different stocks. Data 84 and 102 days after grafting for M.M. and Dc. stocks respectively

Graft-combination	Number of scions		Number of flowering scions	Mean number of days to flower buds
	Generative	Vegetative		
N.s.+/M.M.+	0	3	0	-
N.s.-/M.M.+	8	1	4	58 ± 3.9
N.s.+/Dc.+	0	3	0	-
N.s.-/Dc.+	8	0	5	75 ± 4.3

It is clear that M.M. and Dc. did not differ in their ability to function as donors for N.s. Several scions flowered normally at the end of the experiment; see photo 11. All ungrafted N.s. plants, either defoliated or with leaves, remained vegetative when kept in SD.

This experiment has been repeated with similar results, although the response of defoliated N.s. scions was somewhat slower: only 17 out of a total number of 37 defoliated receptor-scions (*i.e.* 46 %) had formed flower buds at the end of the experiment after 120 days. At the same time completely comparable grafts were exposed to LD. This means that non-induced M.M. plants had to function as "donor" for N.s. When the N.s. scions retained their leaves in LD, they flowered rapidly as they could perceive the inductive daylength directly. However, when the scions were defoliated regularly, so that only very small leaves were present, 18 out of a total number of 37 scions (*i.e.* 49 %) had formed flower buds when the experiment was discontinued. No difference in flowering response was evident between N.s. scions grafted on Dc. and M.M. Likewise the response of N.s. scions to M.M. in SD (induced) and in LD (non-induced) was completely identical. Ungrafted and continuously defoliated N.s. plants remained vegetative in LD, so that it can be assumed that flower formation of defoliated scions in LD was induced by the M.M. and Dc. stocks and not by the inductive daylength. So, we must reach the conclusion that both induced and non-induced M.M. donors can induce flower formation in N.s. Similar results have been obtained by LANG and MELCHERS (77; see also 73, p. 271). These authors assumed that the response of N.s. receptors to non-induced "donors" was "unspecific". According to their interpretation of flowering in LDP (73,

76, 96) the inductive daylength would remove a flower-inhibiting effect. The same result can be achieved under SD conditions by defoliation, provided sufficient "building material" is available for growth and flower formation, as *e.g.* in the storage root of *Hyoscyamus niger*. As stated above defoliation of N.s. does not cause flowering, but LANG and MELCHERS (76, p. 691; 77) assumed this to be due to lack of "building material". However, when such defoliated plants are grafted onto M.M., either induced or not, they will be supplied with the assimilates from the stocks which – in the absence of inhibiting leaves – will lead to the formation of flower buds.

3. DELCREST AS "RECEPTOR"

Delcrest tobacco is day-neutral, so that it flowers both in LD and in SD. In experiments with day-neutral interstocks (p. 68) it was observed incidentally that the flowering of Dc. can be considerably affected by the stock onto which it has been grafted. The following experiment was designed to study this influence quantitatively.

Experiment 48. – In order to influence the flowering of Dc. scions it is necessary to graft them in a very young stage. Otherwise we must take into consideration the possibility that the growing-points are predetermined already at the moment of grafting to initiate flower buds after a fixed number of leaves. Therefore Dc. seedlings were grown in pots until the 7th leaf on each plant had reached a length of 2 to 4 cm. This 7th leaf was marked with a point of paint and all lower located leaves were cut off. Then these plants were planted in the greenhouse (ungrafted controls) or the shoots were grafted onto groups of different stocks.

Plants to be used as stocks were grown in the greenhouse as described previously (160) under LD conditions prevailing during summer. Dc. and N.s. stocks showed flower buds, M.M. plants were vegetative when grafting with the Dc. scions was performed. The stocks retained all leaves, but axillary shoots were removed continuously. The scions which were strictly vegetative at the moment of grafting, were allowed to develop without removal of leaves. The following observations were made:

1) The date at which the first flower bud was macroscopically visible. The mean values given in table 39 are reckoned from the day of grafting.

2) The number of leaves formed before the terminal inflorescence. The leaves were counted from the graft union (the first leaf being given number 7) in upward direction up to and including the first leaf in the axil of which a leafless shoot was located. (In the axils of lower located leaves shoots always develop several leaves before flower buds appear. In the axils of the very highest leaves the shoots bear only flowers).

3) The length of the 15th leaf of each scion when this had become fully expanded. The results are given in table 39, p. 72.

It is obvious that grafting always resulted in a significant decrease of the number of leaves to the inflorescence in comparison with the number formed on ungrafted plants. Dc. and M.M. stocks yielded results which do not differ significantly. N.s. stocks had the greatest flower-promoting effect which is reflected both in the number of days until appearance of flower buds and the number of leaves. Moreover, the leaves formed on the scions in the combination

TABLE 39. *Experiment 48.* Flowering response of day-neutral Dc. as influenced by grafting onto different stocks

Graft-combination	Number of plants	Mean number of days to flower buds	Mean number of leaves to inflorescence	Mean length of 15th leaf in cm
Ungrafted	12	38 ± 0.8	29.9 ± 0.3	82.8 ± 1.3
Dc./Dc.	11	39 ± 0.5	26.8 ± 0.5	70.4 ± 2.8
Dc./M.M.	11	40 ± 1.0	28.2 ± 0.3	67.8 ± 1.5
Dc./N.s.	12	31 ± 1.8	19.7 ± 0.4	35.8 ± 3.4

Dc./N.s. remained very small: the area of a leaf was approximately one fourth of that of comparable leaves in the combinations Dc./Dc. and Dc./M.M.

Similar results were obtained when the bud in the axil of the 7th leaf on each of the plants was forced to develop by excising this leaf and the stem above the 7th node. The mean numbers of leaves formed on these shoots, grafted onto Dc., M.M., and N.s. stocks, were: 17.5 ± 0.3, 19.8 ± 0.4, and 8.5 ± 0.5 respectively.

It does not seem probable that the flower-promoting effect of N.s. stocks on Dc. scions is primarily due to transmission of the floral stimulus produced in the N.s. leaves in LD as we concluded for the combination M.M.–/ N.s.+ in LD (p. 66). For, if we follow this line of reasoning we would expect a delay in flowering after grafting onto M.M. in LD (*i.e.* the non-inductive daylength, so no production of floral stimulus) but in point of fact we observed a flower-promoting effect! In exp. 45 (p. 66) it appeared that defoliated N.s. stocks did not induce M.M. scions to initiate flower buds whereas non-defoliated stocks did, so that it was concluded that the floral stimulus is supplied by the N.s. leaves. A similar experiment was carried out with Dc. as "receptor": the Dc., M.M., and N.s. stocks retained all leaves or they were completely defoliated before grafting. With non-defoliated stocks the results showed the same tendency as those presented in table 39. However, *defoliation* of the stocks always *delayed* the appearance of flower buds and increased the number of leaves formed before the inflorescence. Consequently in the combinations Dc./Dc.– and Dc./M.M.– the scions formed *more* leaves than the ungrafted control plants; but those grafted onto defoliated N.s. stocks formed less leaves than the controls. Therefore it is logical to conclude that the N.s. *roots* or *stems* are the organs which induce early flowering in Dc. scions. It is tempting to compare this effect with the effects of dwarfing rootstocks on precocity of flowering and fruit setting which are widely applied in modern fruit growing. The mechanisms which underlie these effects are still uncertain (*cf.* 123). Our results indicate that for the study of these mechanisms annuals may be preferred above trees as the former are easier to handle and yield results in one growing period (*cf.* also 140).

CHAPTER VII

GENERAL DISCUSSION

In this chapter we shall discuss the results of the present investigations obtained with the aid of grafting against the background of literature and see how far they add new facts to the present knowledge of the physiology of flowering.

1. THE FLORAL STIMULUS

The results presented in the preceding chapters have demonstrated that several plants which remain vegetative under certain light-regimes can easily be brought to flower formation in the non-inductive daylength by grafting with flowering specimens of the same or related species. This suggests that one or more factors necessary for flower formation are transmitted from donors to receptors. These factors are called the floral stimulus. A transmission of the floral stimulus was obtained in intraspecific grafts of the SDP *Perilla*, *Xanthium* and *Kalanchoë*, and the LSDP *Bryophyllum*; further in the interspecific graft of the SDP Maryland Mammoth tobacco and the LDP *Nicotiana sylvestris*, and finally in the intergeneric grafts of the SDP *Kalanchoë blossfeldiana* and the LDP *Sedum ellacombianum* and *S. spectabile*. Together with the graft-combination of the SDP Maryland Mammoth tobacco and the LDP *Hyoscyamus niger* (73) these latter combinations are the only cases of intergeneric grafts which have been completely analyzed with respect to transmission of the floral stimulus. In all these three cases it appeared that the floral stimuli are identical in SDP and LDP because donors of one reaction-type could cause flower formation in the receptors of the other type only if they were induced themselves.

As in the intraspecific grafts with the SDP *Perilla*, *Xanthium*, and *Kalanchoë* the receptors flowered when the whole graft-combinations were kept in LD from the moment of grafting it follows that the induced state or the floral stimulus remained stable for some time in LD. Evidence was presented that in *Perilla* the induced state is retained indefinitely in the leaves which were once exposed to SD. On the other hand induced *Xanthium* plants maintain the induced state in the buds. In *Kalanchoë* no new leaves are formed after appearance of flower buds so that in this plant only two possibilities remain to explain the transmissible after-effect of photoperiodic induction, *viz.* the induced state is retained in the leaves as in *Perilla*, or a certain amount of floral stimulus accumulated during SD treatment does not lose its activity in LD (*cf.* exp. 40, p. 60). The fact that the induced state is retained in different ways in *Perilla* and *Xanthium* offers a satisfactory explanation why the former plant quickly reverts to vegetative growth after transference to LD whereas the latter continues to flower for a very long period (*cf.* 50).

Intraspecific grafts with the LDP *Sedum ellacombianum* and *S. spectabile* yielded negative results. It may be added that a preliminary experiment with the LDP *Trifolium pratense* was unsuccessful as well. LANG (73) lists *Petunia hybrida* as the only case of a LDP in which transmission of the floral stimulus was obtained in an intraspecific graft. However, in a variety of *Petunia hybrida* investigated by the present author flower formation was promoted by LD, but it also occurred in SD.

Transmission of the floral stimulus from an induced to a non-induced branch of the same plant has been demonstrated in several SDP (see p. 45). As far as the author is aware the same has been obtained only in one LDP *viz. Urtica pilulifera* (88). According to CHOUARD (verbal communication) most LDP revert quickly to vegetative growth upon moving back to SD, but *U. pilulifera* continues flowering.

As stated on p. 58 an after-effect of LD treatment is absent in *Sedum*. On the other hand both *Sedum* species induced flower formation in *Kalanchoë* in LD which is evidence for a transmissible stimulus in *Sedum*. As concluded already

above the floral stimuli in *Kalanchoë* and *Sedum* must be identical. However, in view of the absence of an after-effect in *Sedum* it seems that in this plant and in *Kalanchoë different reactions* are involved in the *production* of the *same* stimulus; or it must be assumed that reactions antagonistic to those leading to flowering dominate in *Sedum* in SD. So, it is evident that daylength may affect different processes in different plants but all these processes result in the same final phenomenon, *viz.* flower formation.

A clear flower-inhibiting effect of non-induced leaves was observed after grafting the LDP *Nicotiana sylvestris* as receptor onto the SDP Maryland Mammoth tobacco and the DNP Delcrest tobacco. As flowering was also obtained in LD when the Maryland Mammoth "donor" was non-induced, it seems that the LDP *N. sylvestris* can always flower provided it is supplied with sufficient "building material" and deprived of its own flower-inhibiting leaves. In view of the response after grafting onto non-induced Maryland Mammoth (*cf.* also 73) it seems that the leaves of the latter do not produce flower-inhibiting substances in the non-adequate daylength.

It can be concluded that photoperiodic induction leads to the production of a transmissible floral stimulus in several plants. On the other hand there are indications that – especially in LDP – leaves in the non-adequate daylength produce flower-inhibiting products.

2. THE POSSIBLE SIMILARITY OF THE FLORAL STIMULI IN DIFFERENT PLANTS

In the preceding section it became obvious that a floral stimulus exists in several plants. We shall discuss now the possible similarity of the floral stimuli in different plants.

As extracts of flowering plants have never induced reproducible flower formation in vegetative plants, the floral stimulus can be studied only *in vivo*, *viz.* when only part of a plant is induced, and in grafting-experiments. In the former case the plants must be photoperiodically sensitive and the results apply only to the plant under investigation; in the latter case different varieties or species may be involved, but the method is limited by the condition that the partners must be graft-compatible.

It will be clear that the question as to the similarity of the floral stimuli in all plants is of great importance. Due to the above mentioned reasons this has only been investigated in a small number of plants which are highly specialized regarding their flowering behaviour. It must be admitted that in the few cases studied it appears that indeed the floral stimuli are identical in related species, although our results obtained with the DNP Delcrest tobacco as donor for the SDP Maryland Mammoth oppose this view and warn against generalization. The failure of DNP to function as donor for photoperiodically sensitive plants does not seem to be a common phenomenon: *e.g.* day-neutral strains of soybean could induce flower formation in a short-day strain (58) and the same holds for *Gossypium* (73, see also 80).

It would be of much interest to know whether the transmissible stimuli in *Perilla* and *Xanthium* (two SDP which differ in many respects as to their flowering response) are identical. However, we could establish experimentally that these two plants do not unite after grafting. The scions remained in a good condition in the polyethylene bags for at least 3 weeks but after removing the bags they wilted. The same experience was gained in grafts between *Perilla* and

day-neutral *Salvia*. The receptors always remained vegetative. Similar negative results in grafts between non-related species (*Chrysanthemum* and soybean as donor for *Perilla*) were also reported by OBSIL (108).

In view of the limitations of the experimental approach the question concerning the similarity of the floral stimuli in various plants must remain unanswered for the present. It is not justified to assume *a priori* that they are identical in all plants as is often done in literature.

3. TRANSLOCATION OF THE FLORAL STIMULUS

It is known from literature and it has been observed with various plants in the present investigations that the floral stimulus moves up and down the stem. This seems to be in sharp contrast to the strict basipetal pathway followed by auxin and has been used as an argument that the two are not identical (79, p. 254). However, the polar auxin transport has been demonstrated only by applying auxin to short sections of stems or other tissues, but little is known about transport of the naturally occurring auxins in *intact green* plants (*cf.* 110). Thus, it appears that there is insufficient evidence to conclude on the base of differences in translocation that auxin and the floral stimulus are not identical.

Besides our results an impressive amount of data in literature (*e.g.* 10, 23, 50, 73, 81, 83, 136) indicates that the floral stimulus moves mainly in the phloem with the stream of assimilates. Thus, if in the graft-combinations between donors and receptors the stream of assimilates coming from the induced leaves does not reach the buds on the receptors, no flowering response will become evident.

Unfortunately little is known about the export and import of assimilates by leaves and buds (*cf.* 23), and the mechanism of phloem transport is still poorly understood (8, 27, 35, 154).

Recent studies with the strawberry plant, a SDP, have clearly demonstrated the generation of two different streams of assimilates (different in the sense that they can direct the development of buds into two different ways) by induced and non-induced leaves, *viz.* a *reproductive* and a *vegetative* stream respectively (*cf. Perilla*, p. 50). HARTMANN (55) had shown already that the flower-inducing stimulus can be transmitted from induced parent plants *via* the stolons to non-induced runner plants. This case has been studied further by GUTTRIDGE (45, 46). He observed the opposite, *viz.* that a *vegetative* stimulus moved from non-induced runner plants to induced parent plants which resulted in delayed inflorescence initiation, increased stolon production and increased petiole length in the parent plants. All these responses are characteristic of a LD effect. The effect was proportional to the number of leaves retained on the runner plants in LD. Later GUTTRIDGE (46) observed that "effects of the "vegetative" stimulus were observed in the receptor plants only in the treatment where a large movement of assimilates from donor to receptor plants was likely to have occurred".

CRAFTS (27, p. 256) has pointed out already that anatomical studies on the ontogeny of shoot tips (see *e.g.* 33, 34) "indicate that the protophloem sieve tubes are the only vascular elements that could possibly carry the hormone close enough to the meristem". Recent investigations of growing-points (98, 114, 143) have revealed that after beginning of photoperiodic induction the initial changes occur immediately below the tunica. Assuming that these changes are caused by the floral stimulus it becomes clear that this has to move from the ends of the protophloem strands *via* meristematic cells.

By using dyes, viruses, radioactive substances, and growth regulators as indicators for translocation in the phloem, transport rates between 20 and 180 cm per hour were estimated (8, 27, 35, 110).

The transmission rate of the floral stimulus has been determined only in *Perilla* (17, 18) and *Pharbitis nil* (61, 62). The values found were approximately 2 and 9 cm in 24 hours respectively. These rates are considerably lower than those for phloem transport mentioned above. There are several possibilities to explain these divergent estimations. The only evidence for transmission of the floral stimulus is the appearance of flower buds. It seems that this process is too complicated for calculating exact transmission rates. Moreover, it is probable that a certain threshold value of floral stimulus must be exceeded in the growing-points before these change from differentiating leaf primordia to the differentiation of floral primordia. If more than one substance is involved in the floral stimulus one will in fact measure the time required for exceeding the threshold value of the most slowly moving substance. It is not yet clear whether it is warranted or not, to assume (as done in these studies) that the floral stimulus does not become "diluted" when translocated over a long distance, and is not translocated by preference to buds in the immediate proximity of the donor leaf. It must be recalled that in the present investigations with *Perilla* (p. 41) there were no indications for a slow movement of the floral stimulus. But it is obvious that in grafting-experiments with optimally induced leaves the floral stimulus will be translocated in large amounts as soon as a phloem connection has been established.

For a good comparison of rates of transport in the phloem of assimilates and of the floral stimulus, estimations should be carried out in comparable plants of the same species and under equal conditions.

4. THE NATURE OF THE FLORAL STIMULUS

The term "flower hormone" or "florigen" was originally used (14, 15) for a single substance which is produced in the adequate daylength for flowering and not in the non-inductive daylength. It would bring about flowering throughout the Vegetable Kingdom, thus being a specific organ-forming substance in the sense as used by SACHS (124, 125). It seems that the idea of one single substance being responsible for flower formation has been broadened somewhat. For example, TUKEY *et al.* (146) define "flowering hormones" as organic compounds, other than nutrients, produced by plants "which initiate the formation of floral primordia, or promote their development."

In spite of extensive investigations all attempts to extract "flower hormones" from *flowering* plants with which *vegetative* plants can be caused to flower have so far failed to yield reproducible results. Although it is obvious that this is not yet a conclusive proof for the non-existence of such substances, many workers have abandoned the flower-hormone hypothesis and put forward in some form the flower-inhibition hypothesis (see p. 4). According to LONA (89, 90) flowering would be inhibited in the non-inductive daylength by flower-inhibiting substances produced in the leaves whereas normal nutritive substances would cause flowering in the inductive daylength. VON DENFFER (30) expressed the view that *auxin* is a flower-inhibiting factor. Other authors also assumed that auxin plays an important rôle in the flowering-process. For example flower formation would be regulated by the auxin/antiauxin balance (116, 118), the ratio assimilates/auxin (148), or the auxin/florigen balance (38).

Our results have clearly demonstrated that – at least in some plants – photoperiodic induction cannot be interpreted as the removal of a flower-inhibiting effect, but results in the production of a transmissible stimulus in induced leaves. However, as discussed already on p. 52, the demonstration of such a stimulus is not yet a conclusive proof for the existence of *one* flower-inducing substance. It is probable that in a complicated process as flower formation more than one factor is involved, or as it has been formulated for organ formation in general by THIMANN (144, p. 122): "The formation of organs requires a complex interplay of factors, not just a single one. It must depend on the *relative* rates of a number of processes, and on the supply, removal and destruction of many factors". A similar view has been expressed by SÖDING (139, pp. 278 and 279). His idea that the process of organ formation seems to be regulated in certain cases by one substance, *viz.* by the substance which is the limiting factor in the process on hand, but in fact by many other factors (which, however, are present in non-limiting concentrations) may also be applied to flower formation. Following these suggestions it might be assumed that photoperiodic induction leads to production of a very definite substance – or substances – in SDP, and to the production of another substance – or substances – in LDP. As discussed on p. 73, however, it must be concluded that in the three cases thus far investigated photoperiodic induction leads to production of the same stimulus both in SDP and LDP, although further experimental evidence is necessary before it is warranted to extend these findings to SDP and LDP in general. However, the limiting factor may be the same in closely related species, but it needs not be identical in non-related species. This consideration, combined with the fact that it is impossible for the moment to say whether even in the species studied the limiting factor consists of one or more substances, does not permit the assumption of one flower hormone.

5. GIBBERELLINS AS FLOWER-INDUCING SUBSTANCES

Gibberellins are metabolic products produced by certain strains of the fungus *Gibberella fujikuroi* (SAW.) WR. (142). Recently the application of these substances to non-flowering plants has come into vogue. Short notes appear weekly which, however, will not be reviewed and discussed in detail. Reference will be made only to investigations by LANG (75) because these are the most extensive up to now. Moreover, this author has made an attempt to explain the differences in flowering response among various plants. From his work and other data in literature it appears that a number of biennials and LDP can be brought to flower formation under non-inductive conditions by treating them with gibberellins. So, in these plants application of gibberellin can replace the cold and/or long-day requirement for flower formation. In certain plants the flowering response was comparable with the response evoked by an optimal inductive treatment, but in other plants the response to gibberellin was inferior to such a treatment, whereas other species responded only with stem elongation without flower formation. All SDP thus far investigated remained vegetative in LD after application of gibberellin.

LANG has put forward two possibilities to explain the different responses to gibberellin:

1) Work of PHINNEY *et al.* (113) has shown that a variety of gibberellin-like substances occurs in higher plants. However, these substances are not chemi-

cally identical with the known gibberellins. LANG (75, p. 715) writes "it is conceivable that these different gibberellins have some degree of species-specificity. In that case, a given plant may not respond optimally to a "foreign" gibberellin".

2) It may be that gibberellin is not the only limiting factor for flower formation in biennials and LDP.

In a later paper LANG *et al.* (78) have shown that extracts obtained from the endosperm of *Echinocystis macrocarpa* GREENE (which is very rich in gibberellin-like materials) can induce bolting and flowering in the biennial *Hyoscyamus niger* and in the LDP *Samolus parviflorus* when grown under non-inductive conditions, so that this is the first case that a material extracted from a higher plant can induce flower formation in vegetative plants.

In the preceding sections it appeared that the floral stimuli are probably identical in LDP and SDP. As gibberellin does not induce flower formation in plants of the latter reaction-type, it cannot be the floral stimulus itself. But the possibility remains that it participates in the production of the floral stimulus in biennials and LDP.

CHAPTER VIII

SUMMARY[1])

I. The physiology of flowering in a number of herbaceous plants was studied with the aid of grafting.

II. Graft-combinations between flowering (donor) and non-flowering plants (receptor) were performed and grown under a non-inducing daylength for the receptors. Cleft-grafting was used throughout. Polyethylene bags were employed for maintaining a high air-humidity around the scions.

III. *Experiments with Perilla crispa (SDP)*

1. The sensitivity of leaves to photoperiodic induction was found to be markedly affected by the position of the leaves on the plant and not by the physiological age. At least in the first 5 pairs of leaves the sensitivity increased in upward direction.
2. Flowering response of stocks in LD was maximal when the donor leaves were exposed to at least 30 SD (optimally induced leaves). Leaves with an area of 1 or 2 cm^2 could induce vegetative stocks to flower. Optimally induced leaves which were continuously darkened from the moment of grafting caused LD stocks to flower.
3. With optimally induced leaves the contact period between donor leaves and receptors had to last approximately 10 days for obtaining flowering on all stocks. This period increased when donor leaves were suboptimally induced. Anatomical observations and translocation experiments with labelled sucrose indicated that a phloem connection between donor and receptor was necessary for transmission of the floral stimulus.
4. Induced leaves were grafted and regrafted onto several groups of stocks in LD consecutively; as long as these leaves remained in a good condition, they

[1]) The Roman and Arabic figures in this summary refer to the corresponding chapters and sections.

continued inducing stocks to flower, even when more than 3 months had elapsed since the last inductive cycle. Indirectly induced shoots could not function as donor. The strictly localized induced state and the transmissible floral stimulus were distinguished as separate phenomena. Leaves without buds and/or roots could be induced. The induced state was not destroyed when the leaves were exposed to continuous light of high intensity or to high temperature, or when they were treated with auxin, or enzyme inhibitors. Photoperiodic induction was characterized as an irreversible, localized, non-correlative, and indestructible phenomenon.

5. The floral stimulus was found to move only through living bark. Transmission of the floral stimulus from induced stocks to non-induced scions was inhibited by leaves on the receptors, but in the reciprocal combination LD leaves did not exert a flower-inhibiting effect. The same phenomenon was observed in double grafts when the interstocks functioned as donors. Lateral transmission of the floral stimulus was obtained after removal of half a ring of bark above the receptor shoots and in certain double-stock and double-scion grafts.
6. It was concluded that SD treatment results in the production of a flower-inducing stimulus which is transmitted from induced leaves to growing-points with the stream of assimilates.

IV. *Experiments with Xanthium pensylvanicum (SDP)*

Leaves without buds could be induced. Indirectly induced shoots could function as donor. It was concluded that the floral stimulus is multiplied in actively growing buds.

V. *Experiments with Crassulaceae*

1. Flowering response of *Kalanchoë* (SDP) stocks in LD increased with increasing size of donors. Transmission of the floral stimulus was inhibited by non-induced leaves only in upward direction.
2. Vegetative shoots of the LDP *Sedum ellacombianum* and *S. spectabile* in SD did not respond with flower formation after grafting onto previously induced stocks of the species concerned.
3. Vegetative plants of *Bryophyllum* (LSDP) either grown continuously in LD or SD could be induced to flower by grafting onto previously induced stocks.
4. The LDP *Sedum ellacombianum* and *S. spectabile* flowered normally in SD after grafting onto induced stocks of *Kalanchoë* (SDP). Defoliation of *Kalanchoë* stocks indicated that the leaves were the organs which induced *Sedum* scions to initiate flower buds in SD. Non-induced *Kalanchoë* stocks could not induce flower formation in *Sedum* scions as receptors.
5. Both *Sedum ellacombianum* and *S. spectabile* could bring *Kalanchoë* to flower in LD. It was concluded that the floral stimuli are identical in *Kalanchoë* and *Sedum*.

VI. *Experiments with Nicotiana*

1. The SDP Maryland Mammoth tobacco flowered normally in LD after grafting onto the LDP *Nicotiana sylvestris*, but not on stocks of the day-neutral Delcrest tobacco. When a Delcrest interstock was grafted between a *N. sylvestris* donor and a Maryland Mammoth receptor, flower formation

could occur in the latter. Leaves on the interstock inhibited transmission of the floral stimulus in upward, but not in downward direction.
2. The LDP *N. sylvestris* flowered in SD after grafting onto short-day Maryland Mammoth and day-neutral Delcrest tobacco, provided the scions were defoliated. Non-defoliated scions formed rosettes on the tops of the stocks.
3. The numbers of leaves formed before the inflorescence were decreased significantly on shoots of day-neutral Delcrest tobacco after grafting onto various stocks in LD. Indications were obtained that the roots or stems of the stocks were the organs which caused accelerated flowering.

VII. The possible similarity of the floral stimuli in various plants was discussed. Due to the restrictions of the technique (studies *in vivo*) this problem remains unsolved for the present because non-related species are graft-incompatible. However, in some closely related species, which have thus far been investigated, the floral stimuli seem to be identical. But the non-identity in Delcrest and Maryland Mammoth tobacco warns against generalization.

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SAMENVATTING

ONDERZOEKINGEN OVER BLOEMKNOPVORMING MET BEHULP VAN ENTEN

I. Bij een aantal kruidachtige gewassen werd de fysiologie der bloemknopvorming bestudeerd met behulp van enten.

II. Entcombinaties werden gemaakt tussen bloeiende (donor) en niet bloeiende planten (receptor) en gekweekt in de voor de bloei van receptors verkeerde daglengte. Steeds werd spleetenting toegepast. De bovenstammen werden gehuld in zakken van polyethyleen voor het verkrijgen van een hoge luchtvochtigheid (Foto 1).

III. *Proeven met Perilla crispa, een korte-dag plant.*

1. De gevoeligheid van de bladeren voor de fotoperiodieke inductie bleek sterk af te hangen van de positie der bladeren aan de plant, echter niet van hun fysiologische leeftijd. Tenminste in de eerste 5 bladparen nam de gevoeligheid toe vanaf de basis in bovenwaartse richting.
2. Onderstammen in lange dag (LD) reageerden het snelst met bloemknopvorming als de donor bladeren minstens 30 dagen in korte dag (KD) waren geweest (optimaal geïnduceerde bladeren). (Foto 3). Een bladoppervlakte van 1 of 2 cm^2 was reeds voldoende om bloei te induceren. Werden optimaal geïnduceerde bladeren vanaf het enten continu verduisterd, dan induceerden ze toch bloei aan de LD onderstammen.

3. Om bloei aan alle onderstammen te verkrijgen, moest het entcontact tussen optimaal geïnduceerde bladeren en onderstammen tenminste 10 dagen duren (Fig. 4, p. 22). Anatomisch onderzoek en transportproeven met radioactieve suiker wezen uit, dat tussen donor en receptor een floëemverbinding moest worden gevormd, alvorens de bloeistimulus de entplaats kon passeren.
4. Geïnduceerde bladeren konden achtereenvolgens op verschillende groepen onderstammen worden geënt; zolang deze bladeren in goede conditie bleven, veroorzaakten ze bloei aan de onderstammen, waarop ze werden geënt, zelfs al waren meer dan 3 maanden verstreken na de laatste inducerende cyclus. Indirect geïnduceerde scheuten (dat zijn scheuten van onderstammen, die door enting met geïnduceerde bladeren in LD zijn gaan bloeien) fungeerden niet als donor (Fig. 9, p. 36). Eén onderscheid werd gemaakt tussen de geïnduceerde toestand, die strikt gelocaliseerd is, en de transportabele bloeistimulus. Bladeren zonder knoppen en/of wortels konden geïnduceerd worden. De geïnduceerde toestand werd niet te niet gedaan door de bladeren bloot te stellen aan continu sterk licht, of hoge temperatuur, of door ze te behandelen met auxine, of enzymgiften. De fotoperiodieke inductie werd gekenschetst als een irreversibel, gelocaliseerd, niet-correlatief, en onvernietigbaar verschijnsel.
5. De bloeistimulus werd slechts door levende bast vervoerd. Transport van de bloeistimulus uit geïnduceerde onderstammen naar vegetatieve bovenstammen werd geremd door LD blad aan de receptors, maar in de reciproke combinaties remde het LD blad niet (Fig. 10, p. 42). Ditzelfde werd ook waargenomen bij tussenstamentingen als de tussenstam als donor fungeerde (Fig. 11, p. 46). Zijwaarts transport werd verkregen als boven de receptorscheuten een halve ring bast werd weggenomen, en in bepaalde entcombinaties, waarbij 2 onderstammen of 2 bovenstammen als donors fungeerden (Fig. 12, p. 48).
6. Er werd geconcludeerd, dat KD behandeling de productie van een bloeiinducerende stimulus tengevolge heeft, die met de assimilatenstroom van de geïnduceerde bladeren wordt vervoerd.

IV. *Proeven met Xanthium pensylvanicum, een korte-dag plant*

Bladeren zonder knoppen konden worden geïnduceerd. Indirect geïnduceerde bladeren konden als donor fungeren. Geconcludeerd werd, dat de bloeistimulus in de groeiende knoppen wordt vermeerderd.

V. *Proeven met Crassulaceae*

1. De bloei van *Kalanchoë* (KDP) onderstammen in LD nam toe naar mate de omvang van de donors groter was. Transport van de bloeistimulus werd door LD bladeren slechts in bovenwaartse richting geremd (Foto 5 en 6).
2. Vegetatieve scheuten van de LDP *Sedum ellacombianum* en *S. spectabile* konden in KD niet tot bloei gebracht worden door enting op van te voren geïnduceerde onderstammen.
3. Vegetatieve *Bryophyllum* planten (lang-korte-dag plant) konden zowel bij continu verblijf in LD als in KD door enting op van te voren geïnduceerde onderstammen tot bloei worden gebracht.
4. Beide LDP *Sedum ellacombianum* en *S. spectabile* bloeiden normaal in KD na enting op geïnduceerde *Kalanchoë* onderstammen. Door ontbladering

van de onderstammen kon worden aangetoond, dat de bladeren de organen waren, die de *Sedum* bovenstammen tot bloei brachten (Foto 7). Niet-geïnduceerde *Kalanchoë* onderstammen konden *Sedum* receptors niet tot bloei brengen (Foto 9 en 10).

5. Zowel *Sedum ellacombianum* als *S. spectabile* kon *Kalanchoë* in LD tot bloei brengen (Foto 8). Geconcludeerd werd, dat de bloeistimuli van *Kalanchoë* en *Sedum* identiek zijn.

VI. *Proeven met Nicotiana*

1. De KDP Maryland Mammoth tabak bloeide normaal in LD na enting op onderstammen van de LDP *Nicotiana sylvestris*, maar niet op onderstammen van dagneutrale Delcrest tabak. Wanneer een Delcrest tussenstam werd geënt tussen de *N. sylvestris* donor en de Maryland Mammoth receptor, dan kon bloei optreden aan de receptor. Bladeren aan de tussenstam remden de overdracht van de bloeistimulus naar boven, maar niet naar beneden.
2. De LDP *N. sylvestris* bloeide in KD na enting op korte-dag Maryland Mammoth en dagneutrale Delcrest tabak, mits de bovenstammen werden ontbladerd. Niet-ontbladerde bovenstammen vormden rozetten boven op de onderstammen (Foto 11).
3. Het aantal bladeren vóór de bloeiwijze gevormd op scheuten van dagneutrale Delcrest tabak kon aanzienlijk worden verminderd door enting op verschillende onderstammen in LD. Er werden aanwijzingen verkregen, dat de wortels of stengels de organen waren, die de bloei vervroegden.

VII. De vraag werd besproken, of de bloeistimuli in verschillende planten identiek zijn. Door beperkingen van de techniek (onderzoek *in vivo*) moet dit probleem voorlopig onopgelost blijven, omdat niet-verwante soorten entingsincompatibel zijn. In enkele nauw verwante soorten, die tot nu toe onderzocht zijn, schijnen de bloeistimuli echter gelijk te zijn. Maar het feit, dat dit niet het geval lijkt te zijn bij Delcrest en Maryland Mammoth tabak, waarschuwt voor generaliseren.

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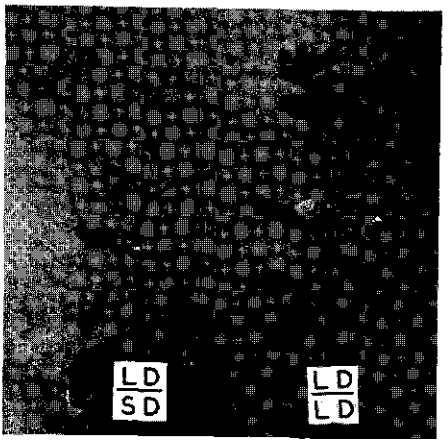

PHOTO 5.
Kalanchoë blossfeldiana. Left: graft-combination LD−/SD+; receptor shoots flowering. Right: LD−/LD+; receptors vegetative. Photo taken 133 days after grafting.

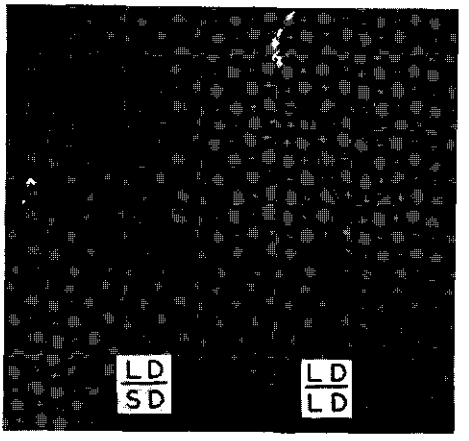

PHOTO 6.
Kalanchoë blossfeldiana. Left: LD+/SD+; inflorescences on receptor show phyllody. Right: LD+/LD+; shoots vegetative. Photo 133 days after grafting. Compare photo 5.

PHOTO 7.
Sedum spectabile grafted onto *Kalanchoë blossfeldiana* in SD. Left: stock with leaves; *Sedum* flowering. Right: stock defoliated at date of grafting; *Sedum* vegetative. Photo 96 days after grafting.

PHOTO 8.
Kalanchoë blossfeldiana grafted onto *Sedum spectabile* in LD. Left: stock with leaves; *Kalanchoë* flowering. Right: stock defoliated at date of grafting; *Kalanchoë* vegetative. Photo 130 days after grafting.

PHOTO 9.
Sedum ellacombianum grafted onto two-branched *Kalanchoë blossfeldiana*. Whole combination in SD. Both *Kalanchoë* donor and *Sedum* receptor flowering. Photo 68 days after grafting.

PHOTO 10.
Sedum ellacombianum grafted onto two-branched *Kalanchoë blossfeldiana*. *Sedum* in SD, *Kalanchoë* in LD. Both *Kalanchoë* „donor" and *Sedum* receptor vegetative. Photo 80 days after grafting.

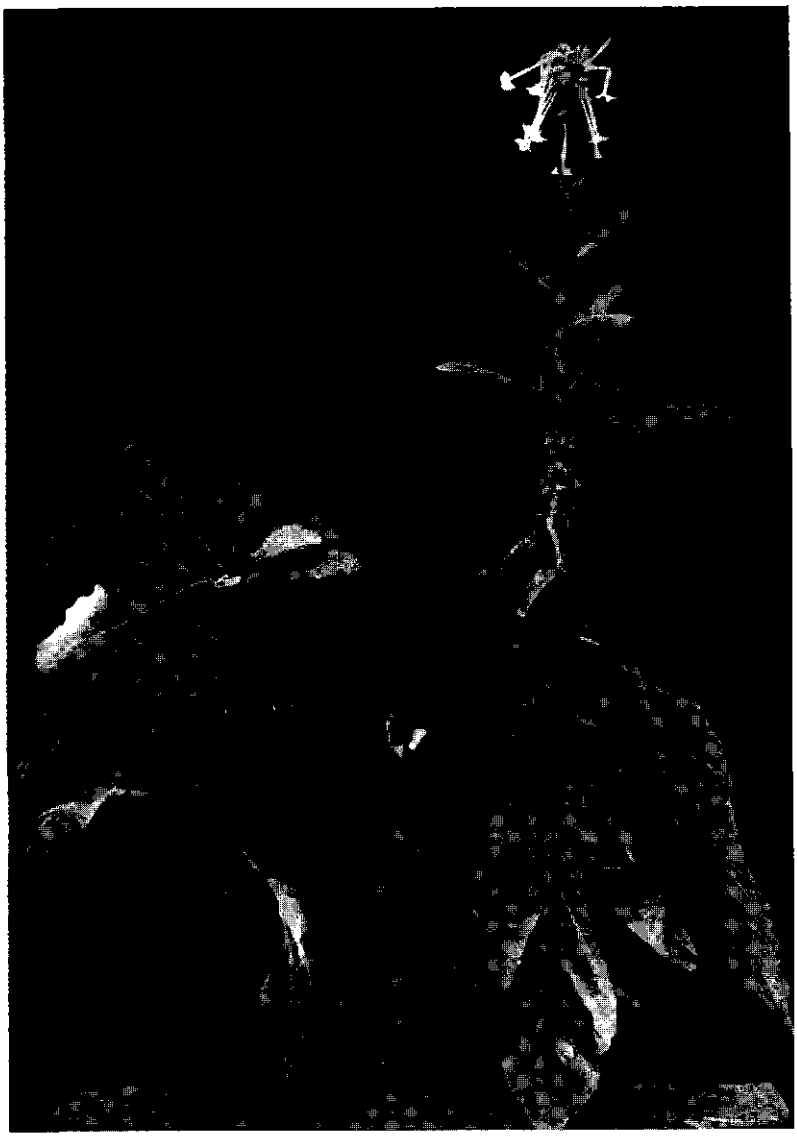

PHOTO 11.
Nicotiana sylvestris grafted onto Delcrest tobacco in SD. Left: *N. sylvestris* non-defoliated, vegetative. Right: *N. sylvestris* defoliated until appearance of flower buds. Photo 101 days after grafting.