PHOTOPERIODISM, FLORAL INDUCTION AND FLORAL INHIBITION IN *SALVIA OCCIDENTALIS*

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635.939.492:581.145.1.035

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(MET EEN SAMENVATTING IN HET NEDERLANDS)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS IR. W. F. EIJSVOOGEL,
HOOGLERAAR IN DE HYDRAULICA, DE BEVLOEIING,
DE WEG- EN WATERBOUWKUNDE EN DE BOSBOUWARCHITECTUUR,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG 16 OKTOBER 1964 TE 16 UUR

DOOR

SURESH CHANDRA BHARGAVA

H. VEENMAN & ZONEN N.V. – WAGENINGEN – 1964

STELLINGEN

Propositions

I

The postulation of LANG that the inhibitory effects of non-inductive daylengths are not transmissible and interfere with the production rather than with the action of florigen, is not justified.

ANTON LANG. Proc. 22nd Biol. Colloq. Oregon State Univ. Oregon, 1961: 68–69.

II

It is untenable that flower inhibiting substance(s) produced under the influence of unfavourable daylengths have physiological properties of auxins and/or gibberellins.

D. VON DENFFER. Naturw. 37, 1950: 296–301, 317–321.
W. W. SCHWABE. J. Expt. Bot. 10, 1959: 317–329.

III

It is not necessary to accept the formation of a specific flower hormone after vernalization.

IV

WASSINK and STOLWIJK's statement to include the kind of light given during the main light period, while defining "critical day-length", is of limited value.

E. C. WASSINK and J. A. J. STOLWIJK.
Ann. Rev. Plant Physiol. 7, 1956: 375–376.

V

The opinion of ADKISSON that an endogenous rhythm is involved in the photoperiodic induction of diapause in the pink bollworm, *Pectinophora gossypiella* Saunders, cannot yet be considered as fully justified.

P. L. ADKISSON. Progress Report 2274. Texas Agr. Expt. Sta. 1963.

VI

The emphasis on birth control through deliberate family planning schemes in most developing countries is not *per se* a panacea for economic ills.

VII

The establishment of an International "Pool of Horticulturists" as part of the International Society of Horticultural Science would be advisable, especially for the benefit of less developed horticultural regions.

S. C. BHARGAVA

Wageningen, oktober 1964

VIII

The observation made by PIEL that "in deployment of her limited physical resources and precious human resources India should take care to distinguish technology from science" is most valid and should be weighed carefully by Indian planners and bursars.

G. PIEL. Nature 202, 1964: 1154–1155.

IX

The structure of the „Studenten verenigingen" in Wageningen needs remodelling to fit the needs of a present day society.

PRELUDE

After completion of my seven years of uninterrupted academic stay at the State Agricultural University, Wageningen, I wish to express my sincere thanks to all those who helped realize my aims and ambitions and to those who guided me to the main path which I often lost in the wilderness of scientific myth.

I owe much to my late Father, whose love and sacrifices I shall never be able to repay in many a births. To you Mother, and brother Girish, your long-term support and regular affectionate briefing made me feel at home away from home, which I shall always cherish.

I am most grateful to my Teacher Professor WELLENSIEK whose inspiring and humane guidance in many respects throughout my stay in the Netherlands I shall always treasure and value. Your very brief but precise comments as a Promotor 'research is never complete' and 'every research can be commented' gave me enough courage to present my own. Your manner of working has set many examples to those who came in close contacts with you which one can but strive to emulate.

I owe considerable debt of gratitude to Professor Dr. Ir. J. DOORENBOS who introduced me to the horticultural teaching in the Netherlands and for his continued interest especially during initial years of my studies.

I am greatly indebted to Professor Dr. J. DE WILDE and Professor Dr. R. PRAKKEN under whom I had the privilege of receiving training in the fields of Entomology and Genetics during my "Ingenieur" study.

This work owes much to my colleagues Ir. J. P. M. BINK, who offered many problems, valuable suggestions and above all his kind friendship. To Ir. G. W. M. BARENDSE I am especially thankful for the statistical analysis and for his generous hospitality.

Thanks are also due to Dr. G. MEIJER of the Philips Research Laboratories, Eindhoven, who not only provided me the plant material, but also many opportunities to discuss this problem with him.

I gratefully acknowledge the help of Dr. Ir. H. C. M. DE STIGTER in the histological work, Dr. Ir. J. BRUINSMA and Mr. J. SWART in making microphotographs of the apical buds and K. R. NARAYANAN M.Sc. for scoring the mitotic counts.

Acknowledgement is due to Mr. G. WAINES and Mr. J. W. KENT, who took considerable interest in correcting the English text. The proofs were read by Miss A. J. B. WOLDA and the Dutch summary was prepared by Ir. H. F. WATERSCHOOT for which I am thankful.

Sincere efforts of Messrs R. JANSEN and H. VAN LENT in preparing the drawings and photographs; of Mr. VAN DE PEPPEL and his colleagues for constructing and running the automatic device and electrical installations, are deeply appreciated.

Thanks are also due to Mr. R. SABARTE BELACORTU and his colleagues, G. VAN

WELIE, A. TIEMESSEN and H. F. VAN DE PEPPEL for their care of the experimental plants.

I sincerely thank Miss MIRJAM WITTEN for her quick-clean approach in typing the manuscript and Mrs JEANNE DE PAUW and Mrs JANSJE ELINGS for their administrative help.

I would especially like to express my appreciation to Dr. D. DE WAAL and Ir. D. W. R. LOS former Dutch Agricultural Attaches at New Delhi for their first introduction to me about Holland; and to Ir. A. H. HAAK and Ir. C. J. VAN BIJLERT of the International Agricultural Centre for their considerable personal and official interest during my stay in Wageningen.

I am extremely grateful to the authorities of the International Agricultural Centre for awarding me the Fellowship and to the State Agricultural University for providing facilities, research grant and for financing the preparation of this manuscript.

CONTENTS

This thesis will also be published as Mededelingen van de Landbouwhogeschool, No. 64–12 (1964) (Communication of the Agricultural University)

CHAPTER 1

GENERAL

1.1. Introduction

The fascinating appearance of flowers on a plant is of basic biological significance, not only because flowers are the last stage in the ontogeny, but also because they are organs of sexual reproduction and therefore responsible for maintaining the species. Moreover, the processes leading to the onset of reproductive development provide us with tools for an understanding of differentiation. The phenomenon of flower formation is characterised by the fact that sometime during development the apical meristem produces, instead of more leaf primordia, floral primordia. The initiation of flower primordia is generally visualised as an interaction between the genetic constitution and the environmental factors as experienced by the plant during its life cycle. Two environmental factors which control the growth and developmental processes in plants in a specific manner are temperature and daylength.

The onset of flowering is not only of theoretical importance, but its control is desirable in many cultivated plants. In horticultural practice some plants are grown in which vegetative parts are used, such as many edible leaf, stem and root vegetables, showy leaf ornamentals etc. This involves suppression of flowering. On the other hand, plants which are grown for their flowers (ornamentals), their fruits (fruit crops and fruit vegetables) or for their seeds (legume or pulse crops) require promotion of flowering. Therefore, both the negative and positive control of flowering have become an integral part of the horticultural industry. It is thus essential that more insight into the fundamental knowledge should be made available for better understanding of the flowering processes.

1.2. Review of literature

Specific, well documented examples of the control of flowering as affected by environmental factors have appeared in many excellent reviews (2, 8, 10, 20, 25, 35, 36, 43, 56, 62, 64, 76, 81, 89, 109, 115 and 116).

Collections of papers presented at Symposia on photoperiodism are now available (21, 29, 79 and 111). Several books (45, 90 and 103), devoted entirely to flowering, have recently been published. A comprehensive survey of flowering and allied topics has been published by a number of investigators in 'Encyclopedia of Plant Physiology' (87). In view of the extensive reports available, only a brief outline is presented to account for both the classical and current concepts as pertaining to the present avenue of investigations.

The classical concept which led to the formulation of systematic studies in the field of physiology of flowering dates back to the late 19th century. Sachs (88, 1865) after working with *Tropaeolum* plants and *Begonia* leaves was the first to have produced experimental evidence that leaves in light generate definite

flower forming substances in minute quantities, which direct the assimilates to the formation of flowers.

VÖCHTING (104, 1892) demonstrated in his transplantation experiments in which *ready to flower* stems of *Beta vulgaris* were grafted on *unready to flowering* roots, that flowering of the former was inhibited. These results led to the conclusion of *flower inhibiting substances* coming from unready to flower beet.

Thus both these investigators were among the first people to recognise the potentialities of *flower forming* and *flower inhibiting* substances.

Currently, the phenomenon by which plants respond to daylength and by which they measure time, thereby controlling growth and development, is termed *photoperiodism*. This *adhoc* discovery is of recent origin. The recognition of its significance is due to the vision of two American scientists, GARNER and ALLARD (30, 1920). Accordingly, plants sensitive to daylengths have been classified as short-day, long-day and day neutral plants.

The first question faced by the earliest student of floral physiology was to elucidate, which plant organ perceives daylength. The problem of site-of-light perception was solved by KNOTT (53, 1934) while working with spinach, a long-day plant. He produced evidence that the foliage responds to a photoperiod favourable to reproductive growth by the production of some substance which is transported to the growing point.This finding was extended with other plants: CHAILAKHYAN (15, 1936) and MOSHKOV (77, 1936) for *Chrysanthemum*, PSAREV (83, 1936) for soybean, and LJUBIMENKO and BUSLOVA (63, 1937) for *Perilla*. MOSHKOV (77. 1936) suggested that the youngest, fully expanded leaves are most sensitive, whereas KHUDAIRI and HAMNER (52, 1954) demonstrated in *Xanthium* that half-expanded leaves are most sensitive to photoperiodic induction. ZEEVAART (114, 1958) concluded that in *Perilla* fully expanded leaves are sensitive to short-day treatment. Quantitative differences in sensitivity were expressed in terms of leaf position rather than in the differences in the physiological age.

Thus it is clear that the flower forming effect of inductive daylength generates a flower hormone which has its origin in the leaf and its action at the apex. However, what is not clear is the flower-inhibiting effect of a non-inductive daylength or a non-induced leaf. The question is put whether this inhibitory effect is due to the production of a specific flower inhibitor or to lack and dilution of a floral hormone.

Accordingly, the interpretations of the hormonal concept of flower control may be divided into three groups, which refer to the concepts of SACHS and VÖCHTING – as cited above – and to a combination of them respectively.

(1) *Flower hormone hypothesis.* At one extreme are those, who advocate that flowering is an over-all inductive process (6, 7, 9, 15, 17, 36, 56, 57, 65, 78, 114). Thus exposure of plants to inductive cycles results in the fomation of an induced state. This is followed by the ability to produce flower stimulus. The former is localised while the latter state is transmissible. This was very clearly distinguished in *Perilla* by elegant grafting experiments, ZEEVAART (114, 1958). Other evidence indicative of the existence of such a hypothetical stimulus emerges from

grafting an induced donor to a non-induced receptor. Flowering takes place in the receptor, provided it is defoliated. Clearly a transmissible flowering stimulus is involved, which is hormonal in nature, as demonstrated by CHAILAKHYAN (16, 1936). KUYPER and WIERSUM (55, 1936), MOSHKOV (55, 1937), LANG (56, 1952), LONA (68, 1949), CARR (13, 1953).

The hypothetical flower hormone governing the developmental processes has been baptised as florigen by CHAILAKHYAN (15, 1936). Since then many new names have been suggested, *anthesin* by CHOLODNY (20, 1939), *anthocaline* by VAN DE SANDE BAKHUYZEN (1, 1947). Very recently CHAILAKHYAN (17, 1958) suggests that florigen consists of two substances, *anthesin* and *gibberellin* but this is disputed by ZEEVAART (115, 1963). However, the transmissible stimulus has an identical nature in many plants, LANG (56, 1952) and ZEEVAART (114, 1958), as has been shown by reciprocal grafting, i.e. a long-day donor on a short-day receptor and vice-versa.

Other information about the characteristics of this stimulus stems from the following experiments. If a single leaf was induced and the rest of the plant kept under non-inductive conditions, flowering took place, HAMNER and BONNER (37, 1938), HAMNER and NAYLOR (38, 1939). If single leaves were induced and periodically cut-off, a gradual movement of the stimulus could be observed, e.g. in *Xanthium*, LOCKHART and HAMNER (65, 1954) and *Pharbitis*, ZEEVAART (115, 1963). The crude extraction of flower inducing substances has been reported in *Xanthium*, LINCOLN *et al* (59, 1961; 60, 1962). This active material can also be obtained from day neutral sunflower, MAYFIELD *et al* (71, 1962), suggesting similarity of these substances in different plants. However, universal action has not yet been found.

Judging from numerous evidences LANG (56, 1952) has pointed out that the existence of florigen is now generally accepted and that this would control floral initiation in a direct and positive manner.

(2) *Flower-inhibition hypothesis.* At the other extreme are those interpretations which support the contention that flowering is due to the removal of an inhibition. LONA (67, 1949; 69, 1959) suggested that exposure of leaves to a non-inductive condition results in the production of flower-inhibiting or anti-anthogenic substances. Removal of leaves in an adverse condition would result in flowering, whereas normal nutritive substances would cause flowering in inductive daylengths. These results have been extended in other short-day plants, *Chenopodium*, LONA (66, 1948), strawberry, THOMPSON and GUTTRIDGE (101, 1960) and in the long-day plant *Hyoscyamus niger*, MELCHERS (75, 1952).

GREGORY (32, 1948, p. 76) mentioned the problem in a more precise manner, stating: 'We must suppose that necessary genes are already present in the fertilized ovum and therefore if external factors are such that no flowers are formed, there must be inhibiting factors at work, in a word the problem may quite well be considered as one of 'failure to flower' as of promoting flowering.'

This concept was further elaborated by VON DENFFER (24, 1950), who believes that plants have a natural tendency to flowering, but when they do not do so, some inhibiting factors prevent their actual flowering. This concept categori-

cally implies that the action of flower promoting factors would consist of negating the flower inhibiting factors. It was suggested that these factors were auxins.

Other investigators have emphasized the importance of inhibitory processes governing floral initiation in studies involving alternation of inductive and non-inductive cycle(s). Prominent among the demonstration of an inhibitory effect of long-day in short-day plants is the work of SCHWABE (91, 1956; 92, 1957; 93, 1959) with *Kalanchoë blossfeldiana*, *Perilla nankinensis*, *Chenopodium amaranticolor*, and Biloxi soybean, who suggests it interferes with the promotive effect of inductive cycles and thus blocks or limits their effect. This inhibition is due to the production of inhibitory substance(s). WELLENSIEK (106, 1958; 107, 1959) further demonstrated with *Perilla crispa* that light inhibits only the origin of the induced state and not the production of a flowering stimulus. Since the 'induced state' in the leaves is not transportable, we would not expect the light inhibition to be either. In other words, the production of the inhibitor is strictly localized in the leaves and hence does not possess a hormonal nature. Inhibitory action of light has also been shown in many other plants, Biloxi soybean by LONG (70, 1939), WAREING (105, 1954), CARR (14, 1955) and SIROHI and HAMNER (95, 1962); *Chrysanthemum morifolium* by POST (82, 1950); *Begonia evansiana* ANDR by ESASHI (26, 1961); *Xanthium* and *Chenopodium* by THOMAS (99, 1962).

However, some studies have shown that the effects of non-inductive daylengths are transmissible and not simply localized. After preliminary remarks by RESENDE (85, 1949; 86, 1955) and by NAUNDORF (80, 1954), this was clearly shown by GUTTRIDGE (33, 1959; 34, 1959). He has obtained evidence that the non-inductive condition i.e. long-day in the quantitative short-day plant strawberry, produces substances which are growth promoting and flower inhibiting. These substances, which are transmissible, have been demonstrated when donor plants either received long-days or a light-break in the middle of a long night, whereas in the receptor, itself in short days, flowering was inhibited considerably. The present author, in a preliminary communication (4, 1963), presented evidence that a transmissible flower bud inhibitor is involved in *Salvia occidentalis*. This inhibitor is generated in continuous light prior to the induced state. The action is due not so much to the position of the inhibited leaves as to the moment at which inhibitory light is administered. This inhibitor has its origin in the leaves and its action at the apex. The present paper presents the details of this work.

(3) *Balance between hormone and inhibitor hypothesis.* Some where between the two are those who assume that flowering involves two processes:
(a) the removal of an inhibitor formed in non-inductive daylengths and
(b) the production of a flower stimulus in inductive daylengths.

This concept as a basis for explaining the mechanism of flower formation, has been widely accepted by many investigators. Valuable information has emerged from the work of SCHWABE (91, 1956), LINCOLN *et al* (58, 1956), WELLENSIEK (106, 1958), DE LINT (61, 1960), BEST (3, 1960), IMAMURA (48, 1961) and RAGHAVAN and JACOBS (84, 1961).

EVANS (27, 1960; 28, 1962) experimenting with the long-day plant *Lolium* and the short-day plant *Rottboellia* has recently concluded that inductive and non-inductive leaves generate specific, transmissible, flower-inducing and flower-inhibiting substances respectively. This inhibitor acts at the apex and hence does not interfere with the formation of the flower-inducing principle, but with its functioning. Flowering in short-day plants takes place as a result of the most favourable balance between these two substances, changing as induction proceeds.

1.3. SCOPE OF THE INVESTIGATIONS

The present investigations were undertaken to study the nature of light inhibition and its role in the flowering processes of the short-day plant *Salvia occidentalis*. This study can be divided into the following two sections:
(1) Describes in general the photoperiodical behaviour of the test plant.
(2) Deals with the characteristics of light inhibition. This was approached by using two methods. Firstly with normal green intact plants endeavour has been made to include a variety of problems, such as: the role of inductive cycles prior to the induced state being reached; the stages in the flowering processes which are sensitive to light-inhibition; its critical length; the manner by which the long days exert their cumulative effect; whether long days act on the preceding and/or succeeding effects of short days; the similarity of light and night-break inhibition. Secondly, with partially defoliated plants, the following problems were studied: the site of inhibition perception, leaf and/or apex; the transmissible nature of a light induced inhibitor; whether or not inhibitor interferes with stimulus formation and its translocation; probable mechanism by which the inhibitor operates.

CHAPTER 2

MATERIAL AND METHODS

2.1. PLANT MATERIAL AND RAISING CONDITIONS

Salvia occidentalis is a member of the family *Labiatae*. It is a sub-tropical weed and supposed to originate in the West-Indies. Certain discrepancies relating to the taxonomy of this species are mentioned in the literature. KRAMER (54, 1923) reported that *Salvia occidentalis* SWARTZ has two forms which differ in their growth habits: (a) an upright form and (b) a creeping form. After close taxonomical examination VAN STEENIS (97, 1936) suggested that these two forms be raised to the rank of separate species and be called *Salvia privoides* BTH and *Salvia obscura* BTH respectively. That these two forms have constant morphological differences is further suggested by HILLE RIS LAMBERS (see VAN STEENIS, 97, 1936, p. 1637). HILLE RIS LAMBERS (personal communication) is of the opinion that the test plant used in the present investigations is not *Salvia occidentalis* SW. but *Salvia privoides* BTH. His conclusions are based on the differences in leaf, flower size and flower colour etc. This warrants further taxonomical investigations to establish the identity of the material in question. However, we shall continue using *Salvia occidentalis* on grounds of its original usage in photoperiodical research by MEIJER (72, 1957) and WELLENSIEK (108, 1960).

Salvia occidentalis is an obligate short-day plant, MEIJER (72, 1957). If raised in short-days of 8 hours of light and 16 hours of darkness, flower buds are initiated, whereas in long-days of 16 hours of strong white light and 8 hours of darkness, the plants will remain vegetative indefinitely.

The plant material was usually raised from cuttings, originally derived from clonal stock kindly supplied by Dr. G. MEIJER of Philips Research Laboratories, Eindhoven, The Netherlands. Repeated stem cuttings from a single original plant were used to obtain homogeneous material.

Stem cuttings with 3-4 leaf pairs and about 4-6 cm long were detached from the top of the vegetative mother plants and transplanted in sand for about 7-10 days for rooting in specially constructed benches with provision for soil heating (temp. 20°-23 °C). The cuttings were raised in long days. Rooted cuttings were grown individually in 9 cm clay pots with fertile soil and sunk in peat benches. They were maintained in long days until the actual start of the experiment, 6-7 weeks after the cuttings.

Plants used in the 'age' experiments were derived from seeds obtained by sowing at weekly intervals.

All plants were raised throughout their life cycle in a glasshouse, heated during winter. Plants were maintained between 18°-25 °C, but during occasional summer days temperatures might rise incidentally to 30°-35 °C.

The installation for short day consisted of a wooden bench in the glasshouse which was covered each day with heavy dark canvas cloth from 4.30 p.m. until

8.30 a.m., so that the short days consisted of 8 hours of natural daylight followed by 16 hours of darkness. Long days were made up of 16 hours light i.e. natural daylight, supplemented with low intensity light from incandescent lamps (Philips 40 W) depending on the time of the year, and followed by 8 hours of darkness.

2.2. The automatic equipment for light regulation

The automatic device for controlling the lengths of light and dark periods in cycles of 24 hours was designed by J. van de Peppel of our technical section for critical daylength experiments carried out by Wellensiek (unpublished). Technical details were published in Dutch in stencilled report No. 64 (1961) of this laboratory. Only the salient features will be described here.

The installation can be broadly divided into four parts: the wooden construction, air ventilation, the electrical installation and the automatic regulator.

The entire wooden construction consists of 7 separated lightproof cabinets. Each cabinet is about 1 meter long, 1.25 meter broad and 60 cm deep. Total length of the construction is 9 meters. The height of the structure from the floor to the top is 3 meters, out of which about 1.20 meter is reserved for raising and exposing the plants. Each cabinet has two doors allowing plants to be put in and taken out. The entire construction is kept in a glasshouse. Temperatures in the cabinets were similar to that of the glasshouse i.e. 18°-25°C. Each cabinet is provided with an efficient ventilation system, and has been installed with 4 Philips fluorescent tubes TL 40 W/29 and except in the control cabinets 5 incandescent lamps of 15 W. The lamps are fixed horizontally at the roof of the cabinet. The total light intensity is 2700 $\mu W/cm^2$.

Both phases of illumination are automatically regulated, so that any desired illumination pattern can be maintained and their effect studied without disturbing the plants.

In the present series of experiments the treatment consisted of:
(a) a constant illumination with TL tubes during 8 or 12 hours;
(b) a subsequent illumination with incandescent lamps which varied in time;
(c) a corresponding dark period which completed the 24 hours cycle.

Experiments with different intensities of white light were performed in the equipment devised and designed by de Zeeuw (113, 1954, p. 6-7). At one end of the installation (from which natural daylight was omitted) 9 fluorescent tubes, Philips TL 40 W/29, were fixed vertically and were burning continuously at a temperature of about 20°C. Decreasing light intensities were obtained by placing the plants at increasing distances from the light source.

Light intensities were measured by an ordinary light meter and the unit is expressed as $\mu W/cm^2$.

2.3. Abbreviations and symbols

Symbols of restricted use are mentioned in the text where they occur. Frequently used abbreviations are:

CL - continuous light
D - total darkness
LD - long day(s)
LDP - long-day plant(s)
SD - short-day(s)
SDP - short-day plant(s)

Some frequently used terms are:

Photoperiodic induction: the process that causes the switch from the vegetative to the generative state, without any visible symptom.

Inhibition: the *state* of a plant or plant parts in which the processes leading to the initiation of flower primordia are retarded - *partial inhibition* - or blocked - *complete inhibition.*

Inhibitor(s): Unknown *substance(s)* of transmissible nature which retard(s) or inhibit(s) the formation of flower primordia.

2.4. General outline of the light inhibition experiments

In studies with light inhibition two types of interruption techniques were employed: (a) with intact plants, (b) with partially defoliated plants.

In the course of the study with intact plants the design of most of the experiments conformed to a similar basic pattern, while others were a mere extension of this design.

The plants were grown under non-inductive conditions before the start of the treatment. They were then exposed to inductive SD-cycles. After varying numbers of SD-cycles varying numbers and durations were given of non-inductive LD or CL cycles with high intensity light, 3500 $\mu W/cm^2$. After this treatment the plants were maintained in continuous SD for the observation of the appearance of macroscopically visible flower buds.

In certain experiments, one inductive SD cycle was alternated either with one LD cycle of various light: dark ratios or with CL of different intensities. In this manner two types of cycles with different modes of action were alternated. This treatment was continued until the plants had received a number of SD cycles which would have been sufficient for flower induction when given continuously. The after-treatment consisted of ordinary SD.

The method used in obtaining partially defoliated single-branched plants has been described already in a preliminary communication (4, 1963).

Details of methods will be described in the respective experiments.

2.5. Recording of observations

The main object has been to assess quantitatively to what extent the non-inductive cycle(s) have a pronounced effect on the initiation of flowering. Since the test plant used is less suitable to furnish flower counts, the estimate was based on the following two observations.

(a) Counting the mean number of days (average of 5 plants) from the start of

the treatment till the appearance of macroscopically visible flower buds. The degree of inhibition is measured by the difference in number of days required for flower bud formation in the SD control and the treated groups.
(b) In certain experiments the method adopted by MEIJER (73, 1959) was applied with a slight modification. The youngest developed leaf was marked at the beginning of the experiment and the newly formed leaf pairs were counted at the appearance of the flower buds. The plants were considered to have been inhibited when the number of leaf pairs of the treated plant was larger than that of the controls in SD at the time of flower bud formation.

CHAPTER 3

PHOTOPERIODICAL BEHAVIOUR

3.1. Introduction

Prior to any investigations on the nature of photoperiodic induction and inhibition of flowering processes, it is desirable to know in some detail the photoperiodical reaction of the plant in question. Meijer (72, 1957) was the first to describe daylength experiments with *Salvia occidentalis*. Higazy (44, 1962) concluded that a juvenile phase (i.e. the period when the plant is insensitive or less sensitive to the relative duration of light and darkness from the start of the seed germination) for flowering exists and that this phase lasts about 3 weeks. Furthermore, he suggested that a preceding CL treatment delays the subsequent induction in SD. These and other problems have been tested in the following series of experiments.

3.2. Types of response to the photoperiod

3.2.1. The effect of short length of day

Experiment 1. - The object of this experiment was to establish the optimal photoperiod which would bring this plant into flower. Plants were exposed to photoperiods of 1, 2, 3, 4, 5, 6 or 8 hrs per day. The TL 29/40W fluorescent lamps (Philips) have been the light source for white light at an intensity of 2700 μW/cm^2. The treatment lasted 60 days. The time of flower bud formation in response to different photoperiods is shown in figure 1.

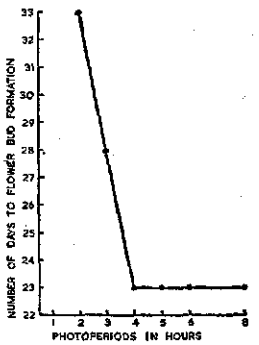


Fig. 1. *Experiment 1.* – Effect of short photoperiods on flower bud formation.

Plants in 1 hr photoperiod remained vegetative for 20-21 days and then died. Flower bud initiation took a long time in 2 or 3 hrs photoperiod, while photoperiods of 4 hrs and longer initiated flower buds simultaneously i.e. after 23 days. The numbers of newly formed leaf pairs from the start of the experiment until bud formation in 2-8 hrs photoperiod were 3, 4, 5, 4, 4 and 4 respectively. Data on the opening of the first flower indicated that no flowers were formed in 2 or 3 hrs, whereas they appeared in 4, 5, 6, or 8 hrs photoperiod after 59, 53, 52 and 49 days respectively.

It is concluded that 4-8 hrs photoperiods are optimal, whereas 2 and 3 hrs are sub-optimal for flower bud initiation.

Experiment 2. - In the preceding experiment it was demonstrated that a sub-optimal region lies in the range 2-4 hrs of light per day. The purpose of the present experiment was to determine whether the delay in the sub-optimal region was due to non-specific or to photoperiodic effects. The experiment was set up in three parts:

(a) Control plants received 35 days of 1, 2, 3, 4, 5, 6 or 8 hrs photoperiods.

(b) Increasing daylength - The first treatment consisted of 5 d. 1 hr light, the second treatment of 5 d. 1 hr light followed by 5 d. 2 hrs light, and so on until the last treatment involved consecutively 5 d. of 1, 2, 3, 4, 5, 6 and 8 hrs light. After these treatments, hence after 5, 10, ---- 35 days, the plants were transferred to LD.

(c) Decreasing daylengths - the procedure was similar to (b) but opposite. Results are shown in table 1.

TABLE 1. *Experiment 2.* Effect of changing daylengths on flower bud formation: from sub-optimal to optimal region and *vice versa* in very short photoperiods. Units of 5 plants per treatment.

Group	Treatment	Mean number of days to flower bud formation
	Consecutive daylengths, each during 5 days followed by LD	
1	1	∞
2	1 – 2	∞
3	1 – 2 – 3	∞
4	1 – 2 – 3 – 4	29
5	1 – 2 – 3 – 4 – 5	28
6	1 – 2 – 3 – 4 – 5 – 6	29
7	1 – 2 – 3 – 4 – 5 – 6 – 8	29
8	8	∞
9	8 – 6	∞
10	8 – 6 – 5	28
11	8 – 6 – 5 – 4	25
12	8 – 6 – 5 – 4 – 3	25
13	8 – 6 – 5 – 4 – 3 – 2	25
14	8 – 6 – 5 – 4 – 3 – 2 – 1	25

The controls point to the same tendency as is found in the previous experiment and therefore have not been mentioned in the table. The results of increasing daylengths indicate that the first 15 days as such are ineffective, but by adding 5 more days of 4 hrs light, flower buds were initiated in 29 days. Thereafter, the days necessary to bud formation remained practically constant. In decreasing daylengths it is seen that the first 10 days (5 days of 8 hrs + 5 days

of 6 hrs) though inductive in themselves, are not completely effective, as the plants remained vegetative after this treatment. However, after the addition of 5 more days of 4 hrs, buds were visible after 28 days. Further addition of decreasing daylength cycles resulted in earlier appearance of the buds.

It is suggested that sub-optimal daylengths have both non-specific as well as photoperiodic effects. In this region processes leading to induction are built up, but rather slowly. Moreover, sub-optimal daylengths could act as optimal when preceeded by a certain number of optimal cycles.

3.2.2. The effect of long length of day

Experiment 3. - The purpose of the present experiment was to separate daylength effects roughly into favourable or inductive and unfavourable or non-inductive for flowering. Plants were exposed to photoperiods of 8, 10, 12, 13, 14, 15 or 16 hrs per day. The light source consisted of 8 hrs TL supplemented with incandescent lamps to complete the various daylengths. The time of flower bud formation in response to these photoperiods is shown in figure 2.

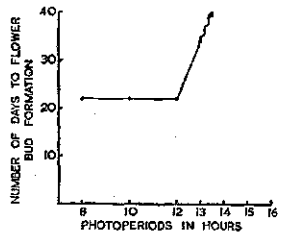


FIG. 2. *Experiment 3.* – Effect of long photoperiods on flower bud formation.

In photoperiods of 8, 10 or 12 hrs flower buds were initiated simultaneously in 22 days. In 13 hrs the appearance of the bud was considerably delayed and took 34 days. Plants remained completely vegetative in 14 hrs and longer daylengths for the duration of the experiment.

On the basis of the data obtained the effects of daylength can be separated into inductive (8, 10, 12 or 13 hrs) and non-inductive (14, 15 or 16 hrs). Stated otherwise, the former daylengths will promote and the latter will prevent flower bud formation when given continuously.

3.2.3. Determination of critical daylength.

Experiment 4. - In order to define the responses of the preceding experiment more sharply, an attempt has been made to determine the critical daylength in strong white light. The plants were illuminated daily in two phases: (a) phase involved 12 hrs of TL 29 given to all groups to facilitate assimilation; (b) involved additional supplementation with incandescent lamps at half hour intervals to achieve and separate photoperiodic effects. The procedure and the results are represented in table 2.

It is evident that photoperiods of 12 or 12½ hrs are fully inductive and initiate buds at the terminal apex. However, in 13 hrs only the lateral buds were ini-

TABLE 2. *Experiment 4.* Determination of critical day-length. TL = fluorescent light; IL = incandescent lamps. Units of 5 plants per treatment. Duration of the treatment 74 days.

Photoperiods in hours			Mean number of days to flower bud formation	Newly formed leaf-pairs at bud formation or at termination of experiment
High-intensity light TL	Supplementary low-intensity light IL	Total photoperiod		
12	0	12	21	6
12	½	12½	21	6
12	1	13	35*	8
12	1½	13½	∞**	>10
12	2	14	∞	>10
12	2½	14½	∞	>10
12	3	15	∞	>10

* only lateral buds were generative.
** one plant had generative lateral buds after 65 days.

tiated, the terminal apex remaining vegetative. In photoperiods longer than 13½ hrs the plants remain indefinitely in the vegetative state, as is also suggested by the relatively large number of newly formed leaves at the time of termination of the experiment.

It is concluded that 13 hrs is supra-optimal for flowering, whereas the critical daylength of *Salvia occidentalis* lies around 13-13½ hrs per day, beyond which the plants will so to say refuse to flower.

3.2.4. Shift in the critical daylength.

Experiment 5. - SCHWABE'S (93, 1959; 94, 1961) proposed hypothesis of the shift in the critical daylength consequent upon partial induction was the subject matter of this experiment. The procedure adopted by SCHWABE (93, 1959, p. 29) was applied. The experiment to be described is similar to expt 2, except that the treatments were changed every 4th day. Table 3 shows the results.

In the control group a trend similar to that of figure 2 was obtained and therefore it is not mentioned in the above table. The results of increasing daylengths point out that the first 8 days as such were ineffective, but by adding 4 more days of 12 hrs, flower buds were initiated in 23 days and addition of subsequent daylengths diminished this number only by one. In the decreasing daylength series the first 12 days, as would be expected, were without any effect, because they constitute daylengths longer than critical. Addition of 8 more days constituting 13 and 14 hrs daylength appeared again to be ineffective, as is also indicated by the increased number of leaf pairs. However, further addition of 4 days of 10 hrs resulted in flower buds after 29 days.

One point should be emphasized, namely events occurring in the sequence (group 7) are much more favourable for bud initiation as compared to the similar but opposite order (group 14), involving the difference of as much as 7 days.

TABLE 3. *Experiment 5.* Effect of changing daylengths on flower bud formation: from inductive to non-inductive region and *vice versa* in long photoperiods. Units of 5 plants per treatment.

Group	Treatment	Mean number of days to flower bud formation.	Newly formed leaf-pairs at bud formation or at termination of experiment.
	Consecutive daylengths, each during 4 days followed by LD		
1	8	∞	>10
2	8 – 10	∞	>10
3	8 – 10 – 12	23	4
4	8 – 10 – 12 – 13	22	4
5	8 – 10 – 12 – 13 – 14	22	4
6	8 – 10 – 12 – 13 – 14 – 15	22	4
7	8 – 10 – 12 – 13 – 14 – 15 – 16	22	4
8	16	∞	12
9	16 – 15	∞	11
10	16 – 15 – 14	∞	11
11	16 – 15 – 14 – 13	∞	12
12	16 – 15 – 14 – 13 – 12	∞	11
13	16 – 15 – 14 – 13 – 12 – 10	29	6
14	16 – 15 – 14 – 13 – 12 – 10 – 8	29	5

Judging as a whole, it may be suggested that a shift in the critical daylength does take place, provided the previous induction is the product of a minimum number of inductive cycles. This minimum number lies between 8-12 days.

3.2.5. Discussion.

The results reported in the preceding section on daylength response are in general agreement with many other SDP. MEIJER's (72, 1957) results are confirmed and extended. Response curves under SD and LD (fig. 1 and 2) are similar to those described by BEST (3, 1960) except that the range of optimum photoperiod is wider and the reactions sharper. Accordingly, daylength response of strong white light when given continuously can be grouped as follows: 1-4 hours sub-optimal, 4-12½ hrs optimal, 12½-13 hrs supra-optimal, 13-13½ hrs critical, and daylengths longer than 13½ hrs are non-inductive to flower bud initiation. The function of light in relation to flowering seems to be at least threefold (a) assimilatory, (b) stimulatory, (c) preventive, (b) and (c) depending on the length of the day.

From the results obtained in table 1 it is obvious that the nature of photoperiodic reactions in the sub-optimal region is both non-specific as well as photoperiodic. That the delay of floral initiation in this region is largely due to lack of carbohydrates is well known, but it is demonstrated that this is also due to the slow build up of inductive products (see increasing daylength series).

However, in the decreasing daylength series it is seen that the optimal photoperiod was shifted as much as 3 hrs in the sub-optimal direction.

The results on the shift in the critical daylength experiment have a somewhat similar trend to that proposed by SCHWABE (93, 1959). However, the shift of the critical daylength in the direction of non-inductive photoperiods seems to have certain limitations - at least in *Salvia* - occurring only when the preceding inductive cycles are of a minimum number. Such a minimum cycle requirement coincides with the completion of induction, as will be shown later on. CUMMING (23, 1963), experimenting with *Chenopodium rubrum* and using a different approach, reached a similar conclusion. A given photoperiod that was optimal with an intermediate R/FR ratio was found to be sub- and supra-optimal with low and high ratios, respectively.

3.3. PHOTOPERIODIC INDUCTION

3.3.1. Morphological changes at the apex during short days.

Experiment 6. – In order to investigate at what moment during the SD treatment the first qualitative changes occur at the apex, the plants were transferred to LD after 1, 2, 3, -----, 25 SD cycles. Inductive effects of varying numbers of SD cycles are represented in photo 1 and figure 3.

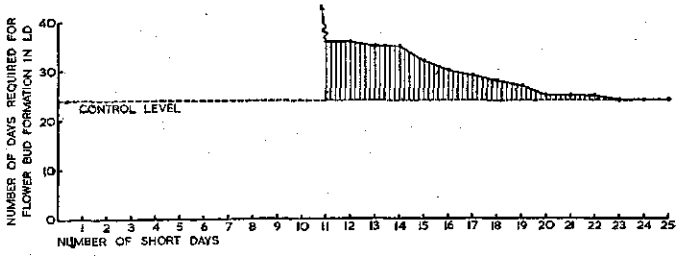


FIG. 3. *Experiment 6.* After-effect of increasing numbers of SD cycles on flower bud formation in LD.

The first macroscopic indication of the generative state appears when dark green needle-like tips of bract-like leaves are visible instead of normal young leaf pairs. It was found that 1-10 SD's were without any effect. A slight effect was obtained by a treatment of 11-14 SD's, indicated by the appearance of some bract-like leaves. However, the buds remained stunted and did not give rise to an inflorescence and the formation of normal leaves was resumed. An induction period of 15–21 SD's was sufficient for the formation of an inflorescence, although its progress towards flowering was prevented in the following LD. Plants reverted to a vegetative state after the laterals had grown from the axils of the uppermost leaf pair. An induction period of 22–25 SD gave rise to an elongated inflorescence of which only the lower axes flowered. Plants kept in continuous SD developed a normal inflorescence and flowered in about 40–45 days.

Experiment 7. – In this experiment the transition of an apical bud primordium

from a vegetative to a generative state was detected under a dissecting microscope. After receiving the required number of SD's the primordium of the main axis, together with surrounding unexpanded leaves, were excised and fixed in 96% ethanol. Before dissection they were transferred into 48% ethanol, in which the tissues were flexible enough to dissect. The growing points were stained in a strong solution of potassium iodide for 1–2 minutes just before examination. Photomicrographs were taken according to the method used by BRUINSMA (12, 1963). Photo's 2 show the state of the bud after 0, 10, 15 or 20 SD respectively. Photo's 2A and 2B represent vegetative growing points which have more or less flat domed apices with young leaf initials arising from their sides. Floral initiation involves the enlargement of the apex and its gradual transformation into a more or less semi-circular dome with reduced leaf initials (photo 2C) to a prominent dome shaped apex (photo 2D).

Experiment 8. – Floral histogenesis. Changes in the shoot apex during SD treatments, as seen anatomically, are illustrated in photo's 3. Shoot apices were fixed in formalin-aceto-alcohol mixtures as described by JOHANSEN (51, 1940, p. 41). All plants were studied by means of paraffin embedded longitudinal sections of 20 mμ thickness, and stained according to the technique used by CHEADLE *et al* (19, 1953). First indication of an effect of SD treatment was evident after 12 SD (photo 3B), when there was slight swelling of the apex accompanied by an increase in cell division, as indicated by spreading of dark zones, as compared to the vegetative apex. On the 14th day (photo 3C) rounding of the apex marked the change in the differentiation pattern, which became more prominent in later stages of induction. The formation of an elongated dome shaped apex appeared after 16 SD (photo not shown) and the appeareance of bract-like leaf initials after 18 SD's (photo 3D).

Experiment 9. – Cell division and induction. This experiment was designed to assess and correlate the mitotic activity at the terminal meristematic shoot apex to the number of SD cycles. At 4.30 p.m. three buds were collected after 0 SD as well as on every alternate SD during a 22 day-period. The Feulgen-squash technique was applied. Early and late prophases, metaphases, anaphases, and telophases were counted. However, the total numbers of cells scored were

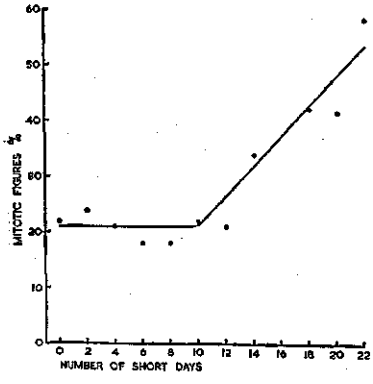


FIG. 4. *Experiment 9.* Effect of an increasing number of SD cycles on the % of mitotic figures in the apex.

relatively low i.e. between 700–1500 cells per treatment. The results are shown in figure 4.

Mitotic figures (expressed as percentage of dividing cells) in the meristematic zones of the apex during 0–10 SD are approximately the same. The first sign of transition from the vegetative into a generative state is accompanied by an abrupt increase in the rate of apical cell division, between 12–14 SD's. This activity is further accelerated by increasing numbers of SD, at least until 22 SD cycles. Results of similar nature have also been reported by JACOBS and RAGHAVAN (49, 1962) and THOMAS (100, 1963) in other SDP's.

3.3.2. Effect of preceding long days on the subsequent induction in short days.

Experiment 10. – In table 2 (p. 15) it was demonstrated that daylengths longer than 13½ hrs are non-inductive when given continuously. It appeared of interest, therefore, to investigate whether LD given before the start of the SD treatment exerted any effect on the subsequent SD induction. If these LD's are inhibitory, flowering will be delayed, and if promotive, flowering will be accelerated in the following SD. Thus plants were subjected to 1, 2 or 3 weeks of LD of various light: dark ratios and transferred to SD. The results are summarized in table 4.

TABLE 4. *Experiment 10.* Effect of preceding LD of various light: dark ratios during 7, 14 or 21 days on subsequent induction in SD. Units of 5 plants per treatment.

Duration of treatment (days)	Light: Dark ratio during treatment.	Mean numbers of days to bud formation in SD – after treatment.
Control	8 L, 16 D	21
7 d.	12 L, 12 D	14
	14 L, 10 D	17
	16 L, 8 D	19
	18 L, 6 D	19
	20 L, 4 D	19
	22 L, 2 D	20
	24 L, 0 D	19
14 d.	12 L, 12 D	7
	14 L, 10 D	19
	16 L, 8 D	21
	18 L, 6 D	21
	20 L, 4 D	21
	22 L, 2 D	23
	24 L, 0 D	22
21 d.	12 L, 12 D	0
	14 L, 10 D	20
	16 L, 8 D	21
	18 L, 6 D	21
	20 L, 4 D	22
	22 L, 2 D	23
	24 L, 0 D	22

The data obtained show in general that preceding LD's of various duration have practically no delaying effect on the subsequent induction in SD. We should keep in mind that 12 hrs L + 12 hrs D has an inductive effect.

Experiment 11. – In this experiment the effect of 1, 2 or 3 weeks of CL (TL) of intensity 3500 $\mu W/cm^2$ preceding the SD treatment was tested on plants 6 weeks old. All groups including the controls took 20–21 SD to initiate flower buds. These results once again show that the preceding LD (in this case CL) does not exert any deleterious effect on the subsequent induction in SD of plants which have been grown in preceding LD.

3.3.3. Discussion.

Let us first discuss the efficiency of induction in SD as a result of preceding LD's of various photoperiods. The results obtained in table 4 point out that preceding LD's do not disturb the inductive processes during the following SD's, when starting with full grown plants. These results are in contradiction to those of HARDER and BÜNSOW (40, 1954) with *Kalanchoë blossfeldiana*, to those of ESASHI (26, 1961) with *Begonia evansiana*, and to those of HIGAZY (44, 1962, p. 41) with *Salvia occidentalis*. However, all these authors started from very young seedlings, and it will be discussed in the next part that in young seedlings no maximal light inhibition has been built up yet, like evidently in my case.

Coming now to the induction in SD itself, my observations on the moment of induction during SD treatment are in harmony with MEIJER (72, 1957). On the basis of experiments, described in section 3.3.1, it can be deduced that vegetative plants in SD must pass, at least, through two distinct phases, each with its own characteristics and requirement for rapid completion. The following two phases are suggested: (1) a preparatory or pre-inductive phase; (2) a realization phase.

The preparatory or pre-inductive phase. In my material the first 10 SD's constitute this phase. Its effects seem to be localized and immobile, for in subsequent LD's no after-effect occurs. It appears to be a phase with low apical mitotic activity. Even though its nature is obscure, this is the most crucial of the two phases, because during this phase certain factor(s) are either built up or removed, the result of which is the induced state. *The realization phase.* During this phase the first qualitative changes take place, while at the shoot apex it coincides with an increase of mitotic activity. The degree of flowering increases with increasing cycles of SD and reaches a maximum beyond which the processes become more independent of photoperiod.

3.4. THE EFFECT OF DIFFERENT FACTORS ON FLOWER BUD FORMATION IN SHORT-DAYS

3.4.1. Plant age.

Experiment 12. – This experiment was carried out to determine the response of plant age to favourable SD cycles. Age series were obtained by weekly sowings, so that after 10 weeks age groups of 1–10 weeks old seedlings were

available. All groups were then simultaneously transferred from LD to SD cycles. Photo 4 illustrates and table 5 summarizes the results.

TABLE 5. *Experiment 12.* Effect of plant age on flower bud formation in SD. Units of 5 plants per treatment.

Age (weeks)	Mean number of days to flower bud formation	Leaf pairs including cotyledons at the start of the treatment	Newly formed leaf-pairs at bud formation
1	25	1	3.0
2	26	2	3.0
3	25	3	3.0
4	23	4	3.4
5	25	5	3.4
6	25	6	5.0
7	26	7	5.0
8	26	8	5.0
9	26	9	4.6
10	27	10	4.0

These results indicate that different age groups initiated flower buds more or less simultaneously namely between 23–27 SD's irrespective of their age. The numbers of leaf pairs at increasing age in the LD-pretreatment exhibit a linear relationship. One leaf pair unfolded each week. However, the trend of newly formed leaves at bud formation was not linear, for 3 new leaf pairs were formed at the time of bud formation in 1–6 week old plants, whereas 4–5 leaf pairs were formed in 7–10 week old plants.

On the basis of the available information it is concluded that very young as well as older plants are equally sensitive to SD treatment. This further suggests that cotyledons and/or primary leaves perceive the SD effect. The existence of a non sensitive or less sensitive period is therefore, not evident.

3.4.2. The sensitivity of leaves of different maturity.

Experiment 13. – The object of this experiment was twofold: (a) to determine the differences in the inductive capacity of leaves of different age and position, and (b) to investigate the effect of defoliation on flower bud formation.

In *Salvia* the arrangement of the leaves is decussate (opposite, with alternate pairs at right angles to each other). At the time of starting the cuttings for this experiment, the uppermost unexpanded leaf pair was marked. At the start of the SD treatment there were about 7–8 newly formed fully expanded leaf pairs (but differing in size and area) on each plant. All leaves below and including the marked leaf pair were removed. The pair of leaves after the marked leaf-pair was designated as 1st pair, the next one as 2nd, etc. Hence, the pairs of leaves are numbered successively from the base upwards. The leaf pairs were then divided into three groups which for simplicity's sake are indicated as:
Mature (M) = 1–2 leaf pairs,

Semimature (SM) = 3–6 leaf pairs,
Young (Y) = 7–8 leaf pairs.

The defoliation was achieved prior to the SD-treatment. New leaves formed during the SD-treatment were continuously removed. The results are indicated in table 6.

TABLE 6. *Experiment 13.* The sensitivity of leaves of different maturity on flower bud formation under SD condition. Units of 8 plants per treatment.

M = Mature leaves
SM = Semi-mature leaves
Y = Young leaves
\+ = leaves present
— = leaves removed

Group	Position of the treated leaves on the plant	Mean number of days to flower bud formation
1	+ M + SM + Y	18
2	+ M + SM — Y	19
3	+ M — SM + Y	19
4	+ M — SM — Y	23
5	— M + SM + Y	18
6	— M + SM — Y	19
7	— M — SM + Y	18
8	— M — SM — Y	18

These results point out that all treatments initiated flower buds simultaneously in 18–19 SD's except in treatment 4 where the presence of mature leaves in an otherwise promoting condition caused a delay in bud formation which took 23 SD. Plants fully defoliated appeared to be as efficient in floral initiation as intact controls. This suggests participation of the stem in perceiving the SD effect. Comparable controls i.e. totally defoliated plants in LD or CL never formed flower buds.

It is concluded that the sensitivity of different leaf pairs towards the SD induction is the same, except that mature leaves partially inhibited the response. Defoliation too does not affect the flowering response, as completely defoliated plants initiated flower buds.

3.4.3. The sensitivity of single leaf pair.

Experiment 14. – The sensitivity of a single leaf pair *in-situ* to the efficiency of the favourable effect of SD was tested. The leaf pair in question was retained and all others removed both at the start of the experiment and during the treatment. In all other respects, the procedure applied was similar to that of the last experiment. The intact plants had 7 leaf pairs at the start of the SD treatment. The results are shown in table 7.

These results indicate that only the 1st leaf pair, which is the oldest, caused a delay, whereas all other leaf pairs irrespective of their position and age took 20 or 21 SD. Completely defoliated plants also initiated flower buds.

TABLE 7. *Experiment 14.* The sensitivity of individual leaf pair *in-situ* on flower bud formation under SD condition. Units of 5 plants per treatment.

Position of individual leaf pairs from base upwards	Mean number of days to flower bud formation
Intact plant	20
1st	25
2nd	21
3rd	20
4th	20
5th	20
6th	20
7th	20
Defoliated plant	20

From this and the previous experiment it can be concluded that all leaves *in-situ* individually or in combination, irrespective of their maturity, position, size and area are sensitive to SD treatment, except the oldest pair of leaves which delayed flower bud formation.

3.4.4. Discussion.

HIGAZY (already cited on p. 12) concluded for *Salvia* that a juvenile phase exists, but lasting only up to 3 weeks. Our results from repeated experiments suggest this not to be true as different age groups (table 5) initiated flower buds more or less at the same time. This difference of results could perhaps be attributed to different growing conditions during the pretreatment. My results point out that the cotyledons and/or primary leaves are fully sensitive to day-length. In this respect *Salvia* is similar to *Pharbitis nil*, IMAMURA (47, 1953) and *Chenopodium rubrum*, CUMMING (22, 1959) and differs from *Perilla*, WELLENSIEK (as cited by ZEEVAART, 114, 1958 p. 9), where young plants, and *Xanthium*, JENNING and ZUCK (50, 1954) where cotyledons are less sensitive or insensitive to SD treatment. The criterion of obligatory vegetative growth prior to the 'ripeness to flower' condition as proposed by HOLDSWORTH (46, 1956) for some SDP's seems less acceptable for *Salvia*, because the plants in our case were already sensitive to the photoperiodic treatment at the cotyledonary stage.

The results of the experiments on the sensitivity of leaf pairs indicated that all leaf pairs were equally sensitive to SD except the oldest one which was studied. However, relatively poor inductive effect of this leaf pair can be masked when accompanied by higher located leaves. It was also found that completely defoliated plants take the same time to initiate flower buds as intact plants in SD. This would oppose the suggestion made by BORTHWICK and PARKER (11, 1938) and HAMNER (35 1948) that the sensitivity to photoperiodic treatment is related to total leaf area. Disagreement on this issue has been pointed out already by LANG (56, 1952) who stated: 'it takes the same number of cycles to induce an intact plant and a plant defoliated to one leaf'. Since defoliated *Salvia* plants in LD or CL will not flower, they differ from strawberry, THOMPSON and GUTTRIDGE (101, 1960).

CHAPTER 4

LIGHT INHIBITION

Whereas the preceding chapter was primarily concerned with the photoperiodic behaviour and consequently describes the changes occurring during different daylength regimes in relation to flower bud formation, this chapter describes and analyses some salient features of non-inductive daylengths in terms of where and when they manifest their inhibitory action in the short-day plant *Salvia occidentalis*.

4.1. CHARACTERISTICS OF LIGHT INHIBITION

4.1.1. Introduction.

Intervention by long-day cycle(s) between the short-day series of an inductive treatment is generally called fractional induction. Studies involving fractional induction have been carried out by numerous workers, and the literature has been reviewed by CARR (14, 1955). There is general agreement that such non-inductive daylengths have inhibitory effects. These effects have been observed and accurately studied by SCHWABE (91, 1956; 93, 1959) in *Kalanchoë blossfeldiana*. WELLENSIEK (106, 1958; 107, 1959) interrupted the SD treatment of *Perilla* at various times by 48 hrs of CL and observed that the inhibitory effect of CL depends largely on the moment of the inductive period at which it is intercalated. He made similar conclusions from work with *Salvia* (108, 1960) using 72 hrs of CL. Similar results have been obtained in *Rottboellia exaltata* by EVANS (28, 1962) and results reported by THOMAS (99, 1963) in *Xanthium* and *Chenopodium*, though somewhat different, point to the same trend. However, in all the previous studies either standard 16 hrs LD or 24 hrs or more CL were intercalated between the SD's. As far as I know, no previous studies except those of SIROHI and HAMNER (95, 1962) have been undertaken to account for the effects of LD of different duration. I come back to this work later.

4.1.2. Periodical interruption of the short day treatment by different photoperiods.

Experiment 15. – This experiment has two treatments. The first one comprises uninterrupted photoperiods of 11, 11½, 12, 12½, 13, 13½ or 14 hrs for 21 days. The second approach was to alternate single SD (8 hrs light and 16 hrs dark) with the single above mentioned photoperiods of varying lengths. This was repeated until the plants received 11 SD, so that the total duration of the treatment lasted 21 days also. In both cases the plants were then removed to uninterrupted SD. The results are presented in figure 5.

During the 21-day treatment, uninterrupted cycles of 11, 11½ and 12 hrs were fully inductive, whereas in cycles longer than 12 hrs this was the case only partially. After transferring the latter group of plants to SD, increasing numbers of SD's were needed as the photoperiods during the pretreatment increased.

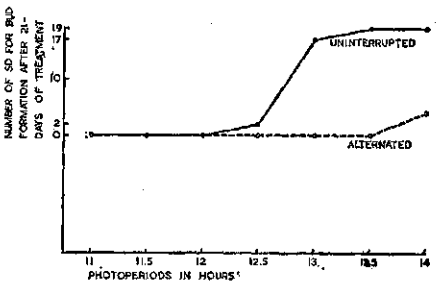


FIG. 5. *Experiment 15.* Number of days required for bud formation in SD (ordinate), when pretreated during 21 days with photoperiods as indicated in abscissa, either uninterrupted or alternated with SD of 8 hrs light and 16 hrs of darkness.

When these different photoperiods were alternated with SD, it was found that all photoperiods were innocuous except 14 hrs which showed a slight floral delay. These cycles on alternation with SD acted like ordinary SD cycles.

It is concluded that the delaying effect of cycles longer than 12 hrs is almost completely absent when these cycles are alternated with single SD.

Experiment 16. – The effect of interruption of a single cycle of varying length of photoperiod after 5, 9, 10 or 15 SD on flower bud formation was studied. After having received the fixed number of SD's, the plants received *one* cycle of 12, 12½, 13, 13½, 14, 14½ or 15 hrs of light, followed by SD. This treatment yielded negative results. In all groups, including the uninterrupted SD controls, the flower buds were visible after 20–21 days.

It is concluded that a single cycle of long-days of varying length between 12 and 15 hrs is not inhibitory.

Experiment 17. – The procedure of this experiment was the same as that of the previous one except that instead of one single cycle, 3 cycles of varying lengths of photoperiod were given as interruption. Table 8 shows the results.

TABLE 8. *Experiment 17.* Mean number of days to flower bud formation when the SD-treatment was interrupted after 5, 9, 10 or 15 cycles with 3 cycles of 12, ---- 15 hrs of light. Units of 5 plants per treatment.

Photoperiod during 3 days of interruption	Mean number of days to flower bud formation when interruption takes place after ---			
	5 SD	9 SD	10 SD	15 SD
12	22.0	21.0	21.4	21.5
12½	22.8	21.0	21.0	21.0
13	24.0	22.0	23.6	21.0
13½	24.0	23.6	23.0	21.0
14	24.0	24.0	24.2	21.0
14½	24.0	24.6	25.2	21.2
15	24.0	25.0	26.2	21.4

In practically all cases besides 15 SD the time for flower bud formation is increased as the photoperiod during intercalated LD is increased. Only in isolated cases, namely with daylengths of 14½ and 15 hrs applied after 9 or 10 SD's, the delay in flower bud formation is larger than the duration of the interruption. Only in those cases we could speak of an inhibition.

The absence of inhibition when the interruption takes place after 15 SD indicates that the inhibition only influences the processes leading to the induced state.

4.1.3. Critical period of light inhibition.

Experiment 18. – This experiment was performed to determine the interrelationships between the effect of varying lengths of photoperiod and the time of application during the SD-treatment. A graduated series of photoperiods ranging from 12-24 hrs at 2 hrs interval was administered once after 9, 10, 11 or 12 SD's. The results are plotted in figure 6.

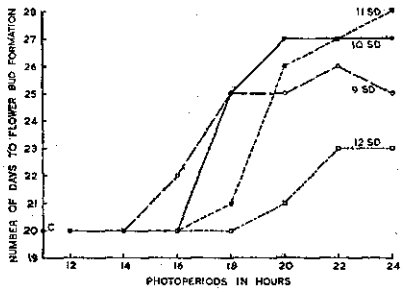


FIG. 6. *Experiment 18.* The effect of interrupting the SD-treatment by 1 cycle of varying duration of light (abscissa), when administered after 9, 10, 11 or 12 SD. Control 'C' in SD (ordinate).

This graph clearly demonstrates an inhibitory effect of certain daylengths. After 9 SD, the maximum inhibition occurs from 18 hrs of photoperiod, after 10 SD the maximum occurs from 20 hrs, after 11 SD also from 20 hrs and after 12 SD from 22 hrs. The graph also shows that the maximum inhibition is largest when the interruption is applied after 10 or 11 SD while after 12 SD it is much less. This means that during the proceding of the induction the length of the inhibitory daylength increases.

4.1.4. Timing and light inhibition.

Experiment 19. – This experiment was designed to test whether the CL (shown to exert maximum inhibition in expt 18) has a different effect when administered at different times during the entire range of SD treatment. SD-treatment of groups of plants was interrupted once by 2 days of CL (high intensity 3500 $\mu W/cm^2$ at greenhouse temperature) after 0, 2, 4 ---- 24 SD respectively. The results are shown in figure 7.

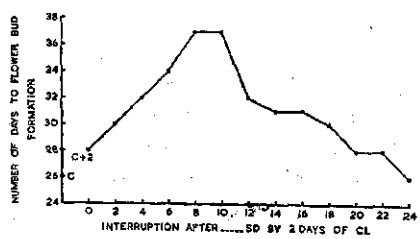


FIG. 7. *Experiment 19.* Effect of the time of 2 days CL interruption. Abscissa: Number of SD after which an interruption during 2 days CL was given. Ordinate: mean number of days to bud formation. 'C' and 'C+2' on ordinate indicate the controls.

The mean numbers of days to flower bud formation confirm that 2 days of CL interruption in a SD series has a marked time effect. The following results are worthy of attention.

CL does not exert any inhibitory effect when given *before* the SD treatment. The difference with the uninterrupted SD control is exactly 2 days which is the duration of CL interruption.

It is equally evident that after a certain number of SD's the CL exerts an appreciable inhibition.

As the CL interruptions are given at later periods during the SD treatment, the effect of the light inhibition gradually increases and reaches its maximum after 8–10 SD's, involving a difference with the control of no less than 11 days. Afterwards it gradually declines to completely disappear after 20 SD.

From this experiment it is concluded that CL exhibits its inhibitory nature only after some SD's. The maximum effect of CL inhibition lies roughly in the middle of the induction period, when the plants are about to enter the induced state.

Experiment 20. – CL-inhibition and mitotic activity. In expt 9 (p. 18) the relationship between the number of SD given to the plants and the rate of cell division in the apex was presented. It appeared of interest, therefore, to study whether the intercalated 3 d. CL administered at different times during SD-treatment has any causal relationship with the rate of cell division in the apex. The experimental method is similar to that of expt 9, except that the buds were fixed after 0 SD + 3 d. CL or 2 SD + 3 d. CL etc. Figure 8 illustrates the results.

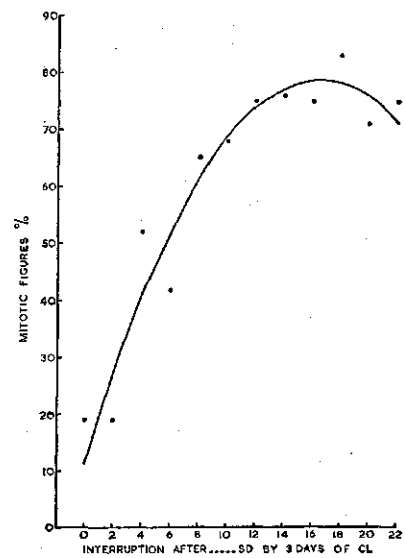


FIG. 8. *Experiment 20.* Effect of 3 days CL interruption after varying numbers of SD cycles on the % of mitotic figures in the apex.

The results indicate that no apparent increase in the mitotic index was evident as a result of 3 d. CL interruption after 0 or 2 SD's. However, between 4 and 6 SD's the rate in cell division was almost doubled and then increased pro-

gressively upto 12 SD, involving a rather high rate of cell multiplication. After 14-16 SD's a decreasing trend was apparent.

These results have thus shown that the intercalated CL creates a rather high physiological activity at the apex only after receiving some initial SD's. However, it should be kept in mind that the method used was rather crude and nothing as yet can be said conclusively.

4.1.5. The cumulative effect of light inhibition.

In the previous experiments, physiological evidence for the existence of flower inhibition by light was established. It was now important to investigate the cumulative effect of interrupting LD or CL periods. Thus the objective of the following two experiments was to determine whether increasing the length of interruption with LD or CL results in increasing inhibition or whether a maximum exists. Simultaneously the inhibitory effects of LD (16 hrs light) and CL are compared.

Experiment 21. – In this experiment 1, 2, 3 —— 7 LD's of 16 hrs light were given after 9, 10 or 11 SD's i.e. the period of SD's after which the LD-inhibition either approaches maximum or is about to decline. After the LD interruption the further treatment consisted of SD. Table 9 shows the results.

TABLE 9. *Experiment 21.* The inhibitive effect of increasing periods of *long-day* (16 hrs light) administered after 9, 10 or 11 SD-cycles and followed by SD. Five plants per treatment.

Time of application and duration in days of LD-interruption	Mean number of days to flower bud formation	Difference with control	Newly formed leaf pairs at bud formation
Control in SD	21.0	—	5.4
9 SD – 1 d	23.2	2.2	5.6
– 2 d	26.4	5.4	6.6
– 3 d	28.0	7.0	7.0
– 4 d	31.4	10.4	7.4
– 5 d	33.0	12.0	8.2
– 6 d	34.4	13.4	8.0
– 7 d	36.2	15.2	8.4
10 SD – 1 d	23.8	2.8	6.0
– 2 d	27.8	6.8	6.8
– 3 d	31.4	10.4	7.8
– 4 d	33.4	12.4	8.0
– 5 d	33.8	12.8	8.4
– 6 d	36.2	15.2	8.4
– 7 d	37.0	16.0	8.8
11 SD – 1 d	21.0	0.0	5.0
– 2 d	28.0	7.0	7.0
– 3 d	28.8	7.8	7.0
– 4 d	33.4	12.4	7.8
– 5 d	36.0	15.0	8.6
– 6 d	37.0	16.0	9.0
– 7 d	36.8	15.8	9.8

The results indicate that the effects of increasing numbers of interrupting LD's increase regularly. Generally speaking, the values of such LD interruptions (column 3) are highest after 11 SD's as compared to 10 SD's, which in turn are higher than 9 SD's.

Gradual increase in the numbers of newly formed leaf pairs at the time of flower bud formation in all the treatments also confirms the increasing degree of inhibition.

Experiment 22. – This experiment is similar to expt 20, except that instead of interrupting LD, CL was given. The results are shown in table 10.

TABLE 10. *Experiment 22.* The inhibitive effect of increasing periods of *continuous light* administered after 9, 10 or 11 SD-cycles and followed by SD. Five plants per treatment.

Time of application and duration in days of CL-interruption	Mean number of days to flower bud formation	Difference with control	Newly formed leaf pairs at bud formation
Control in SD	20.4	—	6.4
9 SD – 1 d	28.0	7.6	8.4
– 2 d	32.2	11.8	9.0
– 3 d	34.2	13.8	8.8
– 4 d	34.0	13.6	9.6
– 5 d	34.8	14.4	9.8
– 6 d	36.0	15.6	9.6
– 7 d	37.6	17.2	9.8
10 SD – 1 d	28.6	8.2	8.4
– 2 d	32.8	12.4	9.6
– 3 d	33.6	13.2	9.2
– 4 d	35.6	15.2	10.0
– 5 d	36.0	15.6	10.2
– 6 d	37.6	17.2	10.0
– 7 d	38.0	17.6	10.0
11 SD – 1 d	28.2	7.8	8.2
– 2 d	33.4	13.0	9.0
– 3 d	34.6	14.2	9.6
– 4 d	36.8	16.4	9.4
– 5 d	37.8	17.4	9.4
– 6 d	38.0	17.6	10.2
– 7 d	38.6	18.2	9.8

The results point to a somewhat similar trend as was shown in table 9, regarding the appearance of flower buds and the formation of new leaves. The CL-inhibition is quantitatively larger than the LD-inhibition. How different both are is shown in the next paragraph.

4.1.6. The quantitative estimation of light inhibition.

The question arises how many SD's are annulled per LD or CL cycle, when such non-inductive cycles are intercalated between the SD inductive treatment. To answer this question use was made of data obtained in tables 9 and 10 and

the comparative effectiveness of LD or CL inhibition after 10 SD's is presented in figure 9.

The CL-inhibition is significantly higher than the LD-inhibition in all the

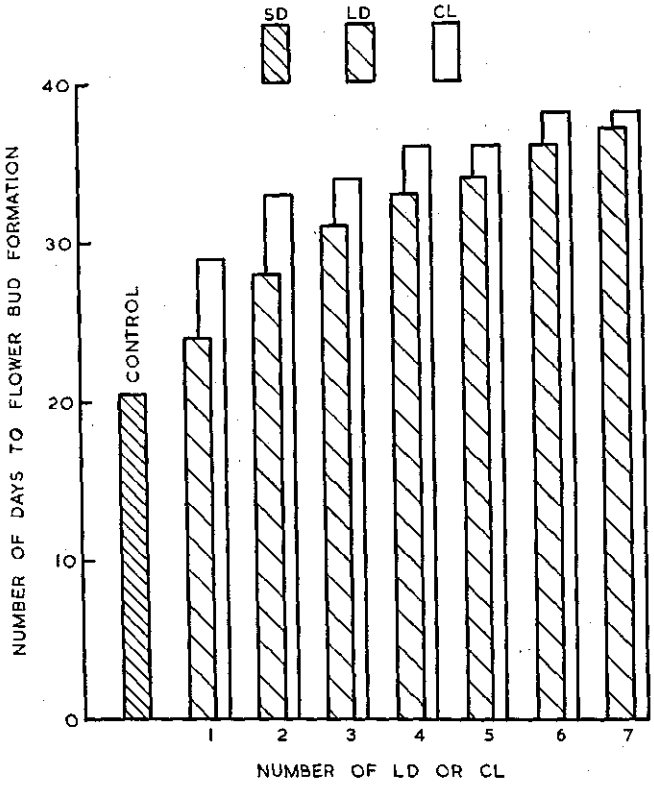


FIG. 9. The quantitative measurement of the inhibitive effect of increasing durations of LD or CL (abscissa), applied after 10 SD-cycles and followed by SD.

cases. Further details from these experiments have been calculated in tables 11 and 12.

In table 11 we see that the mean inhibition per LD (column5) first rises and then

TABLE 11. Quantitative estimation of the inhibitive effect of varying numbers of long-day given after 10 SD (Data from table 9 – see also figure 9).

Time of application and duration in days of LD-interruption	Days to bud formation	Difference with SD-control	Actual inhibition	Mean inhibition per LD	Actual inhibition per day
Control	21.0	—	—	—	—
10 SD – 1 d	23.8	2.8	1.8	1.8	1.8
– 2 d	27.8	6.8	4.8	2.4	3.0
– 3 d	31.4	10.4	7.4	2.5	2.6
– 4 d	33.4	12.4	8.4	2.1	1.0
– 5 d	33.8	12.8	7.8	1.6	– 0.6
– 6 d	36.2	15.2	9.2	1.5	1.4
– 7 d	37.0	16.0	9.0	1.3	– 0.2

TABLE 12. Quantitative estimation of the inhibitive effect of varying numbers of continuous light period given after 10 SD (Data from table 10 – see also figure 9).

Time of application and duration in days of CL-interruption	Days to bud formation	Difference with SD control	Actual inhibition
Control	20.4	—	—
10 SD – 1 d	28.6	8.2	7.2
– 2 d	32.8	12.4	10.4
– 3 d	33.6	13.2	10.2
– 4 d	35.6	15.2	11.2
– 5 d	36.0	15.6	10.6
– 6 d	37.6	17.2	11.2
– 7 d	38.0	17.6	10.6

drops, as the number of days of LD-interruption increases. Table 12, for CL, presents quite another picture: already after 2 d. CL the inhibition is larger than the number of preceding SD.

4.1.7. The effect of light inhibition on preceding and/or succeeding short-days.

The basic point at issue, i.e. whether the intercalated LD's during SD treatment affect the processes during the preceding or during the succeeding SD, has been partly answered by SCHWABE (91, 1956) and WELLENSIEK (107, 1959). Both these investigators found that LD's destroy the effect of future SD's. SCHWABE's conclusion was based on the result that a long period of darkness following intercalated LD neutralizes its inhibitory effect, and does not do so when it precedes the LD.

The inhibitory effect of light is of basic importance, especially in relation to the induced state. For if LD's annihilate the effect of preceding SD's the process of flowering has either to start anew (complete reversal) or from another level in the chain of reactions (partial reversal). If LD's annihilate the effect of succeeding SD's, the preservation and/or fixation of the effect of previous SD cycles is expected.

This phenomenon was studied in the following experiments.

Experiment 23. – Plants were exposed to 10 SD plus 3 days CL (light intensity 3500 $\mu W/cm^2$) 1, 2, 3, 4 or 5 times. After each treatment the plants were kep' in uninterrupted SD. The results are presented in figure 10.

The results clearly show that all treated groups, irrespective of the number of alternations, needed 21-22 SD to initiate flower buds, as also did the SD control. Stated in another way, each interruption of 3 days CL reversed the effect of 10 preceding SD's completely.

The next experiment was designed to find out until what time in the SD-treatment reversibility of the processes by 3 d. CL is possible.

Experiment 24. – Plants were exposed to 3, 5, 7, 8, 9 ---- 16 SD's respectively, followed by 3 d. CL, which treatments were applied 1, 2 or 3 times; the after-

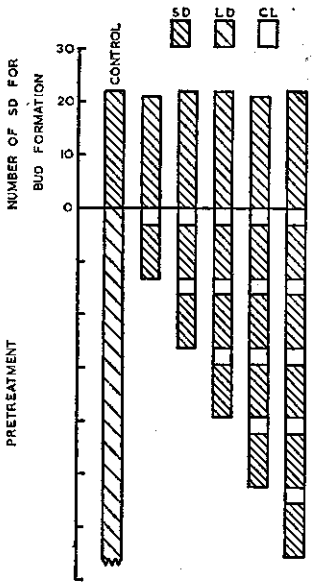


FIG. 10. *Experiment 23.* The effect of pretreatments consisting of alternating 10 SD and 3 d. CL 1×, 2×, - - - - 5× on the flower bud formation in subsequent SD, compared with a control (at the left) pregrown in uninterrupted LD.

treatment, if necessary, was uninterrupted SD. The results are shown in figure 11.

The result of this experiment shows that after 3, 5, 7, 8, 9 or 10 SD cycles the induction was completely negated when followed by 3 d. CL.

The effectiveness of 3 d. CL inhibition when administered after 11, 12, 13, 14, 15 or 16 SD cycles is not evident. It is seen that the necessity of more than one alternation after 11 SD cycles did not arise, for all groups initiated buds before the second alternation had been implemented. At this point this part of the experiment was terminated. It should be noted that the later the 3 d. CL were

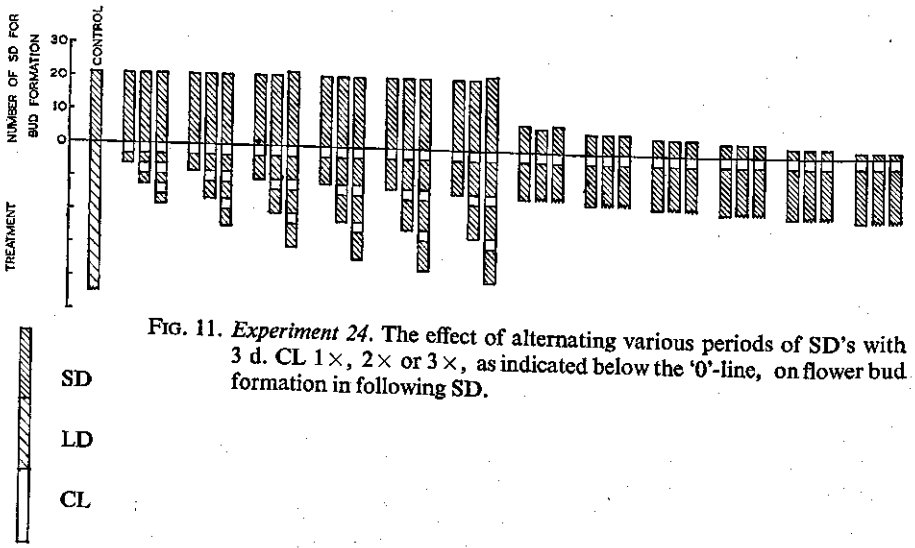


FIG. 11. *Experiment 24.* The effect of alternating various periods of SD's with 3 d. CL 1×, 2× or 3×, as indicated below the '0'-line, on flower bud formation in following SD.

given during the SD treatment, the fewer the number of additional SD's required to initiate flower buds. In all the treated groups, however, the total number of days necessary for bud formation was similar to that of the SD control i.e. 21 SD's.

One unexpected result should be mentioned, i.e. the abrupt fall in light inhibition after 10 SD. In figure 7 (p. 26) it was namely shown that CL-inhibition declines gradually though not abruptly.

The results obtained in this and the previous experiment strongly suggest that intercalated non-inductive CL cycles between SD's strongly inhibit the processes of preceding SD cycles.

Experiment 25. – The question arises whether the inhibitory effect of light can be removed by following darkness. This and the following experiments were designed to test this possibility. The procedure was as follows: after receiving 5, 10 or 15 SD's plants were given four different treatments: 1 LD; 1 day darkness (1D); 1 LD + 1 D and 1 D + 1 LD. Further treatment consisted of SD. The results are summarized in table 13.

TABLE 13. *Experiment 25.* The effect of one day LD, of 1 day Darkness (1 D), of 1 LD + 1 D, of 1 D + 1 LD, applied after 5, 10 or 15 SD. Units of 5 plants per treatment.

Treatment	Mean number of days to flower bud formation	Difference with control
Control	21.0	—
5 SD – 1 LD	22.4	1.4
– 1 D	21.4	0.4
– 1 LD + 1 D	22.0	1.0
– 1 D + 1 LD	23.8	2.8
10 SD – 1 LD	23.6	2.6
– 1 D	21.2	0.2
– 1 LD + 1 D	23.8	2.8
– 1 D + 1 LD	24.0	3.0
15 SD – 1 LD	21.2	0.2
– 1 D	21.4	0.4
– 1 LD + 1 D	21.0	0.0
– 1 D + 1 LD	21.0	0.0

A single LD exerts no inhibitory action when given after 5 or 15 SD and only slightly after 10 SD. A dark period of 24 hrs was found not to be of any effect in all the three treatments. The slight inhibitory effect of a single LD when given after 10 SD's, was not removed by following 1 D. However, it should be noted that the combination 1 D + 1 LD after 5 SD and 10 SD was slightly inhibitory.

Experiment 26. – The experimental design was similar to that of the previous experiment, except that 1 d. CL was employed instead of 1 LD. Table 14 shows the results.

TABLE 14. *Experiment 26.* The effect of 1 day CL, of 1 day darkness (1 D), of 1 CL + 1 D, of 1 D + 1 CL, applied after 5, 10 or 15 SD. Units of 5 plants per treatment.

Treatment	Mean number of days to flower bud formation	Difference with control
Control	21.0	—
5 SD – 1 CL	26.0	5.0
– 1 D	21.4	0.4
– 1 CL + 1 D	24.0	3.0
– 1 D + 1 CL	26.4	5.4
10 SD – 1 CL	28.6	7.6
– 1 D	21.2	0.2
– 1 CL + 1 D	27.0	6.0
– 1 D + 1 CL	28.0	7.0
15 SD – 1 CL	21.6	0.6
– 1 D	21.4	0.4
– 1 CL + 1 D	21.4	0.4
– 1 D + 1 CL	21.0	0.0

One day of CL, as expected, had a marked inhibitory effect when applied after 5 or 10 SD's, no effect when applied after 15 SD's. Darkness of 24 hrs was without any effect, as we have already observed in experiment 25. The inhibitory effect of 1 d. CL after 5 SD was slightly diminished by following 1 D, namely by 2 days, but was not completely removed. However, the combination 1 D + 1d. CL was inhibitory. Similar effects were apparent after 10 SD's.

These results suggest that the inhibiting effect of an interrupting light period cannot be removed by the following 24 hrs of darkness.

Experiment 27. – The purpose of this experiment is similar to that of expt 25. The results are shown in table 15, the text of which also describes the various experimental treatments in detail.

It will be seen from this table that alternation of SD with 24 hrs darkness resulted in delay in bud formation as compared to the SD control. In series A, in which the SD were alternating with LD of varying length, only treatments with 12 and 14 hrs light initiated buds during the treatment, while longer periods of light only formed buds during the aftertreatment in SD and needed more SD, the longer the light period. In series B, where the dark period of 24 hrs was preceding the different long days but following the SD, it was found that only the treatment with 12 hrs L initiated buds during the treatment, whereas 18 or more hrs of light remained ineffective. These results suggest that light periods from 18 L to 24 L completely removed the effect of the pretreatment, so far administered. Series C clearly shows that the 24 hrs dark period following the long day treatment (12 L and 14 L) to a greater extent neutralizes the delaying effect of such cycles, but such dark periods when following LD's of above 16 L do not remove the inhibitory effect of such daylengths completely, as is seen

TABLE 15. *Experiment 27.* Effect of SD; of alternating 1 SD and 24 hrs dark (D); of 1 SD and 1 LD of varying numbers of light (L) (series A); of 1 SD, 24 hrs D and 1 LD of varying numbers of L (series B); of 1 SD, 1 LD of varying numbers of L and 24 hrs D (series C). The first three treatments can be considered as controls for the last two. Duration of the treatment 32 days. Units of 5 plants per treatment.

Treatment during 32 days	Mean number of days to flower bud formation.	
	during treatment	in SD after treatment
Control	20.4	—
SD – D	26.8	—
Series A – (SD–LD)		
SD – 12 L	20.6	—
SD – 14 L	21.8	—
SD – 16 L	—	6.0
SD – 18 L	—	16.4
SD – 20 L	—	18.4
SD – 22 L	—	19.8
SD – 24 L	—	20.6
Series B – (SD–L–LD)		
SD – D – 12 L	28.2	—
SD – D – 14 L	—	3.0
SD – D – 16 L	—	17.2
SD – D – 18 L	—	20.2
SD – D – 20 L	—	20.0
SD – D – 22 L	—	20.0
SD – D – 24 L	—	20.6
Series C – (SD–LD–D)		
SD – 12 L – D	21.8	—
SD – 14 L – D	24.0	—
SD – 16 L – D	—	6.0
SD – 18 L – D	—	15.8
SD – 20 L – D	—	15.6
SD – 22 L – D	—	18.0
SD – 24 L – D	—	17.0

from the relatively low number of SD's required for bud formation in SD aftertreatment as compared to the treatments in series A.

From such an observation it may be concluded that the sensitivity to inhibition is relatively increased, when preceded by darkness, while it is not neutralized by following darkness.

4.1.8. Discussion.

Before discussing this section, the relevant observations will be summarized.

(1) Alternation of SD with LD of varying lengths indicates that not all non-inductive daylengths studied were inhibitory, while the flower inhibiting effect of non-inductive daylengths increased with increasing daylength.

(2) Single long days of 13, 13½, 14, 14½ or 15 hrs intercalated periodically during SD-treatment did not cause any inhibition. However, by increasing the number of such LD's some slight inhibition was evident in isolated cases.
(3) Intercalated LD's of more than a certain daylength caused inhibition, but a critical daylength for inhibition was not well defined. The light period required to inhibit the induction completely increased with increasing numbers of preceding SD's.
(4) A period of one or more days of CL intercalated between two series of SD, has a positive inhibitory action and is not merely passive.
(5) The inhibitory CL cycles have a marked time-effect when given during the run of inductive SD-treatment. The CL manifests its maximum inhibitory potential primarily prior to the beginning of induction.
(6) An increasing number of consecutive intercalated LD or CL cycles at a fixed point prior to induction causes increasing inhibition. This can be measured, one CL cycle showing far greater inhibitory effect than one LD.
(7) More than two consecutive CL cycles following 10 SD's, completely erases the effect of the preceding treatment, so that the flowering processes must be initiated anew.
(8) When a single inhibitory LD or CL cycle is followed by a long dark period, their effect is not completely nullified; a long preceding dark period increases the sensitivity to inhibition.

LONG (70, 1939) for Biloxi soybean and SCHWABE (91, 1956) for *Kalanchoë* have shown that these SDP undergoing alternating SD and LD (16 hrs light) treatments remained vegetative over considerable periods of time. In our experiments where SD alternated with LD of varied light: dark ratio, the results clearly demonstrated the existence of the inhibitory effects of LD's of varied lengths (figure 5, table 15, series A). The degree of inhibition increased with increasing photoperiod. One striking point which emerged from this experiment is the result that when 'near-critical' and certain normally non-inductive daylengths were alternated with SD, they became indistinguishable from SD in their effects on flowering. This would be expected if the role of the SD's is to disperse or reduce the inhibition built up in LD's.

However, it should be noted that not all normally non-inductive daylength cycles came to behave as inductive cycles. Cycles of daylength longer than 16 hrs (table 15, series A) not only were non-inductive in themselves, but also extended their effect to adjacent SD's, as a result of which flowering was completely prevented. Thus non-inductive cycles of various light: dark ratio, alternated with inductive SD cycles, have either promoting, partially inhibiting or completely inhibiting effects. Our results are contrary to those of SIROHI and HAMNER (95, 1962) who with Biloxi soybean found that certain inductive cycles became inhibitory on alternation with SD. The known influence of endogenous rhythm in Biloxi soybean probably accounts for such a difference.

Other investigators, CARR (14, 1955), SCHWABE (91, 1956), WELLENSIEK (106, 1958; 107, 1959) and EVANS (28, 1962) have studied various properties of either

LD (16 hrs light) or CL cycles when intercalated during SD-treatment. However, none of these investigators applied variation in the length of LD.

From my experiments it follows that not all such LD's show their inhibitory influence (expt 16 and 17 p. 25). This provides further evidence for the conclusion already reached in expt 15 (p. 24). This inhibition depends largely on the length of the light period during the intercalated LD, as well as on the number of such LD cycles. Moreover, inhibitory effects of LD become apparent only when preceded by SD's. Since not all intercalated LD's show a non-inductive effect, it may be that a light period above certain length actively inhibits induction. Such a phenomenon does indeed exist (figure 6). However, the minimum daylength for inhibitory action of light is not well defined and is a function of the previous SD-treatment. The maximum inhibition was produced by intercalated CL periods.

Another characteristic of the CL-inhibition is its 'time-effect' (figure 7). For CL inhibits only a part of the inductive process, namely the preparatory phase. After this, inhibition either declines or disappears. These results are in harmony with WELLENSIEK (108, 1960). However, the possibility that CL also acts after induction must not be ruled out, since THOMAS (99, 1963) has shown that CL may have stimulatory effects. A study of cell division (figure 8) indicates a definite stimulatory effect of intercalated CL on mitotic activity of the apex, suggesting a promotion of growth, but somehow its effect may be masked and unnoticeable on flowering.

In table 9, 10 and figure 9 we established that inhibition increases with increasing numbers of LD or CL cycles when such intercalated cycles are given consecutively after 9, 10 or 11 SD's. This observation together with the results of table 11 and 12 allows the conclusion that the capacity to produce inhibitory effects increased, until after a certain number of intercalated LD or CL cycles, maximal inhibitory capacity had been reached. With CL such a maximum is attained after 3 or 4 cycles and with LD it continues to build up to 7 LD's which was the highest number studied. From these results it may be calculated that a single LD is capable of annulling the inductive effect of 1.3 to 2.5 SD's. In case of CL, the first CL cycle has the greatest inhibiting effect, nullifying nearly 7.2 SD's, while 2 or 3 additional CL cycles completely erases the effect of the SD cycles so far given. This finding failed to confirm that of SCHWABE (91, 1956, p. 9) working with *Kalanchoë*, who intercalated an increasing number of long days (0, 1, 2, 3, 4, 6, 8, 12 and 16) between two consecutive series of 6 SD's and stated 'the effect of several long days given consecutively is not cumulative, the inhibitory effect of one long day being nearly, if not quite, as large as that of several', in other words, the inhibitory effect decreased with each additional long day. The reason why SCHWABE failed to find a cumulative effect of LD's probably lies in the fact that such LD's were administered after a plant had already attained a fairly advanced stage of induction. Since interpolated LD's have been shown to be maximally operative prior to induction, it is not surprising that a cumulative effect of such consecutive LD's was not observed. However, it is equally likely that the flowering physiology of *Kalanchoë* differs

markedly from that of *Salvia*. WELLENSIEK (107, 1959) has called attention to the fact that in *Perilla* the inhibitive effect of CL periods are cumulative. The data presented here are in agreement with this result.

The observation with 3 d. CL following 10 SD cycles, repeated several times (figure 10 and 11), suggests that light inhibition destroys the product of previous SD's completely. Combined with the observation in fig. 7 (p. 26) that inhibition becomes operative only after some SD's, this supports the conclusion that light exercises its inhibitory power primarily on the results of the processes of preceding SD's. In the meantime EVANS (28, 1962, p. 296) has found the same phenomenon with *Rottboellia*, without paying much attention to it. This conclusion is in disagreement with the conclusions of SCHWABE (91, 1956) and WELLENSIEK (107, 1959). Evidence deduced from tables 13, 14 and 15, where a 24 hrs dark period following LD or CL did not neutralize their inhibitory effect, further suggest that LD or CL acts by destroying the effect of previous induction.

After surveying the results of this section, it is evident that the effects of the processes taking place during the preparatory phase, are subject to active light inhibition. It seems likely that during the inhibition of this phase hormone-like substance(s) are produced. Later in this section we shall show their existence and discuss where they manifest their action and how they are removed.

4.2. FACTORS INFLUENCING LIGHT INHIBITION

We have demonstrated in the foregoing section the highly inhibitory effect of LD or CL. The question arises whether this inhibitory effect can be reduced or can be made to disappear. To investigate this problem, plant age, light intensity, light quality, temperature and darkness were studied in relation to light inhibition.

4.2.1. Age of plants.

Experiment 28. – One to 10 weeks old plants were available at the start of the SD-treatment as a result of weekly sowing. Plants numbering 250 were selected for their uniformity and were divided into 5 groups of 50 plants each. Each

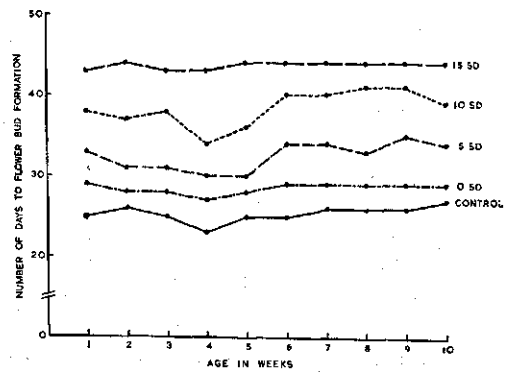


FIG. 12. *Experiment 28.* The effect of interrupting the SD-treatment by 3 days CL after 0, 5, 10 or 15 SD with plants of different ages (abscissa). Control = uninterrupted. Lines '0 SD' etc. = interruption after 0 SD, etc.

group in turn was represented by 10 different ages of 5 plants each. All groups were kept in SD simultaneously. One group was kept in uninterrupted SD as control, whereas in the other four groups the SD-treatment was interrupted by 3 d. CL after O, 5, 10 or 15 SD. The results are expressed in figure 12.

It is obvious that in the *uninterrupted* control group flower buds were initiated almost at the same time, irrespective of plant age. In the 0 SD group the differences with the control were approximately the same as the duration of the interruption, hence no inhibition by CL was observed. However, clear-cut inhibition was evident after 5 SD cycles, increasing when applied after 10 or 15 SD's.

Since the general trend of all the lines is horizontal, increasing plant age does not influence the light inhibition.

4.2.2. Light intensity.

Previous studies have shown that an SDP will initiate flowers in LD or even in CL provided the light intensity is sufficiently low. Such were the results reported in *Perilla*, DE ZEEUW (112, 1953; 113, 1954) and in *Salvia*, MEIJER (73, 1959), WELLENSIEK (108, 1960). In the following four experiments the influence of light intensity on the light inhibition has been studied.

Experiment 29. – In order to determine the effect of continuous light of different intensities the installation described on p. 9 was used. Plants were placed at 12 different distances from the vertical white light source. Table 16 summarises the results.

TABLE 16. *Experiment 29.* Effect of uninterrupted continuous light of different intensities on flower bud formation. Original number five plants per treatment, final number sometimes four. Duration of the treatment 75 days.

Light intensity in $\mu W/cm^2$	Mean number of days to flower bud formation	Newly formed leaf pairs at bud formation
2058	∞	12.0*
1580	56.0	9.2
1378	56.2	9.4
1020	52.6	9.4
800	49.8	7.2
670	45.6	6.2
540	44.7	5.5
380	44.0	4.5
369	40.5	3.7
328	40.0	4.0
292	39.7	3.7
255	39.2	4.0

* after 75 days.

All treatments with the exception of 2058 $\mu W/cm^2$ showed a flowering response within the 75 days of the treatment. The fastest response was found at and below 369 $\mu W/cm^2$. The number of newly formed leaf pairs at flower bud initiation decreased with decreasing intensity.

In conclusion it can be said that flowering can take place in CL provided the light intensity is below a certain range.

Experiment 30. – As seen from the foregoing experiment, high intensity reduces and low intensity increases induction. The present experiment was designed to investigate how the CL-inhibition is influenced by the light intensity. The SD-treatment was interrupted after 10 SD's for 3 days but with CL of different intensities. Table 17 shows the results.

TABLE 17. *Experiment 30.* Effect of interrupting the SD treatment after 10 SD cycles with 3 d. CL of different intensities. Units of 5 plants per treatment.

Light intensity in $\mu W/cm^2$ during CL	Mean number of days to flower bud formation
Control	18.0
2058	26.8
1586	25.6
1378	24.6
1020	24.4
800	21.2
669	20.0
538	20.0
382	20.0
368	20.0
328	21.2
292	20.0
255	19.6

These results indicate that the inhibitory power of intercalated CL decreases with decreasing intensity and is absent at and below 800 $\mu W/cm^2$.

Experiment 31. – In expt 27 it was found that alternation of single SD with single CL (intensity 3500 $\mu W/cm^2$) resulted in complete suppression of flowering. In the present experiment the intensity during the CL was varied. The results are shown in table 18.

TABLE 18. *Experiment 31.* The effect of 25 alternations of 1 SD and 1 d. CL of different intensities, indicated in column 1. Duration of the treatment 50 days. Units of 5 plants per treatment.

Intensity during CL in $\mu W/cm^2$	Mean number of days to flower bud formation	
	during treatment	in SD-aftertreatment
Control	—	22
2050	—	22
1380	—	21
800	—	9
540	42	—
330	34	—
255	33	—

During 50 days of alternating treatment flowering did not occur when the intensity range during CL was between 2050–800 $\mu W/cm^2$. The extent of inhibition is evident from considering the days required for the appearance of buds in SD-after treatment. However, when a SD cycle was alternated with CL with intensity lower than 800 $\mu W/cm^2$ flowering took place during the treatment, the faster the lower the intensity.

It is clear, therefore, that the effect of a single SD is completely lost in an ensuing period of CL of high intensity, but there is only partial loss in its effectiveness with low intensity light.

Experiment 32. – Groups of 5, 10 or 15 SD's were alternated with 1 d. CL of different intensities. The alternations were given 6×, 3× or 2× respectively, so that in all treatments 30 SD were involved. The further treatment consisted of SD. The light intensities during CL are indicated in table 19.

TABLE 19. *Experiment 32.* The effect of alternating 5, 10 or 15 SD with 1 day continuous light of different intensities (column 1), 6×, 3× or 2× respectively, followed by SD and expressed as mean number of days to flower bud formation. Units of 5 plants per treatment.

Intensity in $\mu W/cm^2$ of CL	Alternation with		
	control	5 SD (6×)	10 SD (3×)	15 SD (2×)
	29			
2050		61	56	33
1370		60	42	33
800		60	42	33
538		53	39	33
328		49	39	31
255		44	37	30

As expected, these results indicate that CL exerts its inhibitory action only after 5 or 10 SD's and has very little or practically no inhibitory action after 15 SD's. However, the inhibitions were higher after 5 SD cycles than after 10 SD cycles. This might well be due to the division of 30 SD cycles into smaller sub-units and the increased number of CL exposures, namely 6 as compared to 3 in 10 SD treatment.

From this and the foregoing experiments it may be concluded that the effect of a single or a group of SD's in alternation with a single CL is markedly influenced by the light intensity during the non-inductive CL periods.

4.2.3. Light quality.

Experiment 33. – The data presented in this experiment were originally obtained by WELLENSIEK (unpublished) and have been put at my disposal. The experimental procedure described for *Perilla*, WELLENSIEK (107, 1959) also applies here. In short, the influence of different light quality or wavelengths on the inhibitory effect of CL was studied. After 10 SD cycles the plants were exposed to 4 days of continous light of different qualities and were maintained

afterwards in SD. The results are expressed in table 20 in order of decreasing inhibition by a given light source.

TABLE 20. *Experiment 33.* Effect of interrupting the SD treatment after 10 SD with 4 days of continuous light of different qualities. Units of 12 plants per treatment. IL = incandescent. TL = fluorescent.

Light source	Light intensity in $\mu W/cm^2$ during CL	Mean number of days to flower bud formation	Inhibition in days
controls	—	29	—
3 TL + 2 IL	2281	43	10
Red + infrared	1800	43	10
2 IL 75 W	527	42	9
Red (– infrared)	1800	41	8
Green	1750	40	7
Blue	1500	39	6
3 TL 40 W/29	2068	39	6
White	1500	37	4
Infrared	900	36	3

It is obvious that all light colours administered during 4d. CL either individually or in combination were inhibitory, irrespective of the marked differences in their intensity. Stated in another way, no monochromatic light was found which was not inhibiting.

One striking point is noticeable, namely the slight inhibition by infra-red. Since photosynthesis is negligible in infra-red, these results would eliminate the possibility of an exclusive role of assimilates in light inhibition.

4.2.4. Temperature.

Experiments designed to test the effect of high and low temperatures on the inhibitory action of intercalated CL were performed.

Experiment 34. – The SD-treatment (20 °C) was interrupted during 2 days with CL at either 20 °C or 10 °C. The results are expressed in figure 13.

The results indicate that only CL interruptions at 20 °C are highly inhibitory with a curve which is similar to figure 7. However, at the low temperature interruption of 10 °C, the inhibitory power of CL was completely prevented. In the flat curve at 10 °C the points lie either at or below the control point. This means that CL interruptions at 10 °C do not *suspend,* but actually participate in the photoperiodical reactions. Similar results were obtained with 5 °C.

Experiment 35. – In this experiment the effect of 1, 2 or 3 CL cycles given after 10 SD's but accompanied by temperatures of 30 °C or 35 °C was studied. Table 21 shows the results.

It is seen that CL-inhibition at 30° or 35 °C remains essentially at the level of 20 °C as was found in expt 33.

However, one point should be emphasized, namely that the capacity of CL

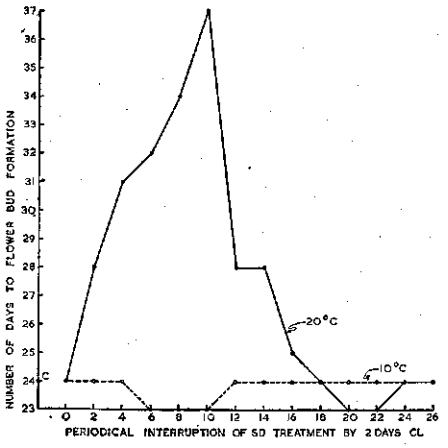


FIG. 13. *Experiment 34.* Periodical interruption of SD-treatment by 2 days CL at two different temperatures.

to inhibit completely the effect of the preceding 10 SD cycles was already reached after 1 d. CL at 30° or 35°C. This can easily be calculated from table 21 by subtracting 10 SD plus the duration of the CL-interruption from the final values. This results in a value which does not differ from the control. To obtain similar effects at 20°C, 2 or 3 CL cycles were needed as was demonstrated in figure 9.

TABLE 21. *Experiment 35.* The effect of interrupting the SD treatment after 10 SD by 1, 2 or 3 days CL at temperatures of 30°C or 35°C. Five plants per treatment.

Duration of interruption	Temperature during interruption		
	control	30°C	35°C
0 d. CL (control)	22	—	—
1 d. CL		32.6	32.0
2 d. CL		34.2	33.8
3 d. CL		35.2	35.2

Experiment 36. – The effect of low temperature following the high temperature during CL was studied to determine whether or not the inhibition can be neu-

TABLE 22. *Experiment 36.* The effect of interrupting the SD-treatment after 10 SD by 2 days of continuous light at 20°C and 0, 1, 2 —— 7 days of continuous light at 10°C.

Duration of CL at 10°C	Number of days to flower bud formation
Control	19
0	31
1	31
2	31
3	31
4	31
5	31
6	32
7	32

tralised. The plants were subjected to the following schedule: 10 SD – 2 d. CL (20°C) – 1 to 7 d. CL (10°C) – SD. The results are shown in table 22.

The results are negative. This lack of effect of 10°C involves that the entire process has to restart after the interruption. This implies that 2 d. CL (20°C) work on the preceding SD's. However, the function of low temperature subsequent to the second SD-treatment is evident: since the resultant values are constant, the number of SD's at 20°C necessary for bud formation was found to decrease with increasing duration of low temperature CL cycles. Hence, during the CL cycles at low temperature an inductive action takes place.

Experiment 37. – The present experiment is similar to expt 36, but reversed, namely the effect of high temperature following the low temperature during CL was studied. The results are shown in table 23.

TABLE 23. *Experiment 37.* The effect of interrupting the SD treatment after 10 SD by 2 days of continuous light at 10°C and 0, 1, 2 —— 7 days of continuous light at 20°C.

Duration of CL at 20°C	Number of days to flower bud formation
Control	19
0	19
1	22
2	29
3	28
4	29
5	29
6	28
7	29

During a 2 d. CL (10°C) interruption – 0 d. CL at 20° – flowering was not inhibited. However, 1 cycle of CL at 20°C resulted in a slight inhibition, whereas 2 or more CL-cycles gave maximal inhibition.

The results obtained in this section can be summarised very briefly as follows: high tempetarures, 20°, 30° and 35°C, all favour the inhibitory action with a somewhat greater effect of 30° and 35°. Low temperatures, 5° and 10°C, prevent inhibition completely.

4.2.5. Darkness.

In this section the effects of complete dark periods of various duration were studied from two different angles: (a) when such periods preceded or were given during the inductive SD cycles, (b) when they preceded or followed the inhibitory intercalated CL cycles.

Experiment 38. – The influences of 1 to 7 days of darkness following the normal LD pretreatment but preceding the inductive SD-treatment were studied. Table 24 shows the results.

It is seen that increasing the duration of dark periods prior to the SD-treat-

TABLE 24. *Experiment 38.* The effect of increasing duration of continuous dark (D), given *before* the SD-treatment. Units of 5 plants per treatment.

Duration of Darkness	Mean number of SD for flower bud formation
Control	22.6
1 d.	21.0
2 d.	20.0
3 d.	18.8
4 d.	17.6
5 d.	17.0
6 d	17.0
7 d.	17.3

ment decreases the number of subsequent SD's necessary to initiate flower buds upto 5 d. D. After this the effect remains constant as the dark periods are extended.

It should be noted that heavy shedding of leaves took place in plants which received 6 or 7 days of total darkness.

The promotive effect of darkness offers the following possibilities: (a) considerable reduction in the inhibitory level arisen during the LD pretreatment, (b) a direct participation in the act of induction, (c) or both.

Experiment 39. – In an attempt to assign the role of darkness more clearly, the SD-treatment was interrupted by 2 d. D at 2 days interval. In this manner plant groups were available which had received SD's ranging from 0 SD till 24 SD's. There were 5 plants per treatment.

The results are that all treated groups had visible flower buds after 20 days as compared to 21 days in SD control.

This difference is too small and uncertain to draw any definite conclusion. In the next experiment darkness of longer duration has been given.

Experiment 40. – The role of increasing duration of dark periods when given after 10 SD cycles was investigated. Table 25 shows the results.

TABLE 25. *Experiment 40.* The effect of interrupting the SD-treatment after 10 SD with darkness of various duration. Five plants per treatment.

Number of darks periods	Mean number of days to flower bud formation
Control	22.6
1 d.	22.0
2 d.	23.4
3 d.	23.0
4 d.	24.2
5 d.	25.3
6 d.	25.3
7 d.	28.0

Up to 3 days darkness the values remain approximately constant. This means that this darkness has had a similar effect as SD and hence promoted the induc-

tion. Soon after, the effect of darkness slowly declines as the dark periods are extended, probably on account of weakening the plants.

Experiment 41. – The present experiment was devised to test the effect of a dark period of various lengths preceding the 3 d. CL interruption. The experimental scheme was as follows: 10 SD – 0 to 7 d. D – 3 d. CL – SD. The results are presented in table 26.

TABLE 26. *Experiment 41.* The effect of interrupting the SD-treatment after 10 SD by 0, 1, 2, —— 7days of darkness and 3 days of continuous light. Five plants per treatment.

Duration of darkness in days	Mean number of days to flower bud formation
Control	22.6
0 d.	36
1 d.	35
2 d.	35
3 d.	36
4 d.	37
5 d.	37
6 d.	37
7 d.	38

According to expectation, 3 d. CL exert a marked inhibition when preceded by 0 d. D. As the duration of the preceding dark periods was increased, the effectiveness of the 3 d. CL remained on the same level.

Experiment 42. – An experiment designed to test the possibility of the removal of CL-inhibition by subsequent long periods of darkness was implemented. The usual 10 SD – 2 d. CL treatment was followed by 0, 24, 48, 56, 64, 72, 80, 88, 96, 104 or 112 hours of total darkness. Aftertreatment consisted of SD. There were 5 plants per treatment.

The results are negative. All treated groups initiated flower buds in 30–31 days as compared to 19 days in SD control.

It may be concluded that the inhibitory effect of 2 d. CL is not removed by following prolonged dark periods.

Experiment 43. – The experiment just described was repeated in a more extensive way. After having received 10 SD's, the plants were exposed to either

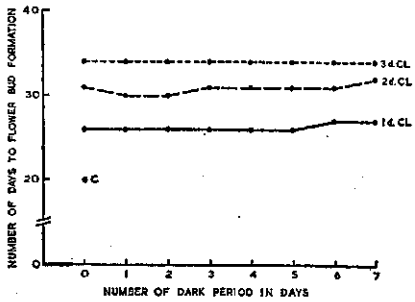


FIG. 14. *Experiment 43.* The effect of interrupting the SD-treatment after 10 SD with 1, 2 or 3 days CL and with increasing numbers of dark periods. C = control.

1, 2 or 3 CL. Each CL-interruption in turn was followed by 0 to 7 d. D. After-treatment consisted of SD. Figure 14 summarizes the results.

As expected, the inhibitory action of CL-interruption increases with increasing CL cycles, as is shown at the point '0' on the abscissa. However, when 1 to 7 d. D follow 1, 2 or 3 CL cycles, it is seen that the resultant value lie horizontally and at the level of approximately 0 d. D. This would indicate that the inhibitory effect of intercalated CL, irrespective of its duration, is not counteracted or faded out by a following dark period upto 168 hours, or 7 days, duration.

4.3. Effect of interruptions during the light and dark periods

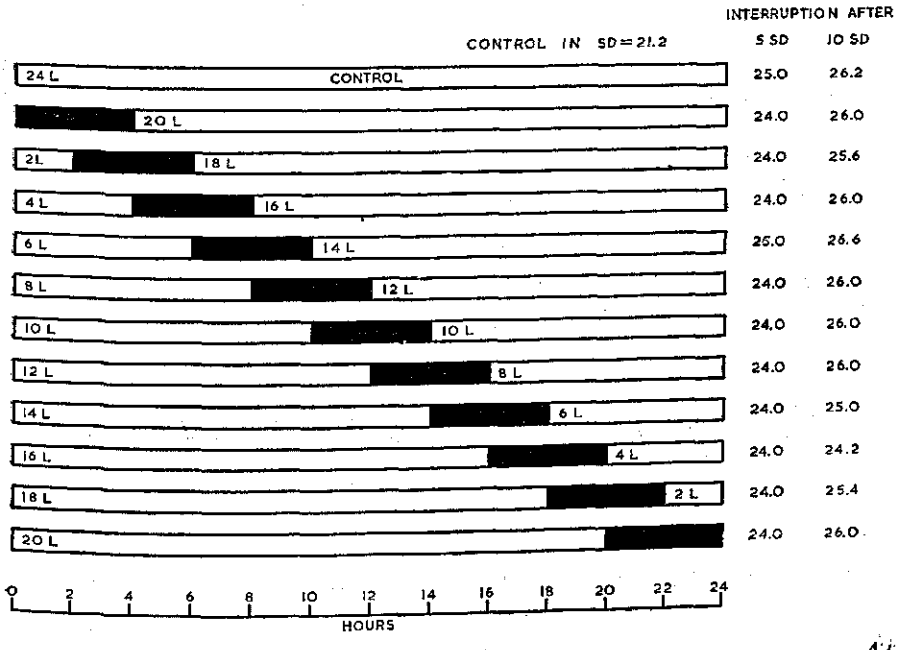
The present section embraces both the effects of dark breaks in intercalated CL and of light breaks in inductive dark periods.

4.3.1. Effect of dark interruption during light.

The object of the following two experiments was to see whether one or more dark-breaks, when given *during* the intercalated CL, reduce its inhibitive effect.

Experiment 44. – Plants which had received 5 or 10 SD's were exposed to 1 d. CL and were placed back in SD. During this single CL cycle, a single dark-break of

TABLE 27. *Experiment 44.* Effect of dark-break of 4 hours duration given at various times during 1 d. CL, which is administered after 5 or 10 SD cycles, on the effectiveness of CL-inhibition and expressed as mean number of days to flower bud formation. The light period is represented by the empty bar and the dark period by the solid bar.

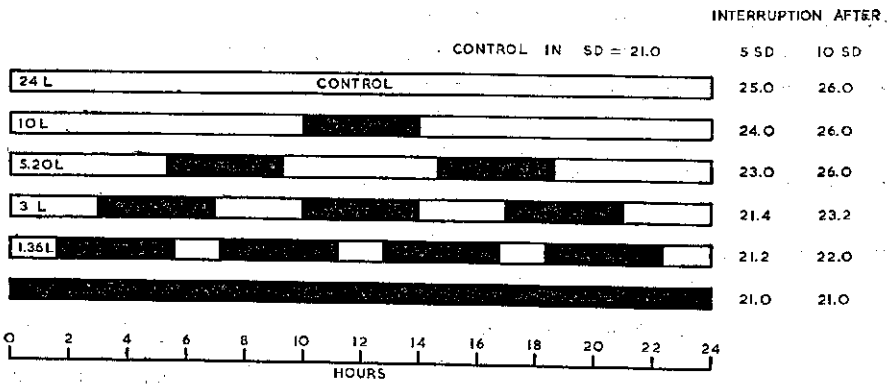


4 hrs duration was inserted, but at various times. In this way the CL period was divided into two parts, usually of different lengths. The effect is shown in table 27.

A single dark interruption of 4 hrs given during intercalated CL, irrespective of its position, was found to be ineffective in reducing the light inhibition. Hence the results are negative.

Experiment 45. – In this experiment the effect of the frequency of the dark period within 1 d. CL was investigated. One, two, three or four dark-breaks, each of 4 hrs duration were inserted during 1 d. CL. The proportion of light and the frequency of dark periods are shown in table 28.

TABLE 28. *Experiment 45.* Effect of one or more dark-breaks of 4 hours duration during 1d. CL, which is administered after 5 or 10 SD cycles, on the effectiveness of CL-inhibition. For the rest see legend of table 27.



It is clear from these results that by increasing the number of dark-breaks within 1 d. CL, the inhibition considerably decreases and disappears completely when replaced by 1 d. D.

In short, it can be concluded that the inhibition of flowering is to a certain extent proportional to the amount of light given within 24 hrs of intercalated CL.

4.3.2. Effect of light interruption during dark.

The prevention of flowering in SDP can be obtained by giving a relatively short daily light-break in the middle of the inductive dark period, as was first reported by HAMNER and BONNER (37, 1938) in *Xanthium*. Thus the importance of the dark period was revealed. Since then this phenomenon was studied in greater detail in many plants by numerous investigators. In *Salvia occidentalis*, MEIJER (72, 1957; 74, 1960) concluded that long-day effect by daily night-breaks during SD depends on the light quality and the intensity of the main light period, and the position of the light-break during the night. SCHWABE (91, 1956) mainly using *Kalanchoë*, compared the effects of occasional nightbreaks and intercalated LD and found both to have similar inhibiting effects. WELLENSIEK

(107, 1959) periodically interrupted the dark period of an SD-treatment and found a similar trend in *Perilla*, although the quantitative night breaks had much weaker effects than intercalated CL periods.

The main aim of the following experiment has been to establish whether short occasional light-breaks have comparable inhibitive power as long periods of intercalated CL, as used in the preceding experiments. Before describing the experiments, it should be mentioned that in preliminary observations 1 or 2 hrs of white fluorescent light interruptions during the long light were hardly effective in preventing flowering. Therefore, 4 hrs light-break have been employed throughout.

Experiment 46. – The effects of a single light break, periodically given in the middle of the dark period of the SD-treatment at 2 days interval were studied. For the sake of convenience the timing of the SD-treatment was altered. It consisted of 8 hrs of TL light of 2700 $\mu W/cm^2$ intensity from 0.30 a.m. to 8.30 a.m., followed by 16 hrs darkness from 8.30 a.m. to 0.30 a.m. With intervals of 2 days groups of 5 plants were transferred from LD into SD, so that after 18 SD's plants were available which had received 0, 2, 4 ---- 18 SD's. All these plant groups received a single light break of 2700 $\mu W/cm^2$ from 2.30 p.m. to 6.30 p.m. simultaneously. Immediately after the breaks, the plants were kept in the darkness to follow the SD routine. The results are summarized in figure 15.

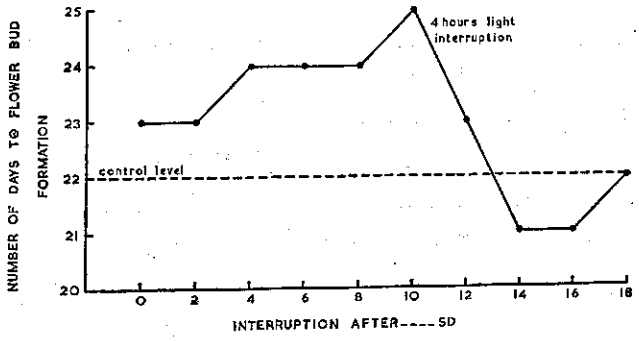


FIG. 15. *Experiment 46.* Effect of a night interruption, given at various times during the SD-treatment, with 4 hours of light-break in the middle of the dark period.

A light break after 0 or 2 SD's showed a tendency towards a slight inhibitive effect. The inhibition increased, showing a peak after 10 SD's and then gradually dropped.

From figure 15 it is seen that the inhibition obtained by a 4 hrs light break is to a certain extent similar to one of CL (expt 19 on p. 26). The inhibitive effect of a light break is quantitatively smaller, but again the peak at 10 SD is striking.

Experiment 47. – The effects of a single light break given at different times during the 5th, 10th or 15th night of an SD-treatment were investigated. Plants were given ordinary SD-treatment with natural daylight. White fluorescent light of intensity 3500 $\mu W/cm^2$ was given during the light-break. The experimental design and the results are plotted in figure 16.

This experiment shows that a night-break inhibition is especially evident

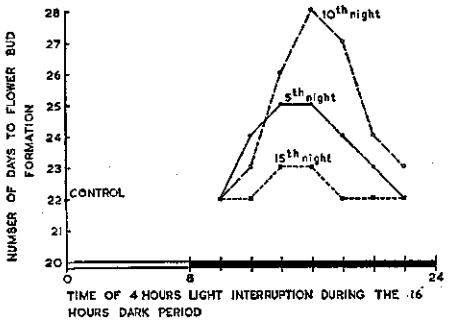


FIG. 16. *Experiment 47.* Effect of 4 hours light-break given at various times during the 5th, 10th or 15th night.

during the 5th or 10th night. This is principally similar to the results of expt 46.

It is also seen that single night-break given at the beginning or the end of the dark period are either less effective or not so at all. They are most effective near the middle of the night. It is quite striking to see that one light-break in the middle of the 5th night inhibits the flowering by 3 days and when given during the 10th night by 6 days.

Experiment 48. – The effects of light-breaks in the middle of dark periods of the inductive SD-treatment during 5th, 5th and 10th, and 5th, 10th and 15th nights were investigated. The experimental conditions were similar to expt 45. The results are summarized in table 29.

TABLE 29. *Experiment 48.* Effect of light-breaks of 4 hours duration given in the middle of the 5th night, 5th and 10th night, 5th, 10th and 15th night. Units of 5 plants per treatment.

Light-break during ---	Mean number of days to flower bud formation	Difference with control
Control	23.0	—
5th night	24.2	1.2
5th and 10th night	28.0	5.0
5th, 10th and 15th night	32.0	9.0

It is clear that when light-breaks are given at an interval of five nights, the effectivity of their inhibitive action invariably increases during an increasing number of exposures.

The delay of only 1 day by a single break during the 5th night is different from a similar treatment in expt 47, resulting in a delay of 3 days. This difference might be attributed to the light source during the light period of the normal SD-treatment. In the present experiment this was TL and in expt 47 it was natural daylight.

Experiment 49. – This experiment was set up to obtain data permitting a comparison with CL-inhibition, as reported in preceding experiments. Plants first received 8, 9, 10, 11, 12 or 13 uninterrupted SD's, followed by 3 consecutive

light-breaks in the middle of the dark period of subsequent SD's. For instance, after 8 SD's the following 9th, 10th and 11th night each received a 4 hrs light-break of intensity 3500 $\mu W/cm^2$ in the middle. After the breaks normal SD followed.

The control had visible flower buds after 21.6 days. The interrupted groups needed 7.4 to 8.8 more days.

From these results it must be concluded that the annulling capacity of short night-breaks is qualitatively similar to that of intercalated CL, as found e.g. in expt 19. This would also suggest that the night-breaks influence the effect of the preceding SD's instead of those which immediately follow them.

The striking features of the experiments reported in this section are: (1) Increasing dark-breaks during intercalated CL considerably reduce the CL-inhibition. (2) Occasional short light-breaks during dark periods are similar in effect to long intercalated CL periods in their inhibitive effects.

4.4. Translocation of the inhibitory effect of light

Many instances of *specific* and *non-specific* effects of non-inductive conditions are known in SDP. A few examples will serve to illustrate the type of specificity, i.e. the production of flower-inhibiting substance(s), as demonstrated by the supporters of the flower inhibition hypothesis (p. 6): (a) Total defoliation may cause flowering under long-day conditions, so that the removal of the source of the inhibitor is sufficient for flower formation (101). (b) Fractional induction studies have shown the production of inhibitory substances under non-inductive conditions (91, 106, 107). (c) Grafting experiments indicate the transmissibility of such substances in donor/receptor plants, just as defoliation experiments (80, 86). On the other hand, the inhibitory action of non-inductive conditions is claimed by the supporters of the floral hormone hypothesis (p. 4) only as a *non-specific* one. They raise the following objections: (a) There is no production of flower-promoting substances in long-days, hence there is no flowering. (b) The principal argument is that the inhibitory effects of non-induced leaves are limited to leaves located *between* the source of floral stimulus and the responding apex (39, 41, 110). Lang (56, 1952, p. 288) stated 'This very action, however, can be accounted for in terms of translocation and is thus the least specific one that could be imagined'. He further comments on the same page 'these effects seem to be directed, not against their functioning, but against the formation of flower-promoting substances'.

It is conceivable that the supporters of the flower hormone hypothesis deny the existence of flower-inhibiting substances; they suggest that evidence in favour of transmissible inhibiting substances is poor and equivocal.

In the course of the present investigation, much of the evidence in the foregoing sections concerning the inhibitory effects of non-inductive intercalated CL was collected while working with whole plants. However, no attention was paid to the ultimate fate of the CL-induced inhibitory effect. Therefore, the possibility of transmitting such an effect was tested, using a selective defoliation technique.

4.4.1. Single-branched plants.

Experiment 50. – The aim of this experiment was to determine the site of action of the inhibitory intercalated CL, whether in the leaves or in the apex. The plants were defoliated except for one expanded leaf pair on the 5th node and the youngest leaves enclosing the apex. These defoliated plants received an SD-treatment. After 9, 10 or 11 SD's either the expanded leaf pair or the apex with unfolded leaves was exposed to 3 d. CL. The newly formed leaves and axillary buds were continually removed before and during the treatment. Five plants per treatment were used. The defoliation patterns and the results are illustrated in figure 17.

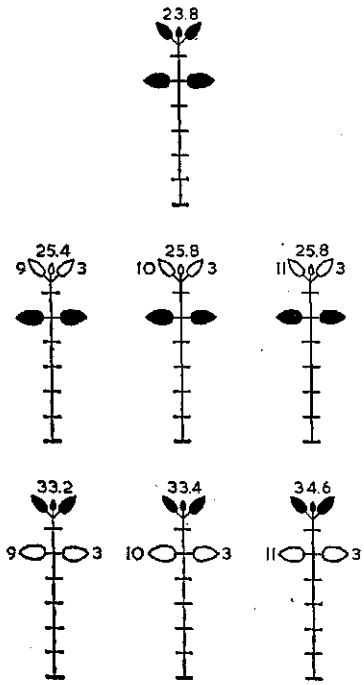


FIG. 17. *Experiment 50.* Effect of intercalated 3 d. CL, given either to leaf or apex, on the localization of CL action.

and = leaves and apex with youngest unfolded leaves always in SD.

and = leaves and apex with youngest unfolded leaves in SD, but exposed to 3 d. CL after 9, 10 or 11 SD and treated with SD afterwards.

Figures on the *left* of the leaf pair or apex are the number of SD cycles given before CL, and on the *right* the number of CL cycles.

Figures at the top of the plants: average numbers of days for the formation of macroscopically visible flower buds.

It is clear that an inhibitory effect of intercalated CL was rather slight when administered during the SD-inductive treatment of the apex. A considerably stronger effect was evident when a similar treatment was accorded to the leaves.

On the basis of these data, it may be safely concluded that the main site of perception of the inhibitory intercalated CL are the leaves and not the apex.

Experiment 51. – The object of the present experiment was to study the position-effect of a single period (1 day) of intercalated CL given after 5, 10 or 15 SD's to either the apical or basal half of a single leaf. The treated leaf was on the 5th node. The apex was always maintained in SD. Five replicates per treatment were used. The results are illustrated in figure 18.

It is seen that the inhibitory influence of intercalated CL was very marked

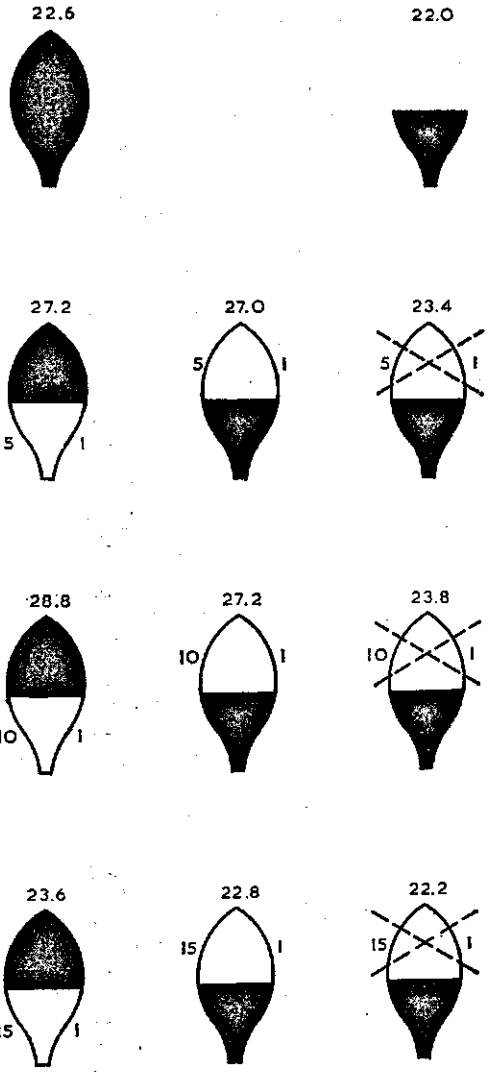


FIG. 18. *Experiment 51.* The effect of the position of CL-inhibition in the leaves. The dark portion of the leaf always received SD, whereas the empty portion received 1 d. CL after 5, 10 or 15 SD's, and was either retained or immediately removed after 1 d. CL. After-treatment consisted of SD's.

 = portion of the leaf removed immediately after CL treatment.

For the rest see the legend of figure 17.

after 5 or 10 SD's, irrespective of its apical or basal position within a single leaf. However, when the apical half of the leaf was removed immediately after 1 d. CL in the case of both 5 and 10 SD's, the inhibition was strongly suppressed, though not rendered completely ineffective.

Two possibilities offer themselves as an explanation. During CL treatment of the apical half an inhibitor is released which passes through the SD-barrier of the same leaf, thereby exerting its effect at the apex, without disturbing the SD-action in the basal half. The alternative seems to be that the inhibitor could partially destroy the effect of the SD in the basal part while moving through it and thus could produce the final delay.

Since it does not seem easy to separate these effects in such single leaf experiments, this problem was further studied in schemes with 2 or 3 leaf pairs and with intact plants.

Experiment 52. – This experiment was conducted to study whether the leaves undergoing a non-inductive treatment below the induced leaves, could be shown to be inhibitory. Plants were defoliated in such a manner that only two leaf pairs were left on the 3rd and 5th nodes, hence in the same rank. The upper leaf pair together with the apex was always kept in SD, whereas the lower leaf pair received one of two different treatments: either 13 d. CL, after which the entire plant received SD, or 10 SD – 3 d. CL – SD. In both cases the lower leaf pair was either retained or removed immediately after the CL treatment. The results are depicted in figure 19.

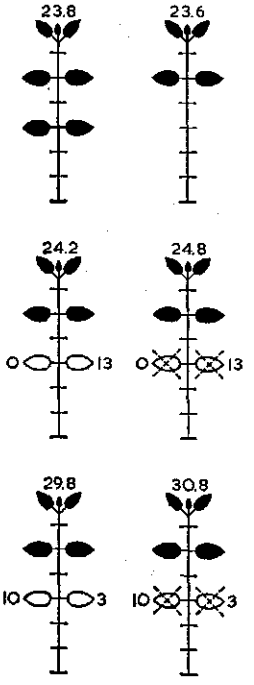


FIG. 19. *Experiment 52.* Two leaf pairs and CL-inhibition. ⋈ = leaves exposed to either 13 days CL or 3 days of CL after 10 SD's, removed immediately after CL-treatment. For the rest see the legend of figure 17.

The results clearly demonstrate that leaves which received continuous illumination for 13 days, but were below the SD leaves, showed no ability to inhibit flower bud formation, when either retained or removed. When the lower leaf pair was exposed to 10 SD – 3 d. CL – SD, a marked inhibitory response was produced. This was also the case when the CL-inhibited leaf was removed immediately after 3 d. CL.

Thus flower inhibition by continuous illumination of the lower leaves was not achieved unless such treatment was preceded by 10 periods of SD's.

It is to be concluded that inhibition of flowering is possible by leaves which are *situated well below the SD leaves* after treating them with 10 SD and 3 d. CL.

Experiment 53. – The experimental design was similar to that of expt 52, save that the lower leaf pair received one of the following two treatments: 10 SD – 3 d. CL – SD or 10 SD – 3 d. CL – 10 SD – 3 d. CL – SD. The lower leaves were either retained or immediately cut-off after the final CL-treatment. Units of 5 plants per treatment were taken. The results are given in figure 20.

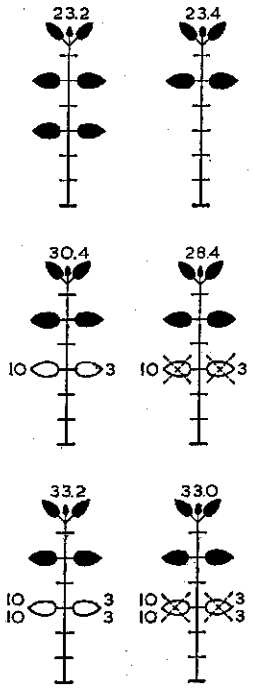


FIG. 20. *Experiment 53.* Effect of intercalated 3 d. CL given once or twice to the lower leaf pair. For the rest see the legend of figure 17.

It is obvious that intercalated 3 d. CL after 10 SD's, given once to the lower leaves, showed again a strong inhibitory effect. However, by giving a similar treatment twice to the lower leaves, the inhibitory effect was increased, though not proportionally.

Experiment 54. – The transmission of 'floral inhibitor' in a three-leaf-pair defoliation pattern was studied. The upper and the lower leaves together with the responding apex received SD's, whereas the middle leaf pair received 3 d. CL after 9, 10 or 11 SD's. Once again the CL-inhibited leaf was either retained or removed immediately after CL, as shown together with the results, in figure 21.

Once again the results (top row) demonstrate the generation of an inhibitor in the CL-inhibited leaf, situated well below the terminal bud and one pair of the SD leaves. From the bottom row it seems that 'inhibitor' migrates from its site of origin towards the apex, soon after its production.

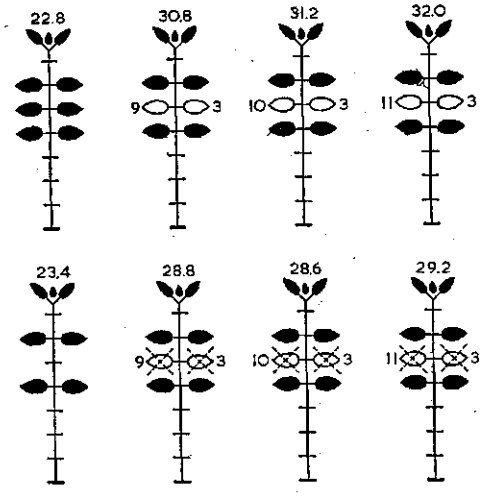


FIG. 21. *Experiment 54.* Three-leaf-pair scheme and the effect of removal of CL-inhibited leaf immediately after CL-treatment.
For the rest see the legend of figure 17.

Experiment 55. – This experiment was similar with expt 53 except that the area of the middle leaf pair was reduced gradually. Five plants per treatment were taken. The results obtained are represented in figure 22.

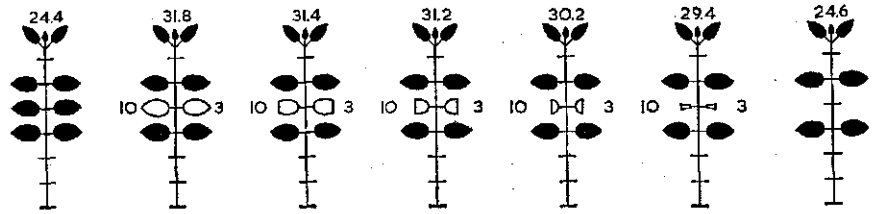


FIG. 22. *Experiment 55.* Effect of CL-inhibited leaf area. Middle leaf pair showing different areas from 100% (17 cm²), 75%, 50%, 25%, leaf petiole and 0%, which were exposed to 3 d. CL after 10 SD.
For the rest see the legend of figure 17.

As the leaf area of the middle leaf pair was reduced, the inhibitory effect of the treatment was decreased. This decrease, however, was not proportional, and the presence of just a petiole with only a small parenchymatous tissue was sufficient to produce a marked inhibition.

After demonstrating the transmission of floral inhibitor in the selective defoliation experiments just described, the question is now raised whether similar effects are attainable with intact plants.

Experiment 56. – The treatment involved intact plants with six leaf pairs. The plants were treated so that 1, 2, 3, 4 or 5 leaf pairs (base upwards) received 3 d. CL after 10 SD's, while the remainder of the plant received continuous SD. After this treatment the plants received SD. Five plants per treatment were taken. Figure 23 shows the results.

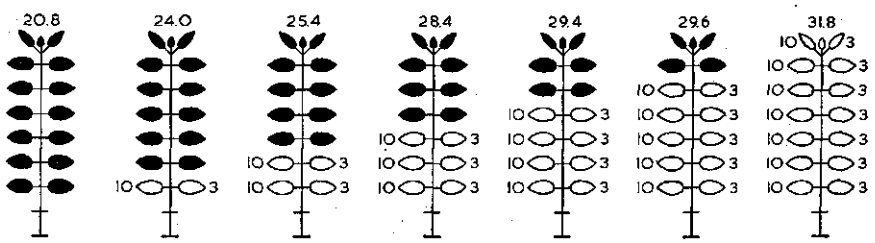


FIG. 23. *Experiment 56.* Effect of exposing increasing numbers of leaf pairs to intercalated 3 d. CL after 10 SD in intact plants. For the rest see the legend of figure 17.

These results reveal two interesting facts. Firstly, a close correlation exists between CL-inhibition and the leaf area exposed to 3 d. CL after 10 SD, the inhibition invariably increasing with increasing leaf surface exposed. Secondly, the presence of one or more inhibited leaf pairs, below the SD leaves, has a marked capacity to inhibit flower bud formation. This was further studied in the following experiment.

Experiment 57. – Plants having six leaf pairs were treated so that the upper three leaf pairs together with the terminal apex were always kept in SD. The lower three leaf pairs received either 13 CL cycles – SD or 10 SD – 3 d. CL – SD.

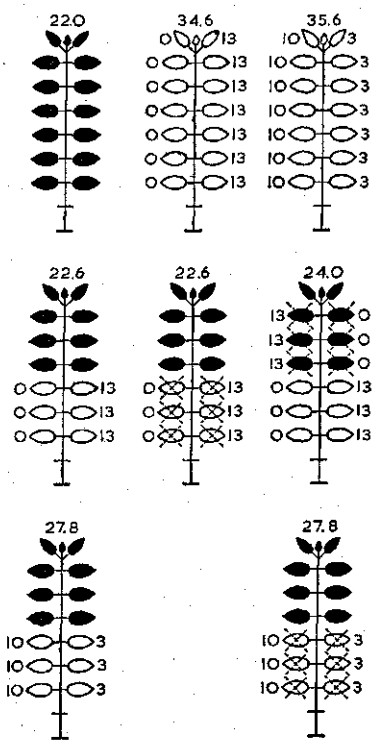


FIG. 24. *Experiment 57.* Intact plant and transmissibility of the CL-inhibition. For the rest see the legend of figure 17.

In certain cases the CL-leaves were removed. Units of 5 plants per treatment were taken. The results are illustrated in figure 24.

From the results in the top row, similarity between 13 CL and 10 SD – 3 d. CL treatment was evident. As expected, in both cases flowering was delayed as compared to the SD control. The middle row presents interesting results. When the lower leaves were treated with 13 CL, while the upper leaves simultaneously received SD, no translocation of the inhibitory influence from the non-induced leaves was observed, same as expt 52, figure 19. This was the case when such leaves were either retained or removed. However, it is interesting to note that removal of the upper SD leaves after 13 days, resulted in the appearance of flower buds with only 2 days difference with the SD control. This means that the product of 13 SD cycles has transmissible properties, resulting in a qualitative change at the apex, but top leaves not removed. The bottom row shows that when the lower leaves were exposed to 3 d. CL after 10 SD's, a clear-cut inhibitory effect of CL was evident. This was also the case when such leaves were immediately removed after 3 d. CL treatment, suggesting that an 'inhibitor' has rapidly been transported in the upward direction.

In conclusion, it may be said that the release of an 'inhibitor' by CL cycles is conditioned by the necessity, that CL periods are preceded by SD cycles. This is the same conclusion as was reached in expt 52 with partly defoliated plants.

4.4.2. Differential treatment of leaves in a two-leaf-pair scheme.

Two possibilities have already been discarded in the foregoing section, namely the non-interference by non-inductive leaves with either the production of a floral stimulus in the SD-treated leaves or with its translocation.

An equally interesting feature of the floral inhibitor, hitherto given less attention, is its activity after it is released from the CL-inhibited leaves. The following experiment throws some light on this aspect.

Experiment 58. – In a two-leaf-pair scheme the upper and the lower leaf pairs were differentially treated, figure 25.

The first row (controls) conforms former results (figure 19). In the second row it is seen that maximum release of an inhibitor from the lower leaf pair gives a delay of about 12–14 days in treatments 7, 8 and 9 when the upper leaves had already received 10, 11 or 12 SD's respectively. This delaying effect was considerably suppressed in the treatment 10, i.e. when the upper leaf received 13 SD's. This is certainly an effect of the inducing influence of the upper leaf pair in SD. Apparently the inhibitor can only effectively express itself before the apex is being changed by the arrival of enough stimulus from the induced upper leaf pair. Comparing the results of this row with the third one, we see similar results, thus showing that the presence or absence of the leaves after the maximum inhibitor was released, is of little importance for the final results.

In the fourth row the upper leaf pair was removed after 10, 11, 12 or 13 SD respectively, while the supply of the inhibitor was continued. Flowering was completely suppressed in all the cases. This is to be expected because, firstly, the source of stimulus supply was removed, hence the apex had to depend on the

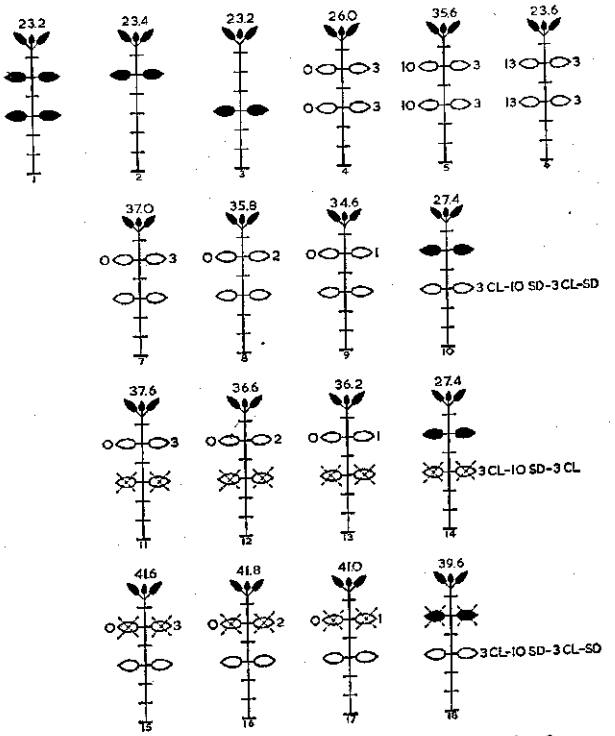


FIG. 25. *Experiment 58.* Differential treatment of the leaves in a two-leaf pair scheme showing the competition between the inhibitor and the flower stimulus.
First row, controls.
Second row, upper leaves received 10, 11, 12 and 13 SD's respectively at the time when the inhibitor was released from the lower leaf pair after the treatment 3 CL – 10 SD – 3 CL – SD. Both leaf pairs were retained.
Third row, similar to second row except that the lower leaf pair was cut-off after the second CL-treatment.
Fourth row, similar to second row except that the upper leaf pair was cut-off after 10, 11, 12 and 13 SD respectively.

supplies from the lower leaf pair. Secondly, the inductive processes in the lower leaf pair are completely reversed by 10 SD – 3 d. CL (p. 31, compare expt 23). So flowering takes place after 23–26 SD of the second SD-treatment of the lower leaf pair.

Judging from these recorded effects, it is to be concluded that morphogenetic changes at the apex are regulated through a competitive system, that is to say, competition between the flower-inhibiting and flower-promoting substance(s), both arising in the leaves and acting at the apex. If the inhibitor arrives first, it delays flowering and thus promotes processes of vegetative character. The dominating role of this inhibition at the apex is disappearing after a certain time so that the stimulus can express it self. Then only the flowering can normally proceed.

4.4.3. Discussion.

In the foregoing section results of expt 50 demonstrate clearly that the main site of the perception of light inhibition is the leaf and not the apex. It may be argued that considerable delay in flowering can be due to the reversal of the effects of the processes leading to induction in the leaves, as already suggested in section 4.1.7 (p. 31). However, this alternative does not account for the profound inhibitory effect of subjecting the lower leaves to intercalated CL-treatment given between two groups of SD-treatment, while the upper leaves together with the apex are always maintained in uninterrupted SD's (figure 19, 20). Since the

CL-inhibited leaves were well below those in SD, their inhibitory effect is unlikely to be due to the role of a 'sink' which dissipates the stimulus in it, HARDER (39, 1948), HARDER *et al* (41, 1949) and LANG (56, 1952). Nor would it interfere with the formation of the stimulus and its translocation in the upper leaves, for only those leaves, exposed to SD will supply both the stimulus (ZEEVAART, 114, 1958) and assimilates, CHAILAKHYAN and BUTENKO (18, 1957, p. 435, fig. 12). This inhibitory effect is more likely to be due to the production of transmissible inhibitory substance(s).

Evidence supporting a direct translocation of the inhibitor produced in the CL-inhibited leaves to the apex is provided by the results in figure 19, where presence or removal of the CL-inhibited leaf suggests the migration of the inhibitor from the leaves to the apex. This would mean that the inhibiting action of light is not localized within the leaves only and is thus transmissible. This inhibitor has the characteristics of a hormone as described by TUKEY *et al* (102, 1954). These results are best interpreted on the assumption that the inhibitor probably interferes with the functioning of the stimulus at the apex. The dominating role of this inhibition at the apex disappears after a certain time as a result of a proper light: dark ratio which enables the stimulus to express itself. Only then the flowering processes can normally proceed.

Evidence in favour of a transmissible inhibitor has also been produced by GUTTRIDGE (33, 1959) in two strawberry runner plants, joined by the stolon and acting as donor/receptor units. Long photoperiods or a light break treatment on the donor plant promote vegetative growth and inhibit flowering in the receptor in SD's. The response of the receptor plants was increased by exposing the donor plants to 3 more hrs of illumination than the receptor. These results suggest the translocation of the flower inhibiting substance, together with the movement of assimilates.

The present author (5) has collected evidence with *Perilla* which is similar in many respects to *Salvia*. Results reported in the present section regarding the transmissible nature of flower inhibitor are in conformity with those of EVANS (28, 1962) in *Rottboellia*. The importance of inhibiting substances has also been demonstrated in LDP such as *Lolium*, EVANS (27, 1960) and even in day-neutral peas, SPRENT and BARBER (96, 1957), HAUPT (42, 1961). Thus it seems that the concept of flower inhibiting substance(s) is of validity in many plants.

Perhaps the interaction achieved by a combination of inhibition and promotion, so common in biological systems (THIMANN, 98, 1956), may serve as a useful model to account for the mechanism of flower control in *Salvia occidentalis* and in other plants.

CHAPTER 5

SUMMARIZING CONCLUSIONS

INTRODUCTION

1. The role of the inhibitory action of light on the photoperiodical control of flower formation in the short-day plant *Salvia occidentalis* has been primarily studied. As an introduction, the non inhibited photoperiodism had to be studied.

DEFINITION

2. An attempt has been made to distinguish between inhibition and inhibitor(s) of flowering. Inhibition is defined as the *state* of a plant or plant parts in which the processes leading to the initiation of flower primordia are retarded – *partial inhibition* or blocked – *complete inhibition.* The inhibitor is defined as substance(s) of transmissible nature which retard(s) or inhibit(s) the formation of flower promordia.

PHOTOPERIODIC RESPONSE

3. With regard to photoperiodic response, day-length between 2–4 hrs were sub-optimal, 4–12 hrs were optimal, 12½–13 hrs were supra-optimal, while 13½ hrs was critical. The sub-optimal region has probably both non-specific and photoperiodic effects. Partially induced plants initiated buds in both decreasing and increasing daylengths, suggesting a shift in sub-optimal as well as non-inductive regions.

4. Transfer experiments suggest that induction occurs in two phases: (a) the preparatory phase, lasting the first 10 SD, without any detectable effect in itself and leading to the induced state; (b) the realization phase, with persistent effects which increase with increasing numbers of SD.

5. During the first 12 SD's the rate of cell division in the apex is relatively slow. The first sign of transition from a vegetative to a generative state was accompanied by an abrupt increase in the rate of apical cell division.

6. Factors affecting induction were studied. Preceding LD's of varying lengths and number have practically no delaying effect on the subsequent induction in SD. Very young as well as older plants were equally sensitive to SD treatment, so that a non sensitive or less sensitive period is not evident. Defoliation during SD does not affect the flowering response. All leaves *in situ* were equally sensitive to SD-treatment, only the oldest pair of leaf showed delay in flower bud formation.

INHIBITION

7. Non-inductive daylengths when intercalated between two series of SD exert an inhibitory effect on the processes leading to induction and hence are not merely passive. However, this inhibition depends quantitatively on the length

and the number of the light periods. Maximum inhibition recorded was by CL. CL does not exert any inhibitory effect when given before the SD-treatment and only becomes operative after some SD's. The maximum effect of CL-inhibition lies roughly near the end of the preparatory phase. The inhibitory effect of LD or CL when given at a fixed point increases with increasing numbers of LD or CL. However, after closer observation a maximal light inhibition is demonstrated after 3 or 4 CL cycles.

8. The maximal CL periods exert an inhibitory effect on the preceding SD cycles and probably partly on the succeeding processes. This inhibition by light was not neutralized when followed by a period of darkness. However, increasing duration of darkness during the CL period decreases inhibition.

9. Occasional short light-breaks during dark periods of normal SD-treatment have inhibitory effects.

10. When light intensity is decreased, the inhibition is decreased and becomes absent at and below 800 $\mu W/cm^2$.

11. At low temperature (10 °C) the inhibition is completely suppressed, but the induction proceeds undisturbed. At high temperature (30° and 35 °C) inhibition is not disturbed.

12. The transmissibility of the light-induced inhibitory effect was studied by the defoliation technique. The intercalation (e.g. 10 SD – 3 d. CL – SD) treatment when given either to a single leaf pair or to the apex suggests that the main site of perception of the light inhibition is the leaf and not the apex.

13. A marked inhibitory response was produced, when the lower leaf pair in a two-leaf pair scheme was given intercalated CL during the SD, while the upper leaf pair and the apex received uninterrupted SD. The same result was obtained, when the CL-inhibited leaf was removed immediately after the CL exposure. This proves the migration of the light-induced inhibitor from its site of origin towards the apex, soon after its production. In other words, CL-inhibition is not only localized within the leaves. It was concluded that an inhibitor is produced in and released from the leaves during intercalated CL-periods, when given prior to the induced state. This inhibitor is then transmitted to the apex without interfering with either the production or the translocation of floral stimulus. It prevents flower bud formation at the growing point. The dominating role of this inhibition at the apex disappears after a certain time so that the stimulus can express itself. Then only the flowering processes can normally proceed.

14. Finally it is suggested that the morphogenetic changes at the apex are regulated through a competitive system: the competition between the flower-inhibiting and flower-promoting substance(s), both arising in the leaves and both acting at the apex.

ACKNOWLEDGEMENTS

I would like to express my profound gratitude to Professor Dr. Ir. S. J. WELLENSIEK for proposing the problem and for granting me the absolute scientific freedom – coupled with the ungrudging help and interest and for perusing the manuscript. This investigation was conducted at the Laboratory of Horticulture of the State Agricultural University, Wageningen, The Netherlands. This study has been made possible by an award of fellowship from the International Agricultural Centre and a grant from the State Agricultural University Wageningen.

FOTOPERIODICITEIT, INDUCTIE EN REMMING VAN DE BLOEI, BIJ *SALVIA OCCIDENTALIS*

Inleiding.

1. Voornamelijk is de remmende werking van licht bestudeerd bij de fotoperiodieke beïnvloeding van de bloemvorming bij de korte-dag plant *Salvia occidentalis*. Daartoe moest eerst de niet geremde fotoperiodiciteit worden bestudeerd.

Definitie.

2. Getracht is onderscheid te maken tussen remming en een remmende factor(en) van de bloei. Remming is de *toestand* van een plant of van delen van een plant, waarin het proces, dat leidt tot de vorming van bloemprimordia, wordt vertraagd of geblokkeerd, resp. *gedeeltelijke* en *volledige remming* genoemd. De remmende factor is de transportabele stof, welke de vorming van bloemprimordia vertraagt of verhindert.

Fotoperiodieke reactie.

3. Een daglengte van 2-4 uren was sub-optimaal, van 4–12 uren optimaal en van 12½–13 uren supra-optimaal; 13½ uur was de kritieke daglengte. Het sub-optimale traject heeft waarschijnlijk zowel niet specifieke als fotoperiodieke effecten. Gedeeltelijk geïnduceerde planten legden knoppen aan bij zowel afnemende als toenemende daglengten, hetgeen wijst op een verschuiving zowel in sub-optimale als in niet-inductieve trajecten.

4. Transportproeven wijzen er op dat de inductie in twee fasen verloopt: (a) de voorbereidende fase, tijdens de eerste 10 korte dagen, zonder enig zichtbaar effect en leidende tot de geïnduceerde toestand; (b) de fase van de realisatie met blijvende gevolgen, welke wordt versterkt met een toenemend aantal korte dagen.

5. Gedurende de eerste 12 korte dagen verloopt de celdeling in de groeitop betrekkelijk langzaam. Het eerste teken van overgang van een vegetatieve tot een generatieve toestand viel samen met een plotselinge toename van celdelingen in de groeitop.

6. De factoren, welke de inductie beïnvloeden, werden bestudeerd. Voorafgaande lange dagen van variabele duur en aantal hebben practisch geen vertragende invloed op de daaropvolgende inductie in korte dag. Zeer jonge, zowel als oudere planten waren even gevoelig voor de korte-dag behandeling, zodat er geen niet-gevoelige of minder gevoelige periode blijkt te bestaan. Ontbladering gedurende de korte dag beïnvloedt de reactie op de bloei niet. Alle bladeren waren even gevoelig voor de korte-dag behandeling, behalve het oudste bladpaar, dat een vertragende werking op de bloemknopvorming vertoonde.

Remming.

7. Wanneer tussen twee series korte dag niet-inductieve daglengten werden ingelast, oefenden deze een remmende invloed uit op het proces, dat leidt tot inductie; deze zijn dus niet zuiver passief. De remming is kwantitatief afhankelijk van de lengte en van het aantal lichtperioden. De maximale remming werd waar-

genomen bij continu licht. Dit licht oefent echter geen enkele remmende invloed uit, wanneer het wordt gegeven vóór de korte-dag behandeling en is alleen werkzaam na enkele korte dagen. De maximale invloed van continu-licht remming ligt ongeveer aan het eind van de voorbereidende fase. De maximale invloed van lange dag of continu licht op korte dag, gegeven op een bepaald punt, neemt toe met toenemende duur van de lange dag of continu licht. Na nauwkeuriger waarnemingen werd echter een maximale lichtremming aangetoond na 3 of 4 continu-licht cycli.

8. De maximale continu-licht perioden oefenen een remmende invloed uit op het effect van de voorafgegane korte-dag cycli en waarschijnlijk gedeeltelijk op de volgende processen. Deze remming door licht werd niet geneutraliseerd wanneer er een periode van duister op volgde. Toenemende duur van duisternis als onderbreking van de continu-licht periode vermindert evenwel de remming.

9. Willekeurige korte onderbrekingen met licht gedurende de perioden van duisternis bij een normale korte-dag behandeling hebben een remmende invloed.

10. Wanneer de lichtintensiteit wordt verminderd, wordt de remming verminderd tot deze afwezig is bij en beneden 800 $\mu W/cm^2$.

11. Bij lage temperatuur (10 °C) wordt de remming volledig onderdrukt, maar de inductie gaat ongehinderd door. Bij hoge temperatuur (30 °C en 35 °C) wordt de remming niet verstoord.

12. Het transport van de remmende invloed, welke wordt geïnduceerd door licht, werd bestudeerd door middel van de ontbladeringstechniek. Onderbreking (b.v. 10 korte dagen – 3 dagen continu licht – korte dag) bij een enkel bladpaar of bij de groeitop geeft aanwijzingen, dat de hoofdzetel van de perceptie van de remming door licht is gelegen in het blad en niet in de groeitop.

13. Een opmerkelijke remmende invloed werd verkregen, wanneer aan het lagere bladpaar in een systeem met twee bladparen continu licht werd gegeven als onderbreking van de korte dag, terwijl het hogere bladpaar en de top ononderbroken korte dag ontvingen. Hetzelfde resultaat werd verkregen, wanneer het blad, dat met continu licht was geremd, onmiddellijk na de continu-licht behandeling werd verwijderd. Dit bewijst transport van de remmende factor, die dan licht geïnduceerd is, naar de groeitop, spoedig na zijn productie. Met andere woorden: de continu-licht remming is niet gelocaliseerd in de bladeren. De conclusie werd getrokken, dat een remmende factor wordt geproduceerd in en wordt vrij gelaten uit de bladeren gedurende continu-licht perioden, wanneer deze vóór de geïnduceerde toestand worden gegeven. Deze remmende factor wordt dan overgebracht naar de groeitop zonder te interfereren met de productie of de verplaatsing van de bloeistimulus. Hij verhindert echter de bloemknopvorming bij het groeipunt. De overheersende rol van deze remming bij de top verdwijnt na een zekere tijd, waarna de stimulus tot uitdrukking kan komen en het bloeiproces normaal voortgang kan vinden.

14. Tenslotte werd gesteld, dat de morfogenetische veranderingen bij de groeitop worden geregeld door een concurrerend systeem: de concurrentie tussen de bloeiremmende en bloeibevorderende stof(fen), welke beide ontstaan in de bladeren, worden getransporteerd naar de groeitop en daar hun werking uitoefenen.

REFERENCES

1. BAKHUYZEN, H. L. VAN DE SANDE: Bloei en bloeihormonen in het bijzonder bij tarwe. I. Versl. Landbouwk. Onderz. **53**, 4 B, 1947: 145–212.
2. BAKHUYZEN, H. L. VAN DE SANDE: Flowering and flowering hormones (one single scheme for both long-day and short-day plants) III A. Photoperiodism in short-day plants. Proc. Kon. Ned. Akad. Wetensch. C **56**, 1953: 164–173; III B 174–184.
3. BEST, R.: Photoperiodism in plants as studied by means of response curves. Proc. Kon. Ned. Akad. Wetensch. C **63**, 1960: 676–691.
4. BHARGAVA, S. C.: A transmissible flower-bud inhibitor in the short-day plant *Salvia occidentalis*. Proc. Kon. Ned. Akad. Wetensch. C **66**, 1963: 371–376.
5. BHARGAVA, S. C.: A transmissible flower bud inhibitor in *Perilla crispa* (in press).
6. BONNER, J.: The photoperiodic process. In 'Photoperiodism and Related Phenomena in Plants and Animals'. (Withrow, R.B. ed.). American Association for the Advancement of Science, Washington, D.C. 1959: 245–254.
7. BONNER, J.: Chemical nature of the inductive processes. In 'Photoperiodism and Related Phenomena in Plants and Animals'. (Withrow, R.B. ed.). American Association for the Advancement of Science, Washington, D.C., 1959: 411–421.
8. BONNER, J. and LIVERMAN, J.: Hormonal control of flower initiation. In 'Growth and Differentiation in Plants'. (Loomis, W.E., ed.). Iowa State College Press. Ames, Iowa, U.S.A., 1953: 283–303.
9. BORTHWICK, H. A.: Daylength and flowering. 1943–1947 Year Book U.S. Dept. of Agri. 'Science and Farming', 1947: 273–283.
10. BORTHWICK, H. A. and HENDRICKS, S. B.: Photoperiodism in plants. Science **132**, 1960: 1223-1228.
11. BORTHWICK, H. A. and PARKER, M. W.: Effectiveness of photoperiodic treatments of plants of different age. Bot. Gaz. **100**, 1938: 245–249.
12. BRUINSMA, J.: Luminar-Objektive im Dienste der Makrophotographie. Zeiss Werkzeitschrift, **12** (47), 1963: 10–11.
13. CARR, D. J.: On the nature of photoperiodic induction. I. Photoperiodic treatments applied to detached leaves. Physiol. Plant. **6**, 1953: 680–684.
14. CARR, D. J.: On the nature of photoperiodic induction. III. The summation of the effects of inductive photoperiodic cycles. Physiol. Plant. **8**, 1955: 512–526.
15. CHAILAKHYAN, M. H.: On the hormonal theory of plant development. Compt. Rend. (Dokl.) Acad. Sci. U.R.S.S. **12**, 1936: 443–447.
16. CHAILAKHYAN, M. H.: New facts in support of the hormonal theory of plant development. Compt. Rend (Dokl.) Acad. Sci. U.R.S.S. **13**, 1936: 79–83.
17. CHAILAKHYAN, K. H.: Hormonal factors in the flowering of plants. Plant Physiol. (U.S.S.R.), **5**, 1958: 547–563.
18. CHAILAKHYAN, M. H. and BUTENKO, R. G.: Translocation of assimilants from leaves to shoots during different photoperiodic regimes of plants. Plant Physiol. (U.S.S.R.), **4**, 1957: 426–438.
19. CHEADLE, V. I., GIFFORD Jr, E. M. and ESAU, K.: A staining combination for phloem and contiguous tissues. Stain Technology **28**, 1953: 49–53.
20. CHOLODNY, N. G.: The internal factors of flowering. Herb. Rev. **7**, 1939: 223–247.
21. CHOUARD, P. (Ed.): Colloq. Intern. Photothermopériodisme. Parma, Italy. Sér. B, **34**, 1957: 1–190.
22. CUMMING, B. G.: Extreme sensitivity of germination and photoperiodic reaction in the genus *Chenopodium* (Tourn.) L. Nature, **184**, 1959: 1044–1045.
23. CUMMING, B. G.: Evidence of a requirement for phytochrome – Pfr in the floral initiation of *Chenopodium rubrum*. Can. J. Bot. **41**, 1963: 901–926.
24. DENFFER, D. VON: Blühhormon oder Blühhemmung? Neue Gesichtspunkte zur Physiologie der Blütenbildung. Naturw. **37**, 1950: 296–301; 317–321.
25. DOORENBOS, J. and WELLENSIEK, S. J.: Photoperiodic control of floral initiation. Ann. Rev. Plant Physiol., **10**, 1959: 147–184.

26. ESASHI, Y.: Studies on the formation and sprouting of aerial tubers in *Begonia evansiana* Andr. V. Antagonistic action of long-days to short-day response. Plant and cell Physiol. **2**, 1961: 117–127.
27. EVANS, L. T.: Inflorescence initiation in *Lolium temulentum* L. II. Evidence for inhibitory and promotive photoperiodic processes involving transmissible products. Austr. J. Biol. Sci. **13**, 1960: 429–440.
28. EVANS, L. T.: Daylength control of inflorescence initiation in the grass *Rottboellia exaltata* Lf. Austr. J. Biol. Sci. **15**, 1962: 291–303.
29. EVANS, L. T. (Ed.): Environmental control of plant growth. Academic Press. New York, 1963: 1–449.
30. GARNER, W. W. and ALLARD, H. A.: Effect of relative length of day and night and other factors of the environment on growth and reproduction in plants. J. Agr. Res. **18**, 1920: 553–606.
31. GIESE, A. C. (Ed.): Photophysiology I. General principles, action of light on plants. Academic Press, New York, 1964: 305–331.
32. GREGORY, F. G.: The control of flowering in plants. Symp. Soc. exp. Biol. Cambridge, **2** (Growth), 1948: 75–103.
33. GUTTRIDGE, C. G.: Evidence for a flower inhibitor and vegetative growth promotor in strawberry. Ann. Bot. N.S. **23**, 1959: 351–360.
34. GUTTRIDGE, C. G.: Further evidence for a growth-promoting and flower-inhibiting hormone in strawberry. Ann. Bot. N.S. **23**, 1959: 612–621.
35. HAMNER, K. C.: Hormones in relation to vernalization and photoperiodism. In 'Vernalization and Photoperiodism' (MURNEEK A. E. and WHYTE, R. O. ed.). Chron. Bot. Cy,. Waltham, Mass., U.S.A., 1948: 63-70.
36. HAMNER, K. C.: The mechanism of photoperiodism in plants. Photobiology. Proc. 19th Ann. Biol. Colloq. Oregon State College, U.S.A., 1958: 7–16.
37. HAMNER, K. C. and BONNER, J.: Photoperiodism in relation to hormones as factors in floral initiation and development. Bot. Gaz. **100**, 1938: 388–431.
38. HAMNER, K. C. and NAYLOR, A. W.: Photoperiodic responses of dill, a very sensitive long-day plant. Bot. Gaz. **100**, 1939: 853–861.
39. HARDER, R.: Vegetative and reproductive development of *Kalanchoë blossfeldiana* as influenced by photoperiodism. Symp. Soc. exp. Biol. Cambridge **2** (Growth), 1948: 117–138.
40. HARDER, R. and BÜNSOW, R.: Über die Wirkung der Tageslänge vor der Kurztaginduktion auf die Blütenbildung von *Kalanchoë blossfeldiana*. Planta **43**, 1954: 315–324.
41. HARDER, R., WESTPHAL, M. und BEHRENS, G.: Hemmung der Infloreszenzbildung durch Langtag bei der Kurtagzpflanze *Kalanchoë blossfeldiana*. Planta **36**, 1949: 424–438.
42. HAUPT, W.: Flower promoting versus flowering inhibiting substances. Recent Advances in Botany, II, University of Toronto press, Toronto 1961: 1285–1287.
43. HENDRICKS, S. B.: Metabolic control of timing. Science **141**, 1963: 21–27.
44. HIGAZY, M. K. M. T.: Shortening the juvenile phase for flowering. Meded. Landbouwhogeschool, Wageningen **62** (8), 1962: 1–53.
45. HILLMAN, W. S.: The physiology of flowering. Holt, Rinehart and Winston, New York, 1962: 164 pp.
46. HOLDSWORTH, M.: The concept of minimum leaf number. J. Expt. Bot. **7**, 1956: 395–409.
47. IMAMURA, S.: Photoperiodic initiation of flower primordia in Japanese morning glory, *Pharbitis nil* CHOIS. Proc. Japan. Acad. **29**, 1953: 368–373.
48. IMAMURA, S.: The nature of inhibition of flowering by the leaves illuminated continuously during the inductive dark treatment of other leaves in short-day plants. Recent Advances in Botany, II, University of Toronto press, Toronto 1961: 1287–1288.
49. JACOBS, W. P. and RAGHAVAN, V.: Studies on the floral histogenesis and physiology of *Perilla*. I. Quantitative analysis of flowering in *P. frutescens* (L.). Britt. Phytomorph. **12**, 1962: 144–167.
50. JENNIGS, P. R. and ZUCK, R. K.: The cotyledon in relation to photoperiodism in cocklebur. Bot. Gaz. **116**, 1954: 199–200.

51. JOHANSEN, D. A.: Plant microtechnique. McGraw-Hill Book Company. Inc., New York and London, 1940: p. 41.
52. KHUDAIRI, A. K. and HAMNER, K. C.: The relative sensitivity of *Xanthium* leaves of different ages to photoperiodic induction. Plant Physiol. **29**, 1954: 437-440.
53. KNOTT, J. E.: Effect of a localized photoperiod on spinach. Proc. Amer. Soc. Hort. Sci. **31**, 1934: 152–154.
54. KRAMER, F.: *Salvia occidentalis* Swartz, een goede bodembedekker. Tectona **16**, 1923: 47–56.
55. KUYPER, J. and WIERSUM, L. K.: Occurrence and transport of a substance causing flowering in the soya bean (*Glycine max.* L.). Proc. Kon. Ned. Akad. Wetensch. **39**, 1936: 1114–1122.
56. LANG, A.: Physiology of flowering. Ann. Rev. Plant Physiol. **3**, 1952: 265–306.
57. LANG, A.: Physiology of reproductive development in higher plants. Proc. 22nd Ann. Biol. Colloquium, 1961. Oregon State University press, Oregon 1963: 53–78.
58. LINCOLN, R. G., RAVEN, K. A. and HAMNER, K. C.: Certain factors influencing expression of the flowering stimulus in *Xanthium*. Part I. Translocation and inhibition of flowering stimulus. Bot. Gaz. **117**, 1956: 193–206.
59. LINCOLN, R. G., MAYFIELD, D. L. and CUNNINGHAM, A.: Preparation of a floral initiating extract from *Xanthium*. Science **133**, 1961: 756.
60. LINCOLN, R. G., MAYFIELD, D. L., HUTCHINGS, R. D., CUNNINGHAM, A., HAMNER, K. C. and CARPENTER, B. H.: Floral initiation of *Xanthium* in response to application of an extract from a day neutral plant. Nature **195**, 1962: 918.
61. LINT, P. J. A. L. DE: An attempt to analysis of the effect of light on stem elongation and flowering in *Hyoscyamus niger* L. Meded. Landbouwhogeschool, Wageningen **60**(14), 1960: 1–59.
62. LIVERMAN, J. L.: Physiology of flowering. Ann. Rev. Plant Physiol. **6**, 1955: 177–210.
63. LJUBIMENKO, V. N. et BUSLOVA, E. D.: Contribution à la théorie du photopériodisme. II. Compt. Rend. (Dokl.) Acad. Sci. U.R.S.S. **14**, 1937: 149–152.
64. LOCKHART, J. A.: Mechanism of photoperiodic process in higher plants. In 'Encyclopedia of Plant Physiology, **16** (Ruhland, W. ed.), Springer, Berlin, 1961: 390–438.
65. LOCKHART, J. A. and HAMNER, K. C.: Partial reactions in the formation of floral stimulus in *Xanthium*. Plant Physiol. **29**, 1954: 509–513.
66. LONA, F.: La fioritura della brevidiurna *Chenopodium amaranticolor* Coste et Reyn coltivata in soluzione nutritiza con saccarosio, in assenza di stimolo fotoperiodico eufloriogeno. Nuovo Giorn. botan. Ital. **55**, 1948: 559–562.
67. LONA, F.: La fioritura delle brevidiurne a notte continua. Nuovo Giorn. botan. Ital. **56**, 1949: 479–515.
68. LONA, F.: L'induzione fotoperiodica di foglie staccate. Boll. Soc. Ital. Sper. **25**, 1949: 761–763.
69. LONA, F.: Results of twelve years of work on the photoperiodic responses of *Perilla ocymoides*. Proc. Kon. Ned. Akad. Wetensch. **62**, 1959: 204–210.
70. LONG, E. M.: Photoperiodic induction as influenced by environmental factors. Bot. Gaz. **101**, 1939: 168–188.
71. MAYFIELD, D. L., LINCOLN, R. G., HUTCHINS, R. O. and CUNNINGHAM, A.: Concentration of a flowering hormone from certain plant extracts. Abstr. 141st meeting. Am. Chem. Soc. Washington, D. C., 1962: p. 31c.
72. MEIJER, G.: The influence of light quality on the flowering response of *Salvia occidentalis*. Acta Bot. Neerl. **6**, 1957: 395–406.
73. MEIJER, G.: The spectral dependence of flowering and elongation. Acta Bot. Neerl. **8**, 1959: 189–246.
74. MEIJER, G. and VEEN, R. VAN DER: Dual effect of night break light. Acta Bot. Neerl. **9**, 1960: 220–223.
75. MELCHERS, G.: The physiology of flower initiation. Dokumentationsstelle der Max Planck Ges. Göttingen, 1952: 1–33.

76. MELCHERS, G.: Einführung. In 'Encyclopedia of Plant Physiology' (Ruhland, W. Ed.), Springer, Berlin. **16**, 1961: p. XIX–XXVI.
77. MOSHKOV, B. S.: The role of leaves in the photoperiodic reaction of plants. Bull. Appl. Bot. Gen. and Pl. Breed. Ser. A **17**, 1936: 25–30. Ref.: Herb. Abstr. **6**, 1936: 204.
78. MOSHKOV, B. S.: Flowering of a short-day plant under continuous day as a result of grafting. Bull. Appl. Bot. Gen. and Pl. Breed., Ser. A **21**, 1937: 145–156. Ref.: Herb. Abstr. **8**, 1938: 135.
79. MURNEEK, A. E. and WHYTE, R. O. (Ed.): Vernalization and photoperiodism. Chron. Bot. Cy., Waltham Mass. U.S.A., 1948: XV + 196 pp.
80. NAUNDORF, G.: Blütenbildende Stoffe beim Kakao. Naturw. **41**, 1954: 340.
81. NAYLOR, A. W.: The photoperiodical control of plant behaviour. In 'Encyclopedia of Plant Physiology', (Ruhland, W. Ed.), Springer, Berlin, **16**, 1961: 331–389.
82. POST, K.: Accumulation of photoperiodic stimuli in Chrysanthemum. Proc. Am. Soc. Hort. Sci. **55**, 1950: 475–476.
83. PSAREV, G. M.: Localization of the photoperiodic stimulus in soybean. Sovet. Bot. **3**, 1936: 89–91. Ref.: Herb. Abstr. **7**, 1937: 326.
84. RAGHAVAN, V. and JACOBS, W. P.: Studies on floral histogenesis and physiology of *Perilla*. II. Floral induction in cultured apical buds of *P. frutescens*. Amer. J. Bot. **48**, 1961: 751–760.
85. RESENDE, F.: Auxin and antiauxin, the hormones responsible for the change of the vegetative into floral phenotypes. Bol. Soc. Portug. Ciênc. Nat. Ser. 2a, **2**, 1949: 174–188.
86. RESENDE, F.: Gradãcao floral e comportamento das PDCL na transissao do estado floral entre 'donor' e 'acceptor' (*Bryophyllum* e *Kalanchoë*). Portug. Acta Biol., Ser. A, **4**, 1955: 272–296.
87. RUHLAND, W. (Ed.): Encyclopedia of Plant Physiology. Springer, Berlin, **16**, 1961: 1-23, 299–330.
88. SACHS, J.: Wirkung des Lichts auf die Blütenbildung unter Vermittlung der Laubblätter. Bot. Zeit. **23**, 1865: 117–121, 125–131 and 133–139 (Ges. Abh. über Pflanzen-Physiologie. Leipzig I, 1892: 229–260).
89. SALISBURY, F. B.: Photoperiodism and flowering process. Ann. Rev. Plant Physiol. **12**, 1961: 293–326.
90. SALISBURY, F. B.: The flowering process. International Series of monographs on pure and applied biology. Plant Physiology division, 4. Pergamon press, Oxford, 1963: XII + 234 pp.
91. SCHWABE, W. W.: Evidence for a flowering inhibitor produced in long days in *Kalanchoë blossfeldiana*. Ann. Bot. NS. **20**, 1956: 1–14.
92. SCHWABE, W. W.: Recent work on the inhibitory effect of long days on the flowering of some short-day plants. Colloque international sur le photopériodisme. U.I.S.B. Publ. **34**, Sér. B, 1957: 95–109.
93. SCHWABE, W. W.: Studies of long-day inhibition in short-day plants. J. Exp. Bot. **10**, 1959: 317–329.
94. SCHWABE, W. W.: Test of a new photoperiodic hypothesis. Recent advances in Botany II, University of Toronto press, Toronto, 1961: 1290–1293.
95. SIROHI, G. S. and HAMNER, K. C.: Floral inhibition in relation to photoperiodism in Biloxi soybean. Plant Physiol. **37**, 1962: 785–790.
96. SPRENT, J. I. and BARBER, H. N.: Leaching of a flower inhibitor from late varieties of peas. Nature, **180**, 1957: 200–201.
97. STEENIS, C. G. F. J. VAN: Hoe heeten de opgerichte en liggende vormen van '*Salvia occidentalis* SW'? De Bergcultures **48**, 1938: 1636–1640.
98. THIMANN, K. V.: Promotion and inhibition: Twin themes of physiology. Amer. Naturalist **90**, 1956: 145–162.
99. THOMAS, R. G.: Promotion and inhibition of inflorescence growth by long days in short-day plants. J. Exp. Bot. **13**, 1962: 268-283.
100. THOMAS, R. G.: Floral induction and stimulation of cell division in *Xanthium*. Science **140**, 1963: 54–56.

101. THOMPSON, P. A. and GUTTRIDGE, C. G.: The role of leaves as inhibitors of flower induction in strawberry. Ann. Bot. NS. **24**, 1960: 482–490.
102. TUKEY, H. B., WENT, F. W., MUIR, R. M. and OVERBEEK, J. VAN: Nomenclature of chemical plant regulators. Plant Physiol. **29**, 1954: 307–308.
103. VEEN, R. VAN DER and MEIJER, G.: Light and plant growth. Philips Techn. Library, Eindhoven 1959: 1–161.
104. VÖCHTING, H.: Über Transplantation am Pflanzenkörper. Tübingen, 1892. DENFFER, D. VON, Naturw. **37**, 1950 (Ref. No. **48**, p. 301).
105. WAREING, P. F.: Experiments on the 'light-break' effect in short-day plants. Physiol. Plant. **7**, 1954: 157–172.
106. WELLENSIEK, S. J.: Photoperiodical reactions of *Perilla crispa*. Proc. Kon. Ned. Akad. Wetensch. C **61**, 1958: 552–560.
107. WELLENSIEK, S. J.: The inhibitory action of light on the floral induction of *Perilla crispa*. Proc. Kon. Ned. Akad. Wetensch. C **62**, 1959: 195–203.
108. WELLENSIEK, S. J.: Les réactions photopériodiques des plantes de journée courte. C.R. Acad. Agr. France **46**, 1960: 607–611.
109. WELLENSIEK, S. J.: The control of flowering. Neth. J. Agri. Sci. **10**, 1962: 390–398.
110. WITHROW, A. P., WITHROW, R. B. and BIEBEL, J. P.: Inhibiting influence of leaves on the photoperiodic response of Nobel spinach. Plant Physiol. **18**, 1943: 294–298.
111. WITHROW, R. B. (Ed.): Photoperiodism and Related Phenomena in Plant and Animals. American Association for the Advancement of Science, Washington, D.C. 1959: 1–903.
112. ZEEUW, D. DE: Flower initiation and light intensity in *Perilla*. Proc. Kon. Ned. Akad. Wetensch. C **56**, 1953: 418–422.
113. ZEEUW, D. DE: De invloed van het blad op de bloei. Meded. Landbouwhogeschool, Wageningen **54**(1), 1954: 1–44.
114. ZEEVAART, J. A. D.: Flower formation as studied by grafting. Meded. Landbouwhogeschool, Wageningen **58**(3), 1958: 1–88.
115. ZEEVAART, J. A. D.: Physiology of flowering. Science, **137**, 1962: 723-731.
116. ZEEVAART, J. A. D.: Climatic control of reproductive development. In 'Environmental control of plant growth' (L.T. Evans, Ed.). Academic press. New York. 1963: 289–310.

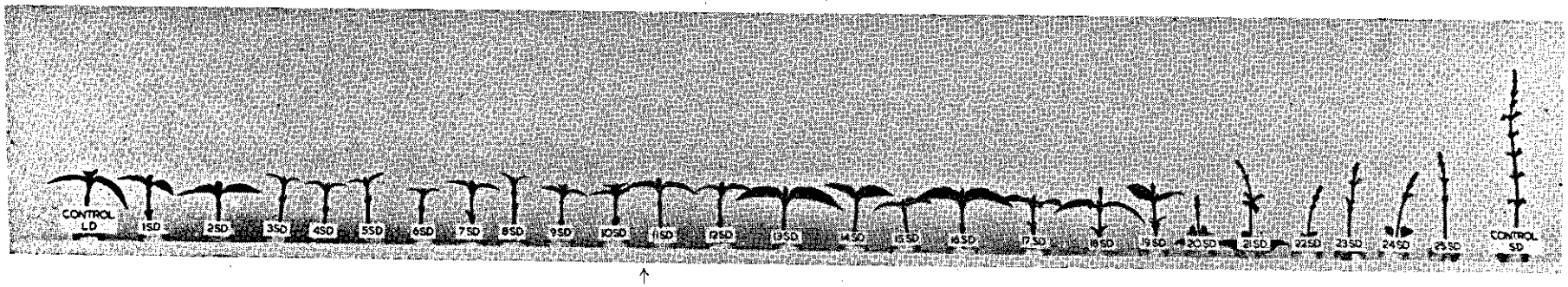


PHOTO 1. *Experiment 6.* Effect of transference from short-day to long-day on flower bud differentiation. Photo shows plants which received from 1 to 25 SD and were then transferred to LD in order to observe macroscopical appearance of flower buds. The arrow indicates the transition from vegetative to generative stage. Photo 45 days after the beginning of SD treatment.

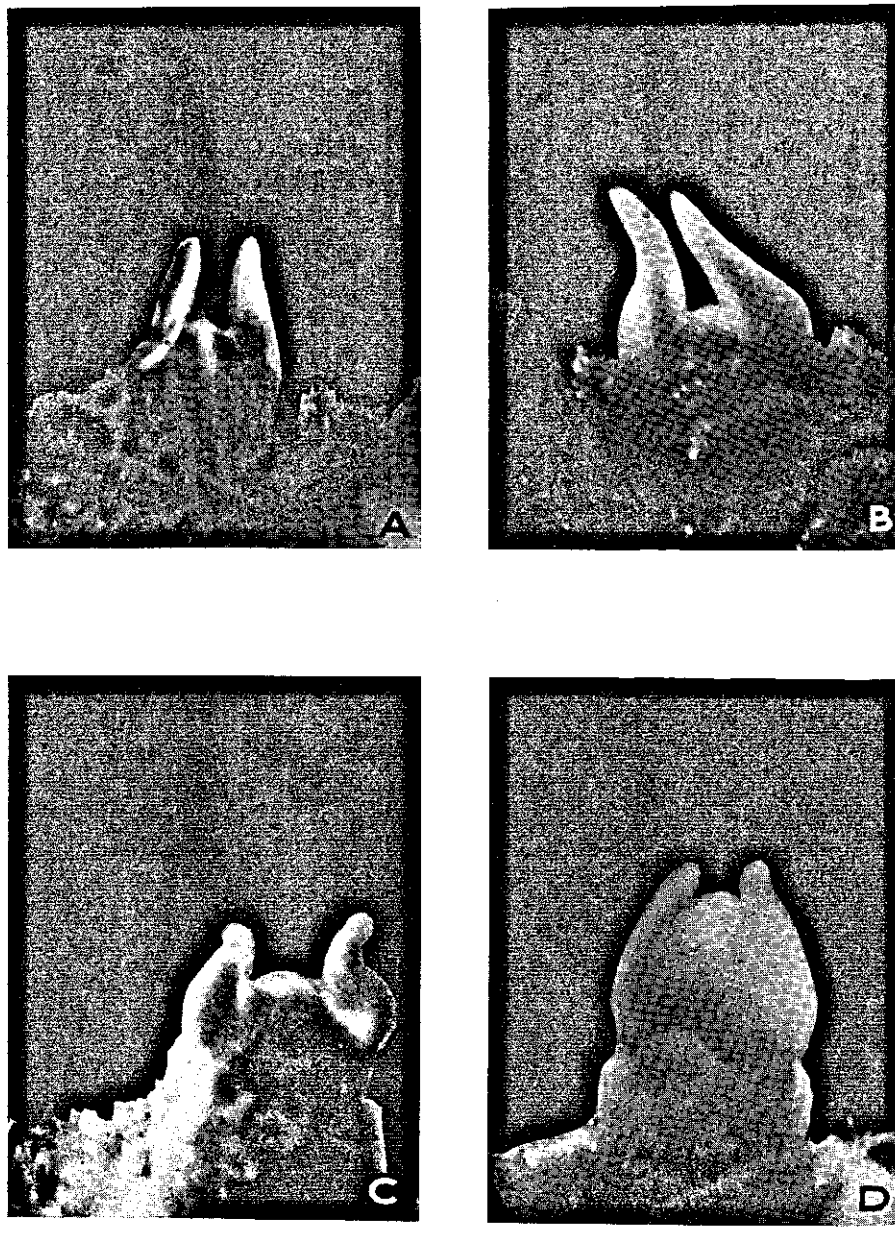


PHOTO 2. *Experiment 7.* Photomicrographs × 70 of an apical bud photo-induced with 0 (A), 10 (B), 15 (C) or 20 SD (D). The photo's show the transition of vegetative apex (A, B) to generative apex (C, D).

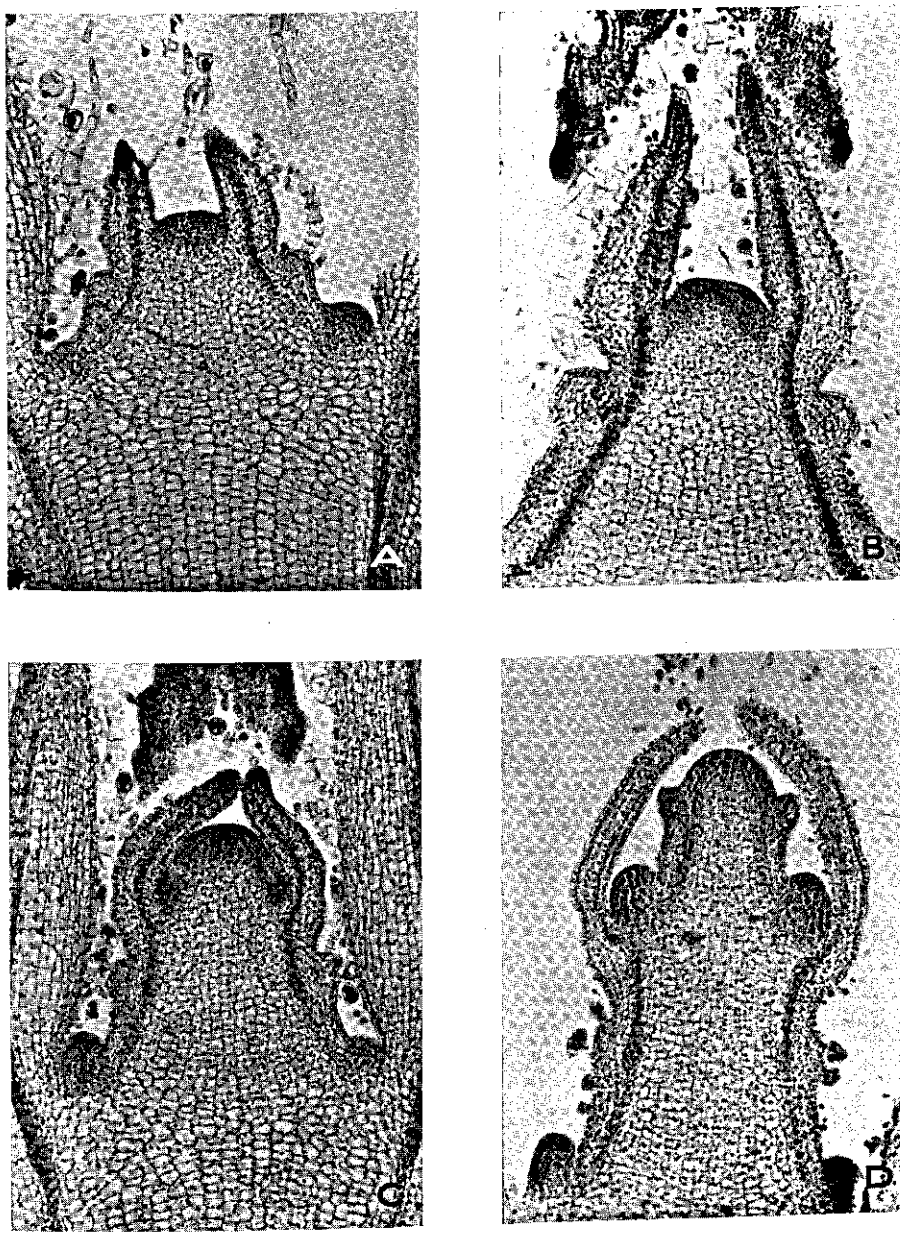


PHOTO 3. *Experiment 8.* Transformation of shoot apex during photoperiodic induction. Photomicrographs of longitudinal sections. × 100. Photo A: shoot apex of the vegetative plant; B: transitional state of shoot apex after 12 SD cycles; C and D: the beginning of the generative state after 14 and 18 SD cycles respectively.

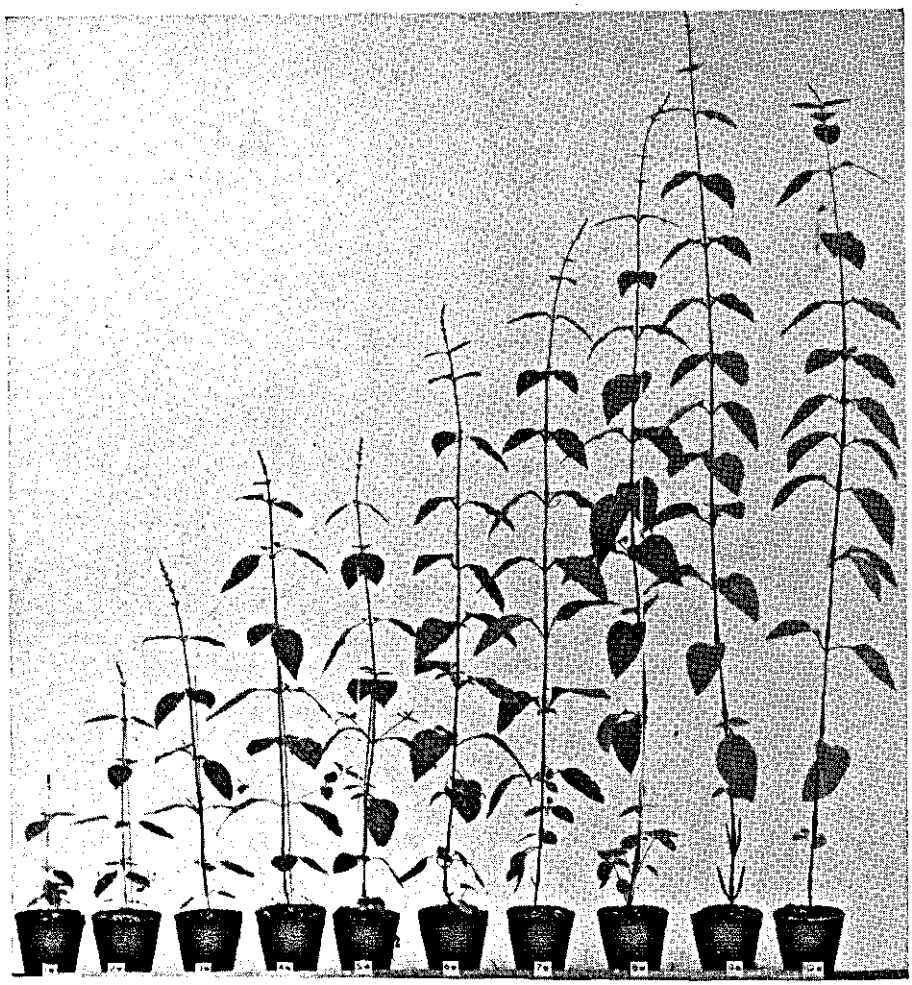


PHOTO 4. *Experiment 12.* Age and flower bud formation: seedlings of *Salvia occidentalis* of various ages from 1 week to 10 weeks (w) old were grown in LD and transferred to SD. All groups flowered simultaneously irrespective of age. Photo was taken 53 days after the SD-treatment started.