THE PHYSIOLOGY AND BIOCHEMISTRY OF FLOWERING

Thesis by

James Leslie Liverman

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DEDICATION

To my wife, Constance, who
has shared all of my sorrows
and my joys during the years
I have been in college, and
to our three children, Carol,
Jane and Bob, who have in
their own way made our lives
more worth living, I cheerfully
dedicate this thesis.

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Finally, the author is indebted to Miss Lucy Mason Clark for her generous financial support of the Lucy Mason Clark Fellowship in Plant Physiology which the author has been privileged to receive during the last two years of graduate study.

ABSTRACT

With the two SDP, Xanthium canadense and Chenopodium Amaranticolor, it has been possible to separate more clearly the partial reactions of the photoperiodic response of SDP and in some degree to associate these processes with particular biochemical or physiological processes of the plant. Thus it has been shown that sugars and Krebs cycle acids are able to replace the high intensity light process. Further investigations have shown that substances formed during an inductive dark process are still susceptible to auxin inactivation even after exposure to several hours of high intensity light immediately following the dark period. It has been shown that the effect of a flash of light in inhibiting the dark process can be reversed by anti-auxins. Further experiments have confirmed earlier work which show that LD leaves on SD plants inhibit flowering. Hypotheses have been advanced 1) to explain the nature of this inhibition and 2) to explain the kinetics of the dark process.

Experiments with a newly discovered LDP, Silene Armeria, have shown that its critical day length is reduced by increasing temperature. Studies on the auxin relations in the flowering of Silene and Hyoscyamus niger indicate that auxin causes flowering of these LDP under conditions in which the controls remain vegetative.

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GLOSSARY OF TERMS AND ABBREVIATIONS

Auxins

IAA	indole-3-acetic acid
NAA	naphthalene-1-acetic acid
2,4-D	2,4-dichlorophenoxyacetic acid
2,4,5-T	2,4,5-trichlorophenoxyacetic acid

Auxin antagonists

TIBA	2,3,5-triiodobenzoic acid
DCA	2,4-dichloroanisole
1-NIBA	1-naphthoxyisobutyric acid
2-NIBA	2-naphthoxyisobutyric acid
PIBA	phenoxyisobutyric acid
2,4-DPIBA	2,4-dichlorophenoxyisobutyric acid
FC, FCM	foot candle, foot-candle-minute (s)
SD, SDP	short-day and short-day plant (s) respectively. These are plants which flower only when given more than a certain daily minimum duration of darkness.
LD, LDP	long-day and long-day plant (s) respectively. These are plants which flower only when given more than a certain daily minimum duration of light.

DN, DNP	day-neutral and day-neutral plant (s) respectively. These are plants which flower over a wide range of day lengths, i.e., those plants which do not require a particular day length before they will flower.
Critical Day Length	(in 24-hour cycles) That length of day in SDP above which flowering is inhibited. In LDP it is that day length below which flowering does not occur.
Bolting	A term used to refer to the beginning of elongation of the stem axis in those plants which grow as a rosette in the vegetative state.
Veg., flr.	Vegetative and flowering, respectively.
Int.	Intermediate. A stage of flower bud development in Xanthium which is intermediate between a vegetative and an easily distinguishable flower bud.
mg./l.	Milligrams per liter.
ppm.	Parts per million.

INTRODUCTION

No single event in the life history of a plant is more spectacular or more important than that which occurs when a plant suddenly redirects its synthetic pathways from the formation of leaves, roots and stems to the formation of flowers. And yet, what do we know about this all-important process of flowering in so far as the biochemistry and physiology of the process is concerned? Why is it that some plants flower only in fall, whereas others flower only in spring or summer? What is this factor in the environment which governs the time of flowering? It was only slightly more than three decades ago that Garner and Allard, two investigators of the U.S. Department of Agriculture, looked at non-flowering tobacco plants and asked themselves this same question. Unlike so many people before them, however, they found a partial answer to this perplexing problem in their discovery of photoperiodism, the response of plants to relative length of light and dark periods. In the course of this work they started a scientific landslide which has never stopped. A whole field of study has been built up as a result of their discovery that day length controls the flowering not only of Maryland Mammoth tobacco, but also the flowering

of a vast number of plants. These studies of the reproduction and growth of plants in response to relative length of day and night have yielded much valuable information both to scientists interested in basic facts, and to persons interested only in practical application of the findings.

Much of this information which has been amassed concerns what we might refer to as the kinetics of photoperiodism, the interplay of light and dark in this process. Thus it has been shown that in the short-day plants, those plants which flower only on regimes in which the night is longer than some critical value, it is exposure of the leaf to the long night which is the decisive factor in regulating initiation of floral primordia. Long-day plants on the other hand are those plants which require a certain minimum duration of the daily light period before they will flower. Indeterminate or day neutral plants are plants in which flowering is not controlled by the length of day.

With most if not all photoperiodically sensitive plants (i.e. long-day or short-day plants) it has been found that vegetative plants exposed for a short time to a photoperiodic regime favorable for flower initiation will subsequently flower even on a regime previously unfavorable to flowering. The changes which have been brought about during this brief exposure to favorable photoperiod we refer to as photoperiodic induction.

The many and varied approaches to the study of flowering, which have been used in the past 30 years to achieve a further elucidation of the photoperiodic response, have piled up a vast backlog of factual data concerning the process. The concept has arisen that flowering is controlled by a specific hormone, florigen, and although the many attempts to isolate it have failed, there seems little reason to doubt its existence. In spite of the vast storehouse of data which has been available, we are only now beginning to be able to arrange these varied facts into a coherent picture of the mechanisms involved in floral initiation of both long- and short-day plants, and to subdivide the photoperiodic response into a series of sequential partial or component processes.

The studies reported in this thesis have been concerned with 1) an attempt to achieve a clear separation of these partial processes and 2) an attempt to associate each of these partial processes with particular physiological or biochemical processes of the plant. These studies, which have extended to the partial reactions of both short and long-day plants, are divided into three major portions.

Part I is concerned with a presentation of preliminary experiments which have served to standardize the plant material used in the physiological and biochemical investigations reported in the remainder of the thesis.

Part II is devoted to a report and discussion of the experimental data gathered in investigations on the partial processes of the photoperiodic mechanism in short-day plants. Finally an attempt has been made to synthesize the presently available data into a coherent scheme which will explain the photoperiodic mechanism in short-day plants.

Part III is concerned with a report of investigations on the temperature response of a newly discovered long-day plant, Silene Armeria, and with a presentation of data which show clearly for the first time that auxin may have a very important role in the flowering of long-day plants.

PART I

MATERIALS AND METHODS

Introduction.

Four plant species have been used in the investigations reported in this thesis. With most of these species, preliminary observations were made to determine the response of the plant under the conditions used in the actual experiment. These experiments, which serve to calibrate and standardize the species used, are reported in the present section.

CHAPTER I. EXPERIMENTS WITH SHORT-DAY PLANTS.

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I. Introduction.

Two short-day plants, Xanthium canadense Mill., the cocklebur, and Chenopodium Amaranticolor Coste et Reyn, a pigweed, have been used in investigations of the photoperiodic response of SDP. Xanthium has been utilized in this country by many different workers and its response has been used as a model for the response of SDP in general. The other SDP, Chenopodium, has been heretofore utilized extensively only by Lona in Italy. The reports herein are the first published accounts of its use in experimental research in the United States. The seed of Chenopodium used in the present investigation were obtained from Dr. Anton Lang, who received them directly from Dr. Lona.

II. Experiments with Xanthium canadense Mill.

The photoperiodic response of Xanthium has been looked into in considerable detail during the past several years and all of the results

have indicated that the plant flowers in response to one or two short-day-long-night cycles. It has been characteristic of Xanthium for the flowering response to be highly uniform, but during early investigations with seed collected locally it became apparent that the local population was far from homogeneous. Thus in one experiment carried out in the controlled conditions of the Phytotron (Earhart Plant Research Laboratory), flowering was observed in only six of fifteen plants after two weeks under what usually would be a photoinductive regime. A further observation made in the field subsequent to this experiment revealed that in the same immediate area part of the cocklebur plants had very large green fruits, whereas dissection of other plants showed them to be completely vegetative. These two observations prompted a quantitative comparison of the photoperiodic responses, especially the critical day lengths, of plants grown from the local seed source with that of plants grown from the Chicago seed source used in the original experiments of Hamner and Bonner (1938).

Special light-tight cabinets and a special time clock were constructed for these experiments in order to achieve the many different day lengths needed for determination of the critical day length. The cabinets were constructed of 3/16 inch masonite, painted black on the

inside to prevent reflection of the light. They were 2 x 2 feet at the base and 8 feet high. Two light baffles, one on the top and one on the side, were installed to permit a free flow of air through the cabinets. The air flow was such that the temperature inside the cabinets seldom exceeded by more than 2-3° C. the 23° C. temperature of the surrounding room. Suspended from the top of each of these cabinets was a single 300 watt bulb which furnished light for extending the day length beyond eight hours.

For the comparative experiments, seed from the local source and from the Chicago source were sown at Orlando Greenhouse. When the seed had germinated, the seedlings were transplanted to a rich sandy loam contained in four-inch, clay pots. The plants were maintained under a 20-hour day until they were approximately six weeks old. The experimental treatments were carried out in Dolk Laboratory where the plants were given a basic eight-hour period of high intensity light plus the supplementary light required to obtain the desired day length. The plants were given four SD and then returned to the greenhouse for two weeks of LD treatment prior to dissection. Table 1 presents the results of these experiments.

The data show that the local strain of cocklebur does include individuals of varying photoperiodic response. In all further experiments only the Chicago cockleburs were used.

TABLE I

Comparison between the response of the local strain and the Chicago strain of Xanthium to the relative length of day and night. X-70, 72, 73, 76, 79.

Strain	Night Length	No. veg.	No. int.*	No. Flr.	%Flr.
Chicago	8 hr.	6	0	0	0
Local	8.5 hr.	4	1	4	45
Chicago	8.5 hr.	0	3	6	67
Local	9.0 hr.	7	0	2	22
Chicago	9.0 hr.	0	0	9	100
Local	10.0 hr.	2	0	7	78
Chicago	10.0 hr.	0	0	9	100
Local	10.25 hr.	2	2	5	56

* Stages after Bonner and Thurlow (1949).

III. Experiments with Chenopodium Amaranticolor.

1) Response to relative length of day and night. Although Lona (1948, 1949 a,b, 1950) has described the response of this plant to photoperiod it seemed desirable to repeat his experiments on a small scale in this laboratory before any major physiological investigations were begun.

One of the first experiments was conducted to determine if the plant would remain vegetative for extended periods of time under continuous LD treatment. Seed were planted (5/23/51) in the Phytotron and following germination were transplanted into plastic dishes (4 inches square and 2.5 inches deep) containing a vermiculite-gravel mixture. On June 26, fifteen plants were moved to Dolk Greenhouse where they were again transplanted to ten-inch clay pots. The plants were maintained on LD conditions - - 20 hours of light daily - - until they had reached an average height of 232.6 centimeters. The plants at this time had been growing under LD conditions for seven months. Dissection of the tips revealed that the plants were all vegetative and that they had developed approximately ninety-one leaves. These results agree with those of Lona (1950) who found that this species would grow for eight months under LD without flowering.

Further experiments were set up to determine the critical day length under the present experimental conditions. Seed were planted in the Phytotron and following germination the plants were transferred to Dolk Greenhouse where they were transplanted to four-inch pots and grown under LD conditions until the beginning of the experimental treatment. Table 2 presents the essential details and results of this experiment.

The data show that the critical night length lies in the region of ten hours. The apparently slight inductive effect of a sixteen-hour photoperiod would never be a practical problem at the Institute since all SD plants are grown in the greenhouses under an eighteen to twenty hour photoperiod. These results are not in complete agreement with those of Lona (1949a) who found that the critical night length was ten hours, but who also observed that the plants would not flower even after eight months under a sixteen hour LD (1950).

2) Age in relation to ability of plant to perceive the flowering stimulus. Experiments were set up to determine the relationships between plant age and ability to respond to photoperiodic induction so that in future experiments plants with an optimum sensitivity could be used. For these experiments seed were planted at weekly intervals in the Phytotron and grown under LD conditions until the oldest plants were twelve

TABLE 2

Response of Chenopodium to the relative length of day and night.

Eight plants per treatment except for 16 hours. LL No. 1.

Photoperiod in hours	No. of plants flowering	Days to bud	Leaf Number	Height * (Final)
8	8	14.0	18.0	6.9
9	8	14.9	18.6	9.9
10	8	14.5	18.3	10.3
11	8	15.9	18.9	12.3
12	8	16.3	17.5	11.4
13	8	19.0	20.0	15.1
14	8	42.1	28.5	27.6
16	2	82.0[a]	55.5	64.5
20	0	-	60.3	65.0
24	0	-	54.0	46.5

* Height given in centimeters.
a Data by dissection. Leaf number for vegetative plants was 61.4.

weeks old. At this time the plants were treated with varying numbers of SD (16 hour nights) and then returned to LD for approximately four weeks from the beginning of the SD treatment. The plants which had not then produced visible buds were dissected to determine the stage of floral development. Only the data on initial height and on flowering are recorded in Table 3. The results will be reported in detail elsewhere (Lang and Liverman, 1951).

These results together with those of the previous experiment indicates that an optimum photoperiodic response can be obtained with six to eight weeks old plants and a twelve hour night.

3) Inhibition of flowering by low intensity light. It was desirable to find out if the flowering of this chenopod is inhibited by low intensity light given during the dark period as is the case with Xanthium.

a. Flashes of light. The first series of experiments were directed toward determining the intensity of a flash of light which would just inhibit flowering when given in the middle of the dark period. For these experiments seed were planted in the Phytotron (6/19/51), transplanted into 4 x 4 x 2.5 inch plastic dishes and then moved to Orlando Greenhouse (7/16/51). The plants were subsequently moved to Dolk Greenhouse where they were used in light flash experiments. A daily basic eight hour period of high intensity light was given in the greenhouse from 0800 to 1600. At 1600 the plants were moved to the special cabinets

TABLE 3

The relation between age of Chenopodium Amaranticolor and its ability to respond to photoinductive treatments. LL No. 2, 1951. Results shown as % flowering.

Age of Plants in Wks.	Initial Height in cm.	Number of Plants	NUMBER OF PHOTOINDUCTIVE CYCLES							
			1	2	3	4	5	7	10	Cont.[a]
0*	0	10-12	-	0	0	0	0	0	100	100
1	1.2	10	-	0	0	0	0	70	100	100
2	3.3	8-10	0	0	0	88	88	88	100	-
4	7.8	5-6	0	100	100	100	100	100	-	-
6	28.0	5-6	83	100	100	100	100	-	-	-
8	65.5	4-5	20	100	100	100	100	-	-	-
10	81.8	4-5	100	100	100	100	100	-	-	-
12	116.0	4-5	100	100	100	-	-	-	-	-

* Experiment begun as soon as these plants had germinated so that the cotyledons were exposed.

a Continuous SD treatment.

described earlier in connection with the experiments with Xanthium. A flash of light was given during the first hour following the middle of the dark period, a time which has been shown to be the point of maximum effectiveness for the flash in twenty-four hour cycles (Harder et al., 1943; Parker et al., 1945, 1946). After the experimental treatment the plants were placed under a twenty-hour day for anadditional two weeks before being dissected. Table 4 summarizes the data obtained in these experiments.

TABLE 4

Inhibition of the dark process in Chenopodium Amaranticolor by flash of light. Results shown as ratio of plants flowering/ plants treated.

Length of Dark Pd. in hours	Number of SD	Length of Flash in minutes 0	10	15	20	30
10	4	0/6	0/6	0/6	0/6	0/6
11	4	5/7	0/7	0/7	0/7	0/7
12	4	7/7	0/7	0/6	0/6	0/6
13	6 *	6/6	5/7	2/7	2/7	0/7
Approx. energy of flash in FCM		0	300	450	600	900

* Given 2 SD at 12 hours by error then given 4 additional SD at 13 hours.

b. Continuous supplementary light. The experiments to be described now were designed to determine the intensity of continuous supplementary light which would just inhibit flowering in this chenopod. In all of the following experiments, different intensities of illumination were achieved by placing the plants at different distances from the small Mazda bulb used as a light source.

A preliminary experiment was run in Dolk Laboratory using a basic eight-hour period of high intensity light plus eight hours of supplementary light from a 7 1/2 watt bulb. Plants at the different stations received 0.15, 0.08, 0.03, or 0.005 FC for the eight hours of additional light. Although none of the intensities were high enough to prevent flowering, they had a marked effect upon the size of the terminal inflorescence. Thus if the size (length x width) at the highest intensity is considered equal to 1, the relative size at the second, third and lowest intensity is 4.4, 6.3, and 8.0 respectively.

This type of experiment was continued in the Phytotron in one of the basement rooms which was converted into a darkroom. In these experiments the plants were grown under LD conditions in the greenhouses and constant temperature rooms until they were ready for experimental treatment. At this time they were moved to the basement darkroom. Here they received a basic eight-hour light

period from a panel of slimline fluorescent tubes which provided an intensity of about 700 FC. Supplementary light was from either a 7 1/2 or a 15 watt bulb which burned continuously. To assure that all of the plants at a particular intensity station received equal illumination from the small bulb the plants were placed on constantly revolving tables. Three experiments were conducted under these conditions, two with a 7 1/2 watt bulb and one with a 15 watt bulb. Table 5 summarizes the data from these experiments.

These experiments show that the threshold intensity lies in the region between 0.3 and 0.15 FC or between 288 and 144 FCM of light when given continuously. The effect of the low intensity supplementary light upon growth is also of interest. At intensities greater than 0.3 FC, which is just at the threshold for flowering, growth in height is markedly greater for the five days treatment than it is at the lower intensities. Thus the lower intensities permit immediate conversion of the plants from the vegetative to the reproductive state and in this way cause a cessation in growth in height.

TABLE 5

Amount of continuous low intensity light given during the dark period which will prevent flowering in C. Amaranticolor. C11, 12, 15. Five days at threshold at 17°C.

Expt. Number	Intensity in FC	Total FCM	Height * in cm.	Increase in Height	No. Flowering/ No. Treated
C-12	9.9	9504	19.0	-	0/12
	1.1	1056	18.6	-	0/12
	0.3	288	19.9	-	0/12
	0.1	96	18.1	-	12/12
C-15	4.4	4224	14.7 [a]	5.7	0/20 [b]
	0.6	576	15.1	5.7	0/20
	0.15	144	12.0	4.6	20/20
	0.05	48	10.7	2.6	20/20

* Height at end of 5 days of experimental treatment. All plants approximately equal at beginning of experiment.

a Remainder of data in this column taken from C-11 where same intensity was used.

b Remainder of data in this column sum of C-11 and C-15.

4) Gross morphological changes of the growing tip as the plant goes from vegetative to reproductive development.

During the course of the investigations described in this thesis it has been necessary to make a great many dissections for determination of the stage of floral development of the bud or buds. It appeared desirable to have a reference standard by which the stages of floral development might be described. Photographs were made of the growing point at different stages during floral initiation and certain of these were arbitrarily designated as standards. For this experiment a group of Chenopodium plants were moved each day from LD conditions to the SD table until sixteen such groups had been transferred. Three days after the sixteenth group had been moved to SD, the plants were dissected and some of the growing tips photographed. These photographs are shown in Figures 1, a, b, c, d, and e.

These photographs show some of the principal stages of development through which the vegetative tip passes as it becomes a well-differentiated inflorescence primordium. No attempt has been made to show all of the intermediate stages. It should be mentioned, however, that these tips have developed under continu-

ous SD induction. If plants are given only two or three days induction and then moved back to LD and allowed to develop there occurs a stage between that shown in Figs. 2c and 2d at which a profusion of very small, almost vestigial, leaves are present. The occurrence of an inflorescence primordium of this type then is probably a reflection of minimal induction since if four or five SD are given before the plants are returned to LD this leafy stage is either much reduced or completely lacking.

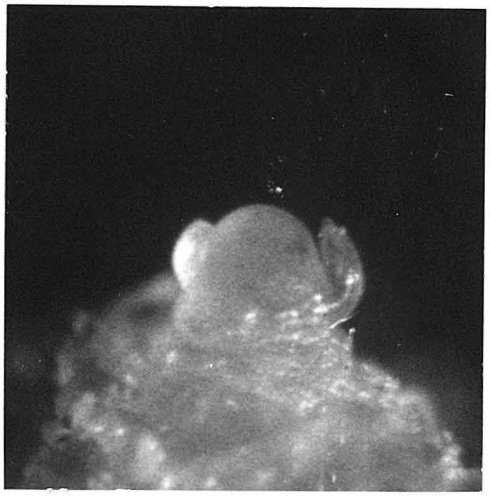


Figure 1 a. A vegetative tip of Chenopodium Amaranticolor.

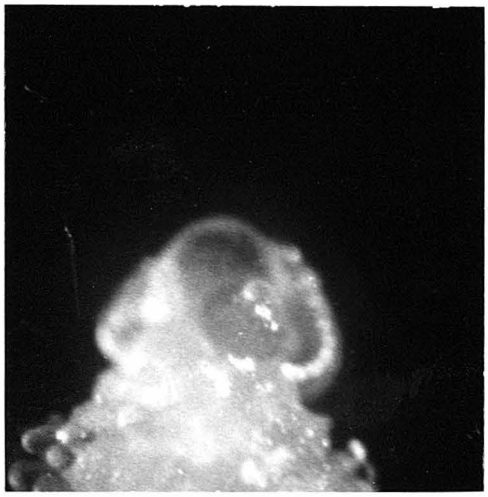


Figure 1 b. First stage in floral initiation.

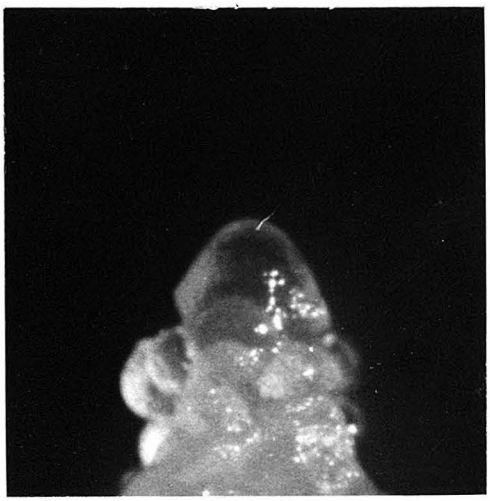


Figure 1 c. Second stage in floral initiation with all leaves removed. Slightly more advanced than 1 b.

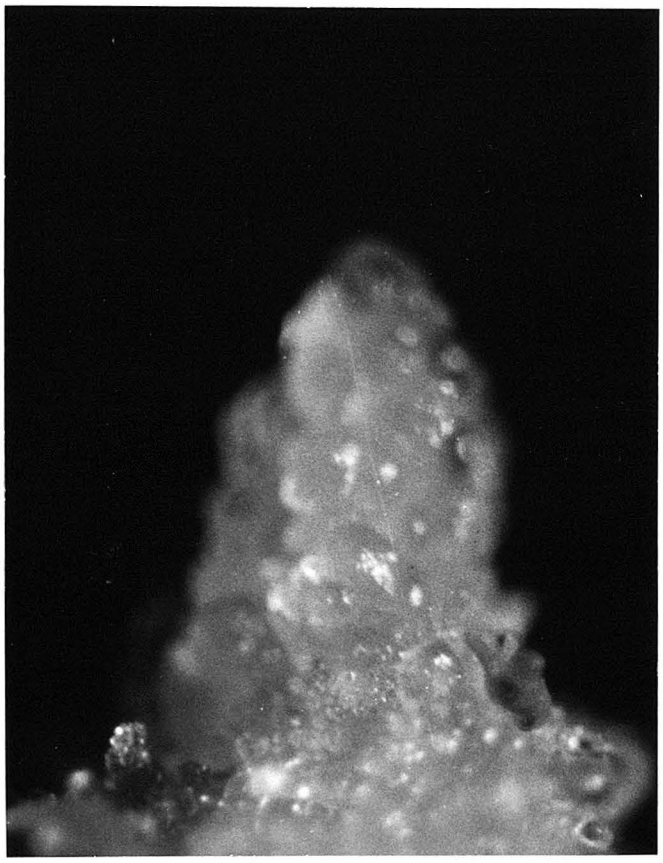


Figure 1 d. Advanced stage in floral initiation.

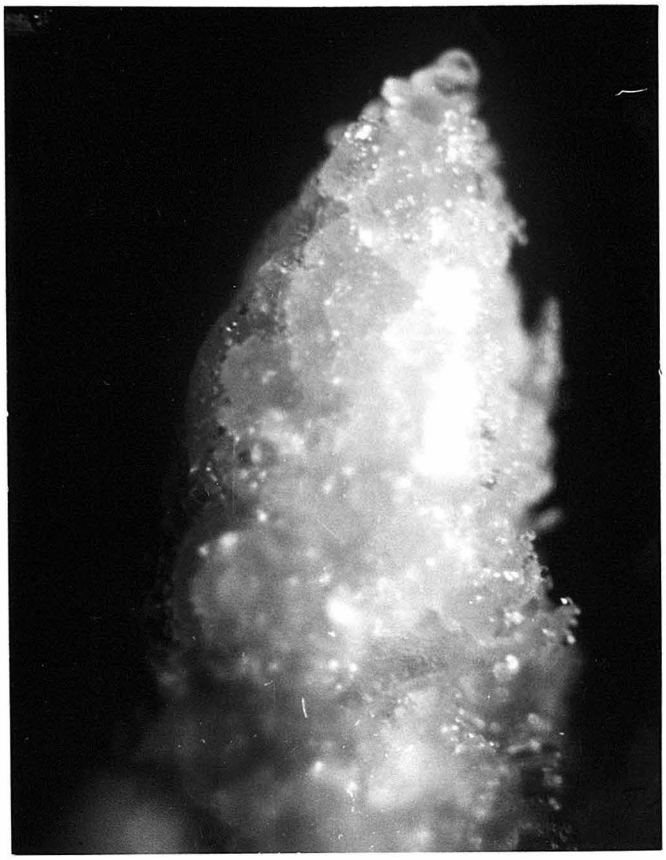


Figure 1 e. Inflorescence primordium just before becoming macroscopically visible.

IV. The site of perception of the photoperiodic response.

The mass of data compiled during the past several years of research on photoperiodism leaves little doubt that the leaves are the principal production sites for the flowering hormone. There have appeared recently, however, two reports (Leopold, 1951, Lona, 1949a) which indicate that plants completely devoid of leaves, or with only very small leaves are able to react to the stimulus afforded by SD and will ultimately initiate flowers in response to photoperiod. Experiments were run to corroborate these results in the same species in which the response had been reported.

1) Site of perception in Chenopodium. In the experiments designed to repeat Lona's (1949a) observation eight-week old Chenopodium plants were subjected to continuous SD treatment consisting of eight hours of light and sixteen hours of dark daily. The degree of defoliation and the principal results of these experiments are shown in Table 6.

These data indicate that the presence of a mature leaf is not necessary for sensitivity to photoperiodic induction in this plant. The experiment reported in Table 7 makes it appear probable

TABLE 6

The response of Chenopodium Amaranticolor to inductive treatment in the presence and absence of mature leaves.

Size of Leaf Remaining	Expt. Number	Number of SD	Number of Plants	% Flower	Infl. size Length x Width
1 cm. or less in length *	C-13	23	4	100	1.0
1 cm. or less in length *	C-14	28	4	100	0.25
1/3 expanded or less *	C-13	15	4	100	2.0
1/3 expanded or less *	C-14	28	5	100	4.0
4.1 sq. cm. of mature leaf [a]	C-14	28	4	100	5.5
9.4 sq. cm. of mature leaf [a]	C-14	28	4	100	13.5
31 sq. cm. of mature leaf [a]	C-14	28	4	100	30.0
1/2 of 1 mature leaf [a]	C-13	15	4	100	10.0
1 mature leaf [a]	C-13	15	4	100	12.0

* Leaves greater than this size trimmed off on alternate days.

a All leaves greater than 1 cm. in length trimmed off on alternate days.

TABLE 7

Further response of Chenopodium Amaranticolor to induction with and without mature leaves.

Treatment of plants	% Flowering	No. of plants	Days to visible buds
Intact plants (8-10 mature leaves)	100	2	11.5
3 recently mature leaves	100	8	13.3
1 recently mature leaf	100	8	14.9
1 one-half expanded leaf	100	8	18.8
1 young leaf	100	8	21.4
Terminal bud only	100	8	21.0
Terminal cut off and axillaries removed daily for 6 days	100	8	18.8

that the stem as well as the leaf is able to perceive the photoperiodic treatment. All of the plants in this experiment received constant SD treatment with daily cycles of sixteen hours of darkness and eight of high intensity light until visible buds were produced.

It appears then that the stem itself is capable of causing induction in one way or another. These results confirm and extend those of Lona (1949a).

2) The site of perception in Xanthium. The experiment discussed below was directed toward corroborating the results which Leopold has obtained with Xanthium (1951). On October 11, 1951, twelve Xanthium plants were moved from LD conditions to the SD table where they received eight hours of high intensity light and sixteen hours of darkness daily. On alternate days throughout the SD treatment all leaves greater than 1 centimeter in length were removed. The inductive treatments were terminated on November 4, 1951 and the plants moved back to the LD bench where the leaves were allowed to develop. Four plants died during the course of the SD treatment, five additional plants died before the termination of the experiment on January 28, 1952. At this time one of the plants had reached anthesis. The other two remain-

ing plants were strictly vegetative. Although it is clear that the leaves are much more effective than the stems in Xanthium at least, there appears to be no evidence to contradict the notion that the stems are also able to perceive the stimulus in either Xanthium or Chenopodium.

CHAPTER II. EXPERIMENTS WITH LONG-DAY PLANTS.

Contents.

I. Introduction.

Two LDP, Silene Armeria, L. and Hyoscyamus niger, L. (annual race) have been used in the present work for investigations of the photoperiodic responses of LDP. Silene Armeria (Sweet William Catchfly) is a new plant to the photoperiodic world, having been discovered and introduced to the field by the author in 1950.

Preliminary accounts of its response have been made (Liverman and Lang, 1951) but this is the first detailed published account of its behavior. Hyoscyamus niger has been used extensively in investigations by Melchers and Lang (1941, 1942, 1948) and others (Lang and Melchers, 1943; Claes and Lang, 1947; Parker et al., 1950). Therefore, its response is so well known that no really preliminary experiments have been carried out with it.

II. Experiments with Silene Armeria.

1) Discovery of a new long-day plant. In the fall of 1950 the severe smog conditions in Pasadena proved highly deleterious to most of the LDP then in general use. A search was begun for a LDP resistant to smog and small enough to permit mass greenhouse culture. Several plant species were brought to the Orlando Road Greenhouse and grown on regimes which included eight-hour days or twenty-hour days. Among the plants whose response was checked under these regimes was a wild species of mustard, Early Scarlet Globe radish, Brassica niger and B. arvensis, Nobel spinach, a variety of opium poppy, and Silene. All of the plants except Silene were either quite smog sensitive or were undesirable for other reasons, so only the experiments with Silene will be described.

On December 1, 1950, several seedlings of Silene were dug up from the waste area in the front yard of the author's residence in San Gabriel, California and brought to the Orlando Road Greenhouse. The seedlings were transplanted to rich sandy loam contained in 4 inch pots and divided into the two groups outlined above. By the 25th day the plants which were placed on LD had produced open flowers whereas those under SD were still in a basal rosette. The plants were allowed to continue under LD or SD until February 23, 1951 when the experiment was terminated. By this time the plants which had been on LD had flowered profusely and died whereas those on SD were still in a basal rosette indicating that they were vegetative.

Part of the short-day series was continued under SD until December 10, 1951 when they were dissected to observe the state of the growing point. The plants were still strictly vegetative, even though during the one year they had been growing under SD they had reached a height of 9.4 centimeters and had produced approximately 234 leaves. It appears therefore that the plants will remain vegetative essentially indefinitely when kept on SD.

Silene has then at least four qualities desirable in a good LDP, i.e., it responds rapidly, it is small, it remains vegetative

under short-day conditions and it is not severely damaged by smog. In view of these desirable characteristics, further experiments were set up to characterize its response.

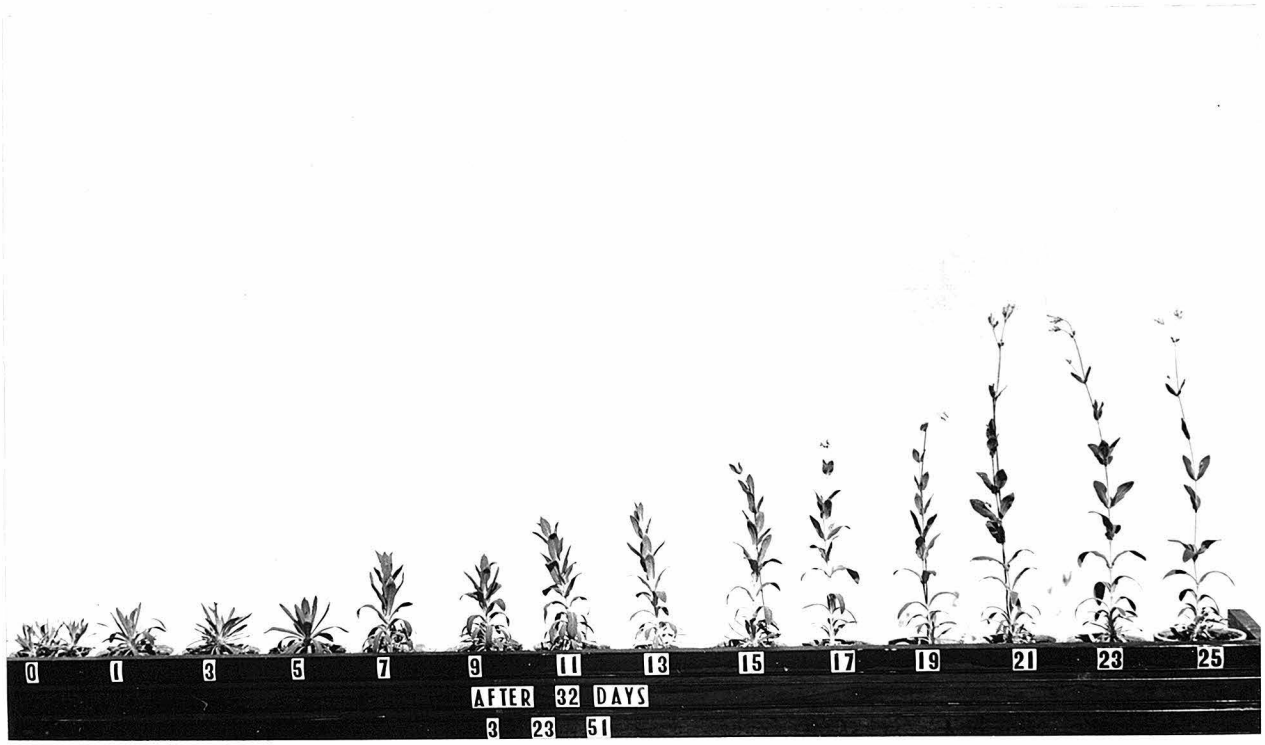


Fig. 2 Response of Silene Armeria to various number of LD. Plants were given from 0 to 25 LD and then returned to SD. Photographed 32 days after beginning of treatment.

The plants from the preliminary experiment outlined above which had remained on SD for the 3 months period were put under LD conditions. After differing numbers of LD they were returned to SD and their rate of further development followed. Fig. 2 pictures the results after 32 days. It is clear from the photograph that approximately 7 LD are required for induction under the conditions of this experiment. The height of the plant increases almost linearly with the number of LD to which the plant has been subjected. All plants which received seven or more LD had produced visible buds by March 14, 1951.

The investigations on Silene had now reached a stage where it appeared desirable to obtain further seed for more extensive experiments. The plants which grew in the author's front yard appeared to be a single isolated introduction and were in any case not common in the locality. The landlord was questioned concerning the origin of the planting. He revealed that he had observed the plant growing as an ornamental in New Jersey and had liked it well enough to bring seed with him to California in 1945. The plants from these seed had reseeded themselves each year and had gradually increased in number. This information was relayed to Campbell's Seed Store of Pasadena who obtained further seed, reputedly of the

same species. These seed were planted in the Phytotron and after germination the seedlings were removed to Orlando Road Greenhouse, transplanted into 3 inch pots and were allowed to develop under SD until May 8, 1951. Their response was compared with plants of the native variety by giving plants of each variety different numbers of LD and then returning them to SD where their development was observed. No essential difference was detected in the photoperiodic response of the two varieties under these experimental conditions. All plants which had received as many as 4 LD flowered, the other plants remained vegetative.

2) Determination of the critical day length in 24, 48 and 72 hour cycles. These very preliminary experiments indicated that Silene would be a desirable plant for use in further studies of the biochemical and physiological nature of the photoperiodic response of LDP. Before these experiments were begun however, it was important to have some knowledge of the critical day length and the response under photoinductive cycles longer than 24 hours. To this end, experiments were set up in Dolk Laboratory. Plants produced from the seed collected locally by the author (designated: Silene N) and seed obtained from Campbell's (Silene C) were

utilized to compare further the response of these two varieties. The plants were grown under SD in Orlando Road and Dodk Greenhouses prior to beginning of the 78 day experimental period. During this period the plants were given daily light periods of 9, 10, 11, 12, 13, 14, 16, 20 and 24 hours in the 24 cycles, light periods of 9, 10, 12, 14, 16, 24 and 36 hours duration in the 48 hour cycles and light periods of 9, 10, 12, 14, 16, 24, 36, and 48 hours in the 72 hours cycles. Only Silene C has been used in the 48 and 72 hour cycles. Data for the 24 hour cycles are shown in Table 7.

The data show that the critical day length under the conditions of this experiment lies between 10 and 11 hours for Silene C and between 12 and 13 hours for Silene N. The response of Silene N was much more uniform than that of Silene C, probably because of the 5 year isolation and resulting inbreeding which had been achieved by the local strain. Because of the apparent heterogeneity of Silene C, only Silene N has been utilized in the further experiments described in the main part of this thesis.

The data in Table 7 also show that, irrespective of the variety considered, there is a marked reduction in the number of

TABLE 7

The response of Silene Armeria (N and C) to relative length of day and night in 24 hour cycles. Silene C, 6 plants, Silene N, 3 plants.

	Length of Ppd.	Number Flr.	Days to Elong.	Days to Vis. Buds	Number of leaves Flowering	Number of leaves Veg.	Height of * Flowering	Height of * Veg.
Silene C	9	0	17.0	-	-	77.6	-	3.0 [a]
	10	0	16.5	-	-	76.7	-	2.6 [a]
	11	2	14.5	78 [b]	97.0	92.5	7.0	3.0
	12	5	14.4	63 [c]	76.0	94.0	18.1	5.0
	13	5	15.4	63 [d]	78.8	80.0	18.2	3.0
	14	6	11.7	27.2	46.3	-	22.8	-
	16	3	8.7	19.0	43.3	-	22.8	-
	24	3	7.7	17.0	43.3	-	29.0	-
Silene N	9	0	-	-	-	78.0	-	2.9 [a]
	10	0	18.7	-	-	76.7	-	2.8 [a]
	11	0	18.3	-	-	98.7	-	8.1
	12	0	18.0	-	-	88.0	-	3.8
	13	3	17.3	65.0	88.7	-	29.3	-
	14	3	14.3	26.7	49.3	-	14.0	-
	16	3	11.7	26.0	46.7	-	22.2	-
	24	3	9.0	16.0	38.7	-	19.5	-

* At appearance of buds or end of experiment.
a Terminated at end of 58 days.
b By dissection at end of experiment.
c 2 plants by dissection.
d 3 plants by dissection.

leaves formed by those plants which initiate flowers as compared to ones at the same day length which remain vegetative. Not only is there a decrease in leaf number with increasing day length, but the number of days until the beginning of elongation and until the appearance of visible buds is also markedly reduced by the increase in day length. These considerations will be discussed in detail in connection with the temperature study reported in the section on LDP.

The above cited response of Silene in 24-hour cycles agrees very well with the results reported for other LDP. What is its response in cycles longer than 24 hours? Table 8 summarizes these data.

These results show that in Silene C the critical day length in 48-hour cycles is much shorter than that in 24-hour cycles. Thus in a 24-hour cycle the plants remain vegetative at day lengths shorter than eleven hours whereas in 48-hour cycles they flower in both a 9- and a 10-hour day. This acceleration appears to be a specific effect upon flowering. In 24-hour cycles the numbers of leaves at 11, 12, 13 and 14 hour daily photoperiods is 97, 76, 78 and 46.3 respectively. Not at a single photoperiod

TABLE 8

The response of Silene Armeria (C) to relative length of day and night in 48- and 72- hour cycles. 3 plants per treatment. LL No. 1.

Length of Photoperiod	Number of Plants Flr.	Veg.	Days to Elong.	Vis. Buds	Number of leaves on Flr. Plants	Veg. Plants	Height of Plant Flr.	Veg.
48-HOUR CYCLES								
9	1	2	18.0	67.0	56	73	11.0	4.5
10	1	2	16.0	38.0	50	78	9.0	2.5
12	1	a	16.0	38.0	46	76 [a]	10.5	a
14	3	0	13.0	28.0 [b]	45.3	-	4.7	-
16	3	0	12.0	30.0	44.0	-	10.5	-
24	3	0	9.7	26.0	44.0	-	13.5	-
36	3	0	7.3	20.0	40.0	-	18.5	-
48 [c]	3	0	6.7	15.3	42.0	-	25.5	-
72-HOUR CYCLES								
9, 10, 14, 16, 24, 36 . All plants in this series dead within three weeks.								
12	1 [d]	-	17.0	27 [e] plus	48	-	2.5	-
48	3	0	10.0	23 [e] plus	42.7	-	6.0	-
72 [c]	3	0	6.7	15.3	42.0	-	25.5	-

a 2 plants dead, 1 after 72 days b 2 plants flowered normally, 1 dissected before buds appeared.
c Continuous light d By dissection, flowering somewhat dubious, other two dead.
e All by dissection after number of days indicated.

in 48-hour cycles was the number of leaves so numerous. Thus at 9, 10, 12 and 14 hours the numbers of leaves were 56, 50, 46 and 45.3 respectively. This represents a decrease of some 30 to 50 leaves in the 48-hour cycle series. These results agree with those of Claes and Lang (1947) for Hyoscyamus. The data for 72-hour cycles are not complete enough to permit any pertinent conclusion about flowering.

3) Morphological changes which occur in the plant during the conversion from vegetative to reproductive development. The first externally visible response to induction in Silene is the elongation of the stem. This growth in height is characterized by visible elongation of internodes which prior to LD treatment are very reduced in size and usually are not detectable even by removal of all leaves.

The next noticeable change in morphology occurs in the shape of the leaves. As shown in Figure 3 a, b, c and d, there is a general change in the relative lengths and widths of the leaves with a change from the vegetative to the reproductive state. All vegetative leaves are characterized by being relative-

ly long and narrow. As the change from vegetative to reproductive state occurs the developing leaves become wider in relation to their length than is the case with vegetative leaves of the same age. This change in leaf shape on an intact plant can be seen clearly in Figure 4.

Concurrent with these externally visible changes, other changes have occurred in the growing tip. These changes are shown in Figures 5 a through 5 i. The vegetative tip (Fig. 5 a, b) is small (100-150 microns wide x 75-100 microns high) and relatively flat. Soon after the floral stimulus has reached the growing point the tip begins to enlarge in all directions so that it protrudes above the adjacent leaf primordia (Fig. 5 c). The further stages of development into macroscopic flowers as shown in the remaining figures occur rapidly so that under optimum conditions flowers become visible to the unaided eye by the 12th to the 15th day. It is clear that other stages must exist between those shown in Figures 5 c and d, but attempts to find these stages and photograph them have not been successful.

Figure 3. Gross morphology of Silene leaves as the plant goes from vegetative to reproductive development.

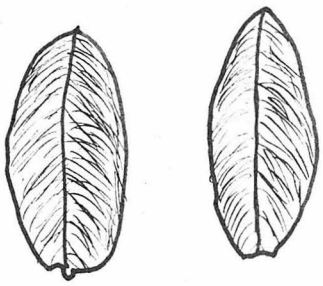
3 a

Figure 3 a. A mature vegetative leaf which is characterized by being long and narrow. This form is typical of all vegetative leaves regardless of age.

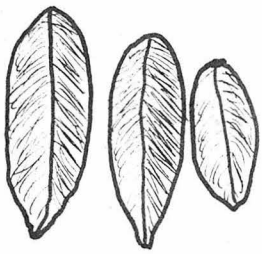
Figure 3 b. Leaf which was about 1/2 expanded at beginning of LD treatment.

3 b

Figure 3 c. and Figure 3 d. Shape of leaves which have expanded since LD treatment began. These were much younger than 3 b when the plant was in the vegetative state.



3 c



3 d

Figure 4. Silene in flowering state showing various stages of change in leaf morphology as the plant goes from vegetative to reproductive development. Mature leaves at base of plant are comparable to those shown in Fig. 3a. Other leaves in various stages of transformation from those shown in Fig. 3b to 3d.

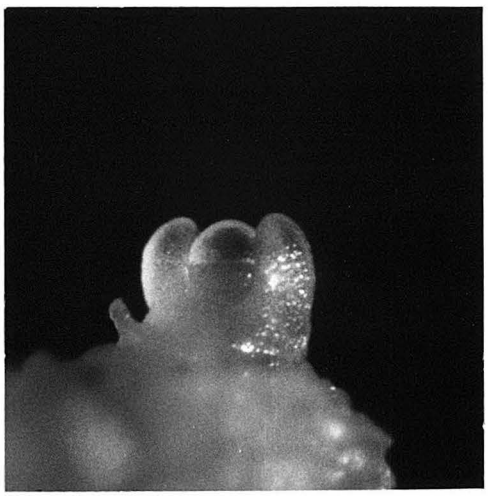


Figure 5 a. Vegetative tip (4 lvs.)

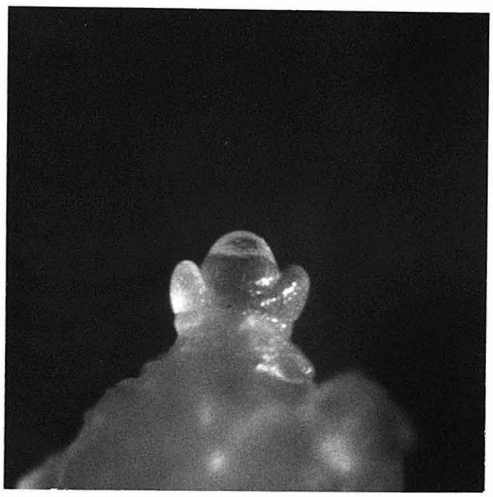


Figure 5 b. Vegetative tip (2 lvs.)

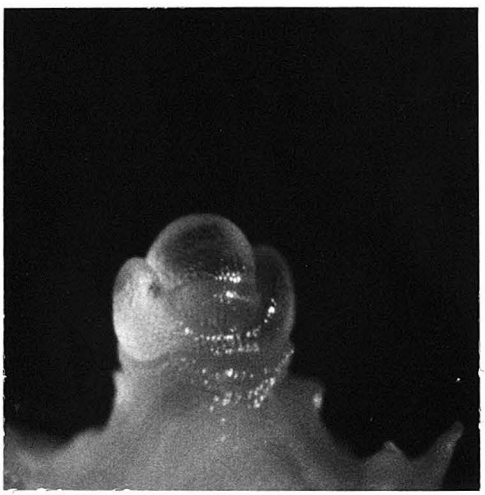


Figure 5 c. First stage in floral development (4 lvs.). Five days after first LD.

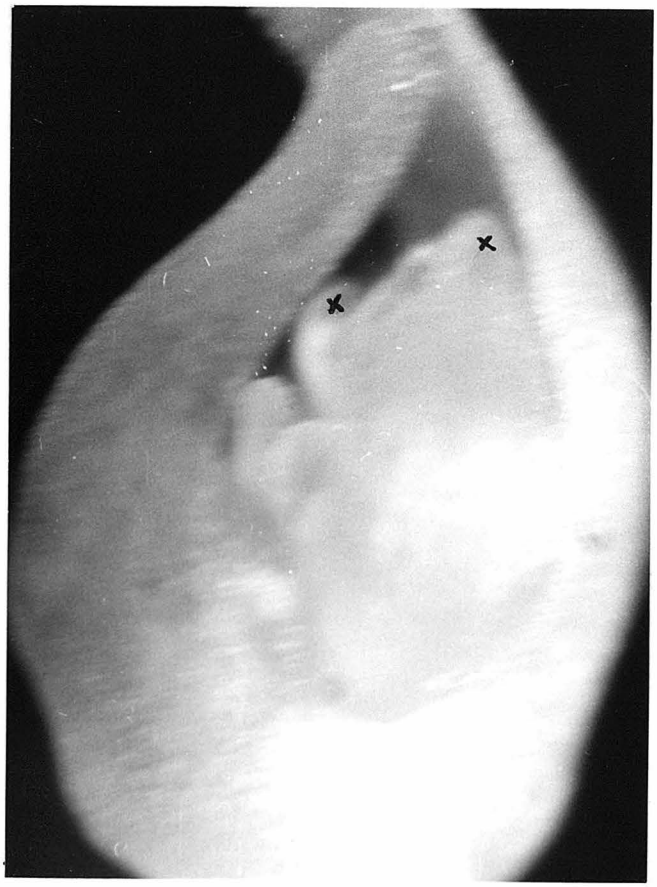


Fig. 5 d.

Figures 5 d and 5 e.
Silene growing point 6-8 days after first LD. Microscopic flower primordia present along with bracts (x) and primordium of another inflorescence.

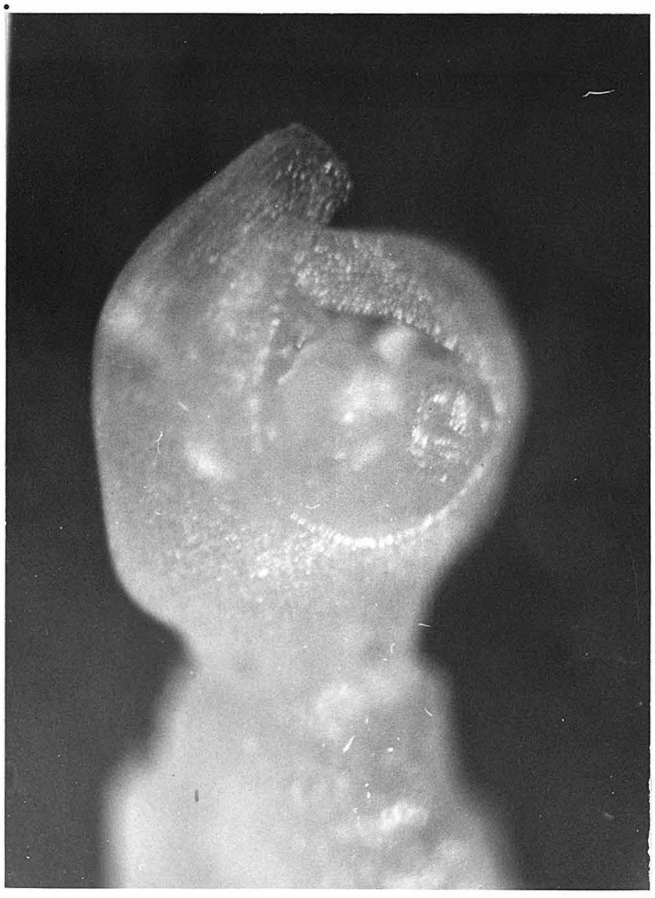


Fig. 5 e.

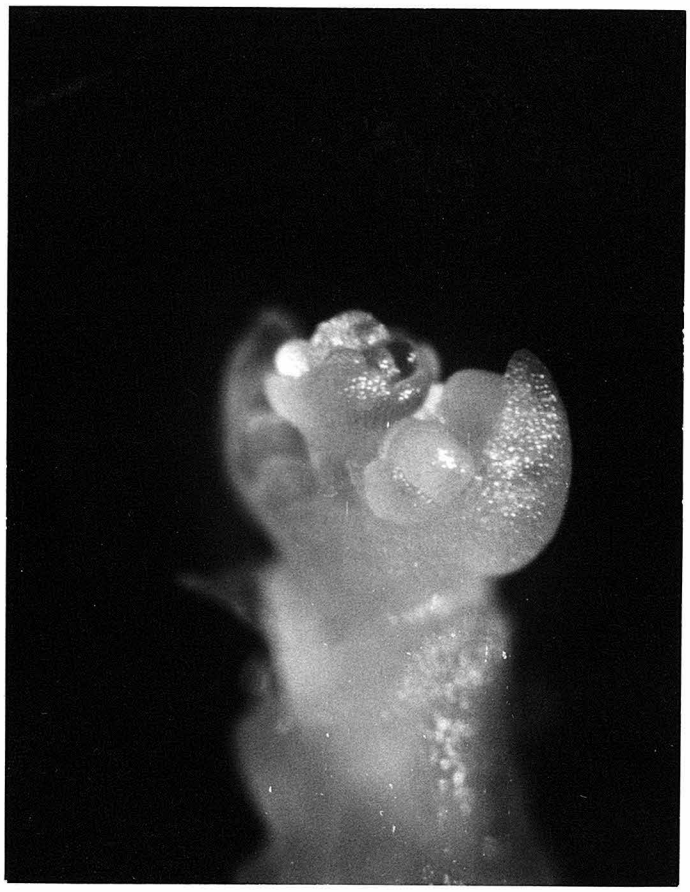


Figure 5 f. Early stages in development of a single flower. After 8-9 LD.

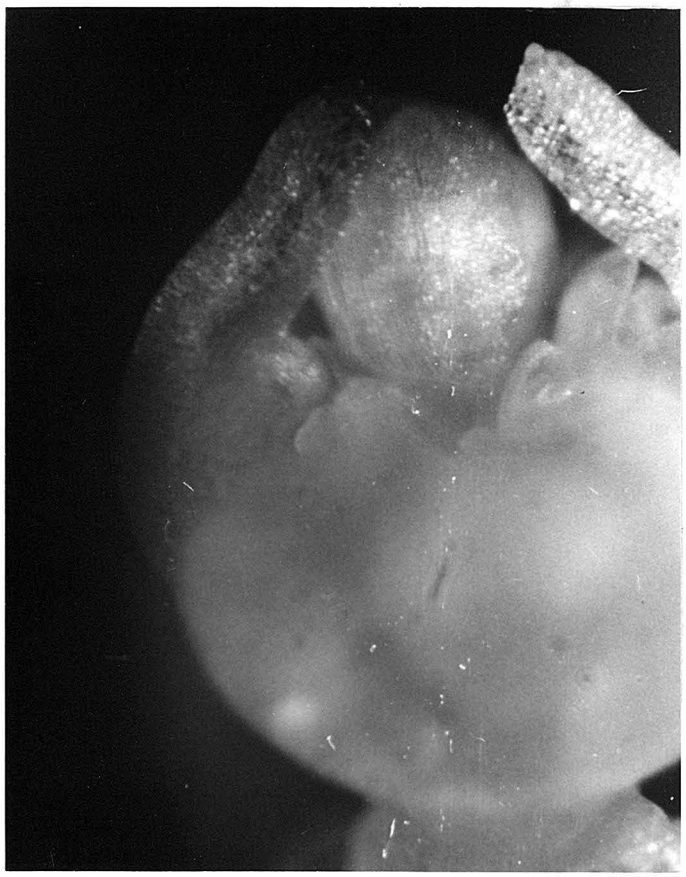
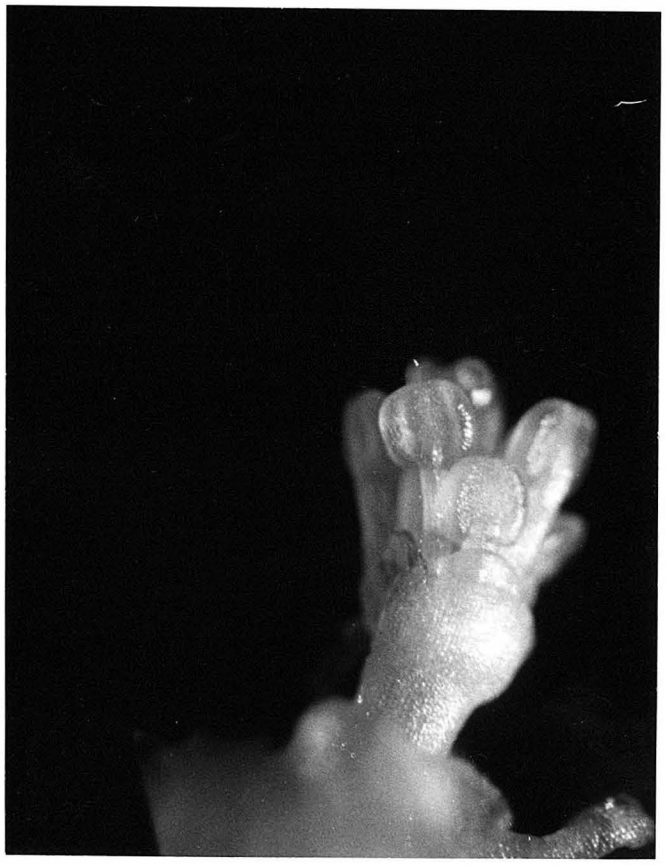


Figure 5 g. Further stage in development of individual flower. After 9 days.

Figures 5 h and 5 i.

After approximately 13 days and just before visible buds appear. All except single flower has been removed. 5 i shows the stage of development of flower parts at this time.

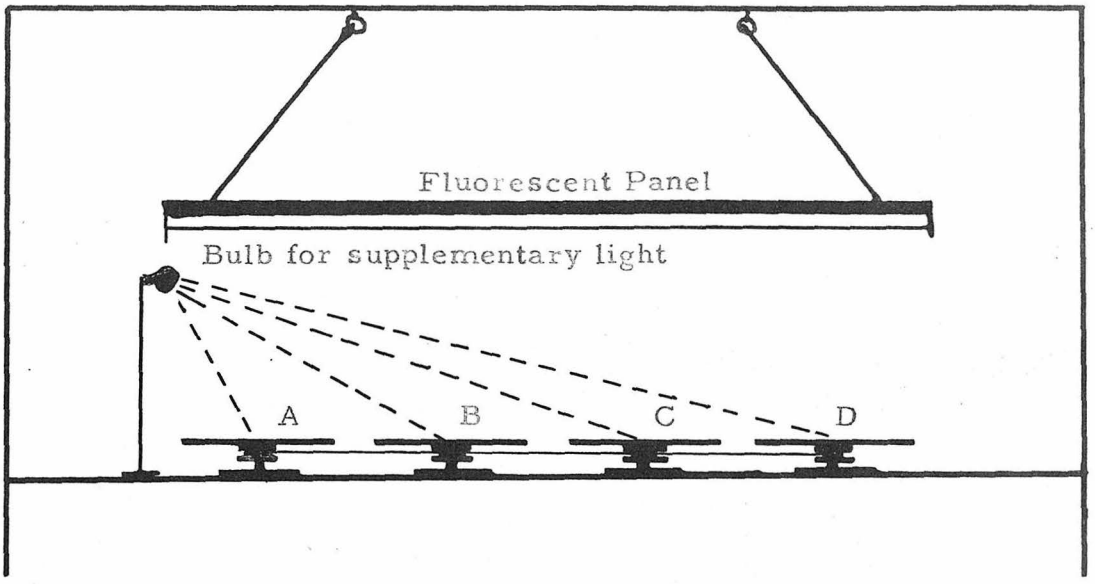


4) Promotion of flowering by low intensity light. Many workers have shown that qualitatively responding LDP need not a given intensity of light but a given duration of light before they will flower. If these plants receive just enough high intensity light for photosynthesis then the remaining portion of the light requirement can be satisfied by light of very low intensity. It appears then that the low intensity light is the controlling factor in the flowering of LDP. For this reason, it was highly desirable to have the proper equipment and conditions for studying this important process.

a. Construction of special equipment. Most of the threshold experiments with Silene have been run in the Phytotron under conditions of constant temperature and uniform high intensity light in order that the results of one experiment might be compared with those of another. Because of the space limitations in the laboratory it was necessary to construct a special device to obtain the threshold conditions. A basement room kept at 23°C. was completely darkened and black curtaining hung along the walls to prevent reflection of light. Four 14 x 14 inch plywood tables were then attached to

four pulleys which were set on axles. The axles were attached to a 1 x 10 inch board 10 feet long in such a way that they were approximately 30 inches apart. A slow speed motor was fastened to one end of the board so that a small wheel on its axle pushed against the pulley of the end table. A leather belt connected this pulley with the remaining pulleys so that when the end table turned all tables would turn. These tables revolved continuously in order that all plants at a particular intensity station would receive equal illumination from both the high intensity light during the daytime and from the low intensity light during the night. The high intensity light was furnished by a light panel containing four Slimline fluorescent tubes and these were supplemented by a 60 watt bulb over each center table and a 100 watt bulb over each of the two end tables. The Mazda bulbs gave the necessary spectral quality for good growth of the plants and in addition they brought the intensity of the end tables up to that of the two center tables. This lighting setup furnished light of approxmately 500 FC at the plant level.

The low intensity light to bring the plants near the threshold for flowering was furnished by a 15 watt bulb placed approximately 18 inches from the center of the first table. Figure 6 shows the essential details of this apparatus.



Top view of an individual table. Space for 9 four inch square x two and one-half inch deep containers.

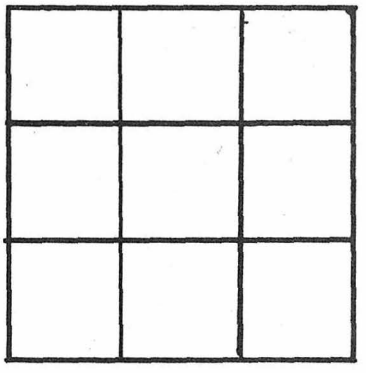


Figure 6. Details of method used to secure threshold conditions with Silene. Top part of figure, gross details. Lower part of figure shows individual table.

b. Preliminary experiments. The equipment described above was used in preliminary experiments to determine the intensity of light needed for threshold experiments with Silene. Plants for all of these experiments were grown under SD conditions prior to the beginning of the low intensity light treatments. Table 9 summarizes the data of three of these experiments.

TABLE 9

Promotion of flowering in Silene N by low intensity light given during the dark period.

Expt. Number	Approx. Intensity in FC	Total FCM	Duration of LIL in hours	Days at Threshold	% Flr.
8	4.4	4224	16	10	100
11	2.0	1920	16	24	100
7	0.9	432	8	20	0
8	0.7	670	16	10	0
7	0.3	144	8	20	0
7	0.1	48	8	20	0
7	0.04	19	8	20	0

It appears that the threshold lies somewhere between 0.7 and 2.0 FC of continuous light. In experiments reported later in this thesis the highest intensity used has been approximately 2.0 FC.

PART II

STUDIES OF THE PHYSIOLOGY AND BIOCHEMISTRY OF FLORAL INITIATION IN SOME SHORT-DAY PLANTS

Introduction.

It has been pointed out in the general introduction that this work will concern plants whose flowering is controlled by photoperiod. We recognize two principal categories of photoperiodically sensitive plants: the LDP in which flowering occurs when the plant is subjected to day lengths above a certain critical and the SDP in which, within certain limits, flowering occurs only when the plant is subjected to day lengths below a certain critical. Since the photoperiodic response is a complex one it has proved advantageous to resolve it, for purposes of experimental study, into a series of component or partial processes. Part II of this thesis is devoted to an analysis of our present knowledge of the flowering of SDP and to a study of these component or partial processes.

During the past three decades evidence has accumulated which permits us to recognize the following partial processes in

the steps leading to floral initiation in SDP:

1. The High Intensity Light Process.
2. The Dark Process.
3. The Low Intensity Light Process.
4. The Process of Hormone Synthesis.
5. The Process of Hormone Translocation.
6. The Process of Bud Differentiation.

A portion of the evidence for the separation and recognition of these particular processes is described below. Garner and Allard (1920) first showed that relative length of day and night is the controlling factor in the flowering of SDP. It has become clear more recently that with SDP the night length is ordinarily the most important factor and that SDP flower only on regimes in which the night is longer than a certain critical. Xanthium, for example, may remain vegetative for several years provided only that the night lengths never exceed 8 hours. However, if this plant is given a single night longer than 8-8 1/2 hours it will subsequently initiate flower primordia (Hamner and Bonner, 1938). In Xanthium as in some other SDP, flowering may be achieved by transferring the plant to continuous darkness (Hamner, 1940, for Xanthium; Lona, 1949 a, for Perilla and Chenopodium).

Other evidence which supports the view that night length is the governing factor is produced by experiments which show that interruption of an inductive dark period by a flash of low intensity light completely nullifies the effectiveness of this dark period in causing floral induction (Hamner and Bonner, 1938; Hamner, 1940; also see section on low intensity light process). By such a light flash the dark period is essentially divided into two dark periods each shorter than that required for the process of photoperiodic induction.

All of the above data are consistent with the view that night length is the factor which ordinarily controls the flowering of SDP. Hamner (1940), however, has been able to show in simple yet elegant experiments that light is required for the flowering of SDP. In these experiments it was demonstrated that a long night causes photoperiodic induction only if it is preceded by an appropriate period of high intensity light. To demonstrate this requirement with the cocklebur, it is necessary to make use of the flashing light experiment which has been mentioned above. Cocklebur plants are removed from a regime of long days and short nights and subjected to a long continuous dark period interrupted at frequent (3 hr.) intervals by (3 min.) flashes of light. During

this period the plants are apparently depleted of substances produced during the preceding light period, but they are not induced because of the interruptions in the dark period. When plants which have been subjected to such flashing light for periods of 12 to 24 hours are subsequently given a long uninterrupted dark period, they do not respond by flowering. If, however, after exposure to flashing light, the plants are exposed for a short period of time (2 to 4 hours) to intense light, and then returned to a period of uninterrupted dark, they do respond by flowering.

That dark periods to be inductive must not only be longer than the critical but must also be preceded by exposure to a longer or shorter period of high intensity light has been shown directly with species such as the soybean which require more than one cycle of light and dark (Borthwick and Parker, 1938 a, b).

The observations summarized above provide the evidence for the first three component processes outlined earlier, all of which are involved in the perception of relative length of day and night.

Further components of the photoperiodic process may also be recognized. During the course of photoperiodic induction, changes are brought about in the leaf which result in the production of a floral stimulus. This stimulus, which appears to be formed after exposure to one long night in the case of cocklebur, is itself stable in both light and dark. Thus, cocklebur plants exposed to one long dark period are subsequently able to flower whether kept in continuous light or in continuous dark (Hamner, 1940). Once photoperiodic induction has been achieved, the flowering response of the plant does not appear to depend upon light conditions and the plant has become photoperiodically insensitive with the reservation that there is a quantitatively stronger response with a greater number of SD. The production in the leaf of this light stable floral stimulus constitutes then a further component part of the photoperiodic process.

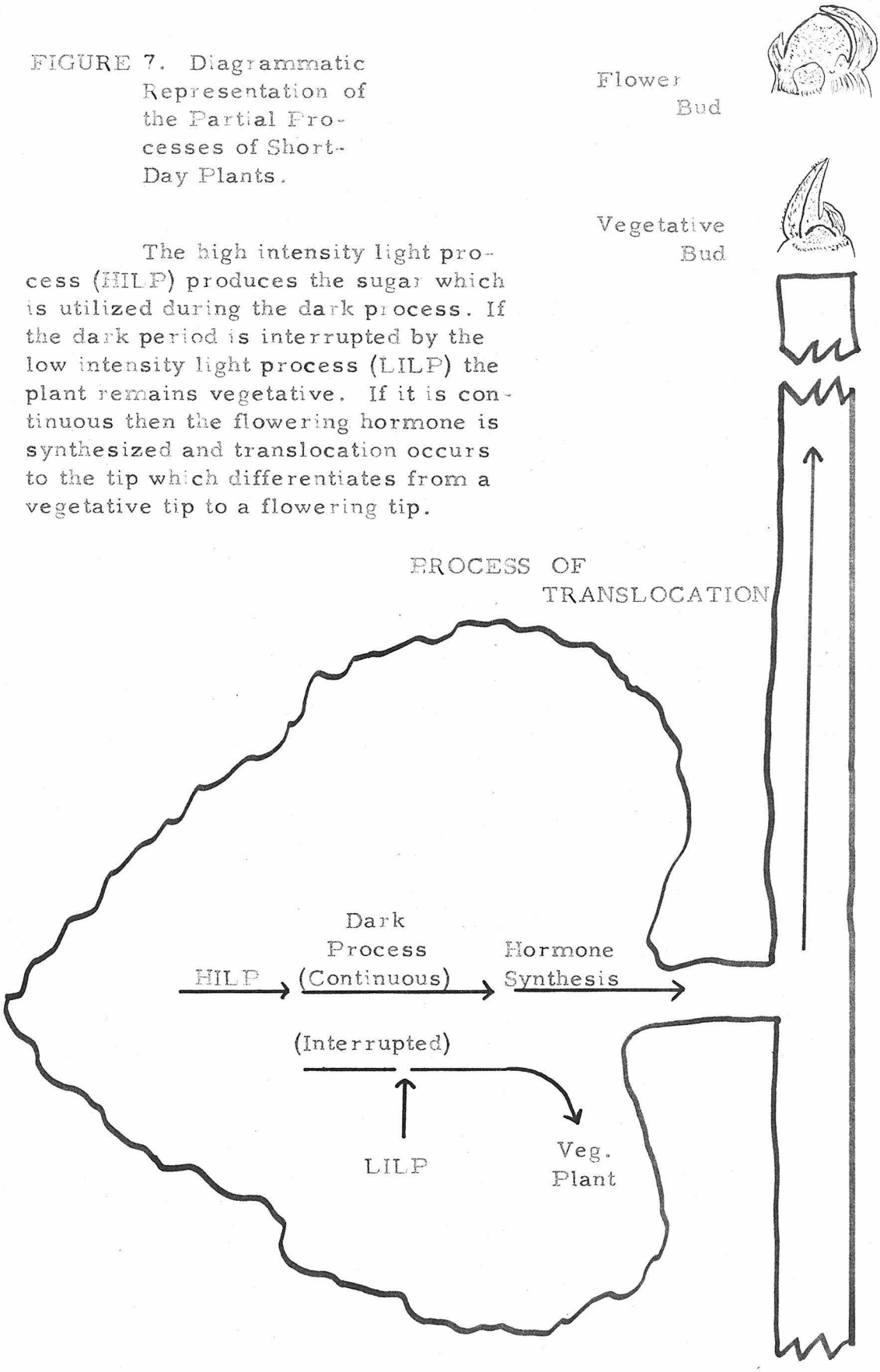
The production in the leaf of this light stable floral stimulus as the final result of the interplay of light and dark in a photoperiodically sensitive plant such as cocklebur can as yet be studied only by determining the effectiveness of such an induced leaf in bringing about floral initiation. This involves trans-

location of the floral stimulus from leaf to bud. Translocation of the stimulus constitutes then a fifth partial process of the photoperiodic response. Finally as the last partial reaction we must include the process by which the light and dark stable substance, or condition, formed in the leaf as a result of photoperiodic induction and transported to the bud, brings about conversion of the vegetative bud into the floral bud. We have then resolved the photoperiodic response into a total of at least six component and separable parts, whose interpretation is shown in Figure 7.

This thesis is concerned directly with the first five of these component parts. The sixth process has been studied only in so far as it has served as a biological assay for the other processes. In the remainder of this part of the thesis our present knowledge of each of these partial processes will be analyzed and evaluated. The original experiments of the author will be described in the appropriate sections, and finally on the basis of the earlier information and these new experiments an attempt will be made to present a coherent picture of the mechanism of floral initiation in SDP.

FIGURE 7. Diagrammatic Representation of the Partial Processes of Short-Day Plants.

The high intensity light process (HILP) produces the sugar which is utilized during the dark process. If the dark period is interrupted by the low intensity light process (LILP) the plant remains vegetative. If it is continuous then the flowering hormone is synthesized and translocation occurs to the tip which differentiates from a vegetative tip to a flowering tip.



CHAPTER I. THE HIGH INTENSITY LIGHT PROCESS

Contents.

I. Introduction.

It appears clear from the literature reviewed above that the photoperiodic response is a response to the cyclical alternation of light and dark and depends upon a regular succession of light processes and dark processes. Appreciation of these facts has come to

us gradually; in particular the concept of a requirement for high intensity light as a component of the photoperiodic process is relatively recent. This section will be concerned with the high intensity light requirement of the photoperiodic response of SDP.

1) Experiments which show the magnitude of energy required for consummation of the high intensity light process.

The following statement is taken from one of the early papers of Garner and Allard (1923).

> "It appears that the daily illumination period not only influences the quantity of photosynthetic material formed but also may determine the use which the plant can make of this material."

This statement implies that light may have two separate functions in the flowering of plants 1) the conduct of photosynthesis and 2) the regulation of other specific processes controlling development, however, it was not until the late 1930's that attempts were made to follow up Garner and Allard's suggestion.

Experiments of Borthwick and Parker (1938 a, b) with the SDP, Biloxi soybean, appear to have been the first to show conclusively that a light period of minimum intensity and minimum duration must precede a dark period of minimum length before floral

initiation will occur. Their experiments were based upon the fact that soybean requires at least two photoinductive cycles to induce flowering. It is relatively simple, therefore, to show that high intensity light must be supplied between the two dark periods. The results of these experiments show that the required light energy could be given with low intensity light over a relatively long period (8 hours at 100FC) or with relatively high intensity for a short period (2 hours of sunlight). Hamner's data (1940) for soybean show a linearity between the number of nodes flowering and the total incident energy (FCM).

With Xanthium, which requires only one photoinductive cycle, it was necessary to use the special technique already discussed in the introduction to show a need for high intensity light. Hamner (1940), who developed the technique, found that Xanthium required approximately 6×10^4 FCM of artificial light or two hours of sunlight before a subsequent long dark period would be effective in floral induction. Hamner's students, (Mann, 1940; Snyder, 1940) have worked out the high intensity light requirement in more detail and have found that the energy requirement for Xanthium is slightly less than that reported by Hamner but still of the order of magnitude

found for soybean. Table 10 summarizes the data available in the literature on the magnitude of the light energies needed to satisfy the requirements for high intensity light in SDP.

It is quite clear with the exception of Kalanchoe that the amounts of light energy required are similar. It is likewise clear that these light energies are such as to be capable of producing a considerable amount of photosynthesis.

2) Requirement for CO_2 during the light period. About the same time that the above experiments were being carried out, Parker and Borthwick (1940) also designed experiments to study the relation of photosynthesis to induction. In these experiments soybean plants were given six photoinductive cycles consisting of daily cycles of eight hours of high intensity light and 16 hours of dark. The light period was given either in the presence or absence of CO_2. They found that CO_2 must be present during the light period for photoperiodic induction to occur. A similar conclusion was arrived at almost simultaneously by Harder et al. (1941) in Germany with the SDP, Kalanchoe blossfeldiana. These results were extended (Harder

TABLE 10

Energetics of the high intensity light process.

Minimum energy (FCM) requirement	Hours supplied	Plant	Source
4.8×10^4	8 hrs. at 100 FC	Soybean	Borthwick et al, 1938
(1.2×10^4)	2 hrs. sunlight	Soybean	Borthwick et al, 1938
(1.2×10^4)	2 hrs. sunlight	Xanthium	Hamner, 1940
6×10^4	10 hrs. at 100 FC	Xanthium	Hamner, 1940
4.5×10^4	5 hrs. at 150 FC	Soybean	Hamner, 1940
5.0×10^4	5 min. at 10^4 FC	Xanthium	Mann, 1940
1.3×10^4 *	12 hrs. at 18 FC	Xanthium	Mann, 1940
$0.1 - 0.17 \times 10^4$ a	1 sec. at $6\text{-}10 \times 10^4$ FC	Kalanchoe	Harder et al, 1947

3.3×10^4 FCM average for all experiments.

* 10 photoinductive cycles needed before induction occurred.

a 65 photoinductive cycles given.

et al., 1944) to show that the CO_2 must be present in the atmosphere surrounding the particular leaf which is receiving the photoinductive treatments. Thus if CO_2 is fed to the leaves either above or below the one being induced, but not to the treated leaf itself, flowering does not occur.

II. Experimental determination of the nature of the products formed by the high intensity light.

1) Introduction and experimental approach. The literature cited above has shown that in the flowering of SDP there is a requirement for high intensity light which cannot be satisfied in the absence of CO_2. Thus it appears certain that at least part of this light requirement is directly concerned with the production of photosynthates. In spite of this obvious conclusion, however, no attempts were made to further unravel this light process until the summer of 1948 when J. Bonner (with Xanthium) conducted three experiments aimed directly at discovering the biochemical nature of the high intensity light reaction. These experiments conducted in a manner described below, indicated that the requirement for high intensity light can be re-

placed by feeding the leaves of the plants with sucrose. This was the status of the problem in 1950 when the author undertook to define more precisely the biochemical nature of the high intensity light process in SDP.

The present study of the high intensity light reaction has made use of the flashing light technique developed by Hamner (1940). This technique, which was mentioned briefly in the introduction, is illustrated in detail in Figure 8.

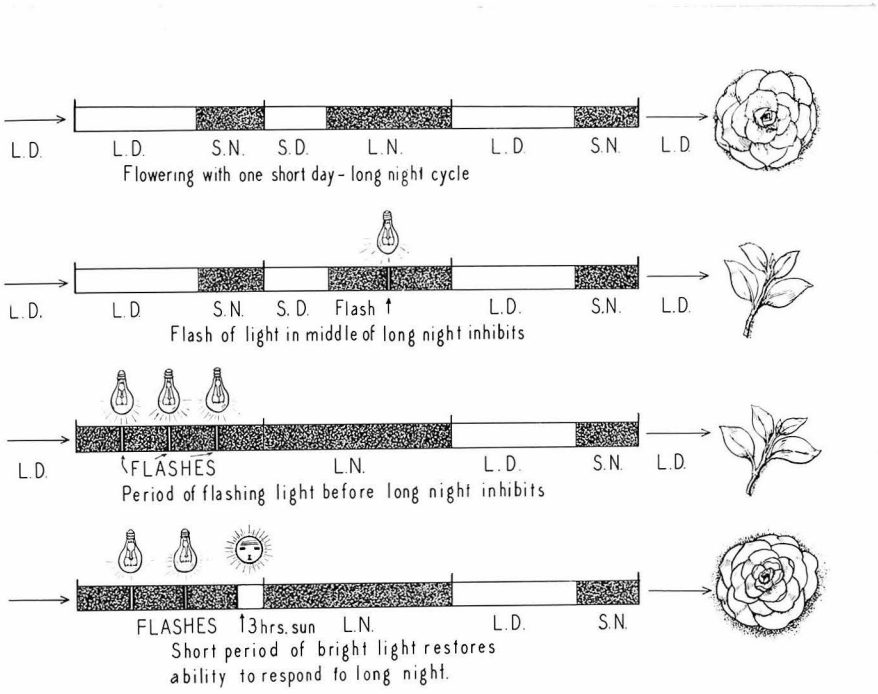


Figure 8. Illustration of the flashing light technique developed by Hamner (1940).

As pointed out earlier, if a cocklebur is kept on cycles of LD and SN, the plants will remain vegetative for several years. If this sequence of LD and SN is interrupted by a single cycle of SD and LN as shown in the top part of the figure, the plants will subsequently respond by flowering. The effectiveness of this LN in causing flowering can be negated by a single flash of light given in the middle of this dark period (second part of Figure 8). Suppose now that several flashes of light separated by short periods of darkness (3 minutes of light, 3 hours of darkness) are given (part 3 of Figure 8). After a sufficient number of such cycles the plant loses its ability to flower in response to a long interrupted dark period. It has apparently been depleted of some light-formed essential substance. If instead of giving the plants the LN immediately following the flashing light treatment they are first given 3 hours of bright sunlight and then given the LN, they are again capable of being induced (part 4 of Figure 8). These results make it clear that a continuous dark period must be preceded by a period of high intensity light before photoperiodic induction can occur. It should then be possible to determine the nature of this light formed substance by maintaining the plants in the dark and supplying them

with varied materials until a substance is found which can obviate the need for light.

In the experiments described below the experimental technique illustrated in part 4 of Figure 8 was used except that the leaves were fed substrates to replace the high intensity light period.

2) Experiments with substrates. For all of these experiments the plants were grown at the Orlando Road Greenhouse until they were approximately 6-8 weeks old, and were then moved to Dolk Greenhouse for the experimental treatment. A typical experiment is outlined below.

At 10:00 A.M. on a bright sunny day Xanthium plants were carried to the basement darkrooms where they received a period of flashing light. At the end of this period of flashing light the plants were treated in the following manner:

One group of plants was moved directly to the greenhouse bench where they remained on LD conditions. This was a control group to assure that the short cycle treatment had kept the plants vegetative.

A second group was moved to the greenhouse for a period of high intensity light (sunlight) after which they were re-

turned to the darkroom for a long dark period. This group gives a yardstick by which to measure the effectiveness of the different substrates in replacing high intensity light.

A third group was kept in the darkroom as a control group. If the duration of the short cycle treatment was insufficient to deplete the substances produced in the previous light period these plants would flower and the whole experiment would have to be discarded. In most cases 24 hours of short cycle treatment were sufficient to insure that this group would remain vegetative.

Further groups of plants in this particular experiment were treated with various substrates supplied to the base of the plant. In other experiments the leaves were dipped by the technique shown in Figure 9. Both methods of treatment are quite effective.

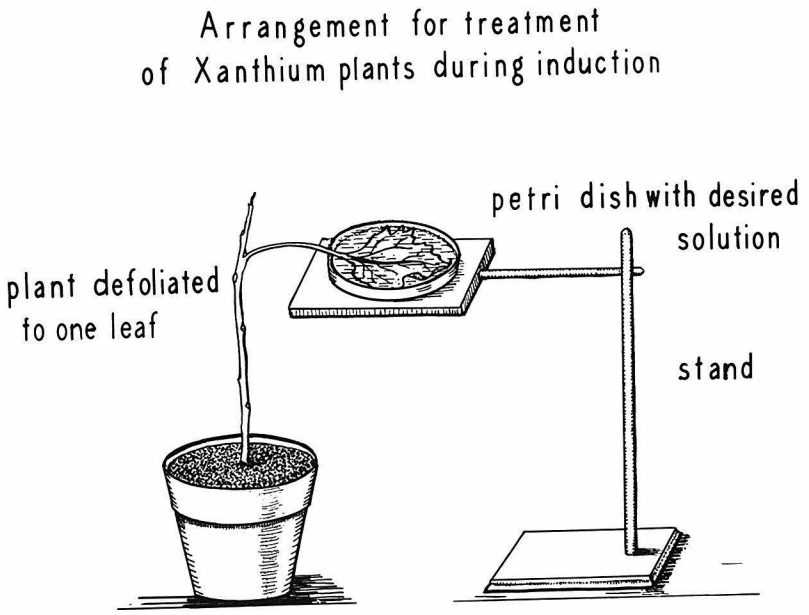


Figure 9. Method of applying substrates to leaves of Xanthium.

After a dark period of 24 hours the plants were moved to LD conditions in the greenhouse where the solutions were poured out, the bases of the plants washed off and placed in Hoagland's solution. Dissections of the stem apices were made 16 days later. Table 11 presents the results of this experiment.

TABLE 11

Substitution of respiratory substrates for the high intensity light process in Xanthium.

Treatment	Number of Plants	No. Intermediate	No. Flr.	% Flr.
Flash Control (LD)	10	0	0	0
Flash Control (LN)	10	0	0	0
7.5 hrs. sun	20	1	14	70
5.5 hrs. sun	9	1	5	56
Sucrose $1x10^{-1}M$	11	1	9	81
Sucrose $3x10^{-2}M$	20	4	5	25
Na Citrate $3 \times 10^{-3}M$	20	0	2	10

These data show that the high intensity light process can indeed be replaced by substances which would arise as a result of photosynthesis. Thus when the plants are given 7.5 hours of high intensity light in the greenhouse, 70% of them were in-

duced as a result of the subsequent long dark period. Plants kept in the dark but supplied with 1×10^{-1}M sucrose flowered even slightly better than did those given 7.5 hours of sunlight. There is a decreasing effect of the sucrose at lower concentrations and citrate appears to be not quite so effective as sugar.

It should be pointed out that this type of experiment is difficult to complete successfully, especially if smog conditions are unfavorable. There are also problems concerned with penetration of the applied substances into the plant which cannot always be overcome. In spite of these problems, however, it has been possible to demonstrate the activity of sucrose in replacing light in a total of six experiments and in 5 other experiments to extend the list of active compounds as shown in Table 12.

In addition to the eleven successful experiments, ten others have been completed which were not successful principally because of the smog problem mentioned above. Attempts to substitute aconitate, succinate and fumarate for this reaction have also not been successful. Such substances as sulfanilamide, Carbowax, and Dreft also do not appear to have a positive effect upon flowering.

TABLE 12

Compounds which are able to replace light in the high intensity light reaction.

Treatment	% Flr.	No. Vegetative	No. Intermediate	No. Flr.
LD control	0	6	0	0
LN control	0	6	0	0
Sunlight (6 hrs.)	83	1	0	5
Sucrose 10%	33	3	1	2
Sucrose 3%	16	5	0	1
Sucrose 1%	33	2	2	2
Glucose 1%	16	5	0	1
Glucose 0.1%	16	5	0	1
Glucose 0.01%	16	4	1	1
Galactose 0.1%	33	4	0	2
Citrate 2.25%	16	3	2	1
Citrate 1.0%	16	4	1	1
l-Malic 100mg/l	16	4	1	1

As a result of these experiments and those of Bonner (1948, unpublished) it appears that the high intensity light process of the photoperiodic response of SDP is concerned directly with the substances produced by photosynthesis. It would further appear that reserve substances which the plant has synthesized earlier during its period of vegetative growth are not able to substitute for sucrose in the flowering response of the plant. The relation of this high intensity light process to the flowering of SDP as a whole will be discussed in more detail in the section on the mechanism of the photoperiodic response.

CHAPTER II. THE DARK PROCESS IN SHORT-DAY PLANTS

Contents.

IV. A new tool for studying the nature of the dark process.

1) The need for a new method of approach.

2) Experiment with auxin.

I. General Introduction.

It has been pointed out in the previous chapter that SDP require approximately 10^4 FCM of light in order to flower in response to a subsequent inductive dark period. Although this light reaction is important, and in fact essential, under the usual environmental conditions the dark period exerts the controlling influence over the flowering of SDP. This has been shown directly in a variety of ways, the simplest and most direct of which has been with Xanthium. Xanthium grown under 20 hour photoperiods has remained vegetative for more than three years, however, if a single night longer than 8.5 hours is introduced into the regime the plants respond by flowering (Hamner and Bonner, 1938). The critical day length for Xanthium then lies between 15.0 and 15.5 hours of light daily. Table 13 shows the critical day length for several other species of SDP.

TABLE 13

The critical day length of several SDP.

Plant Species	Critical Day Length	Source
Xanthium pennsylvanicum	15-15.5	Hamner and Bonner, 1938
Glycine soja, Biloxi	14-15	Borthwick et al., 1938 b
Cosmos sulphureus, Klondike	12-13	Garner and Allard, 1925
Euphorbia pulcherrima	12-12.5	Allard and Garner, 1940
Chrysanthemum indicum, Queen Mary	14-14.5	Moshkov, 1940
Chenopodium Amaranticolor	14-16	Lang and Liverman, 1951
Bidens bipinnata	10-12	Allard, 1948

The critical day length in SDP is then that day length above which the plant will remain vegetative indefinitely. In actual fact in SDP one might better speak of a critical night length and say that this is that night length below which the plant remains vegetative. Evidence already discussed has shown that the dark period must be continuous and that a single flash of light given in the middle of the dark period may inhibit floral initiation. In contrast to the dark reaction which is strongly inhibited by low temperature (Parker and Borthwick, 1939, 1943; Hamner and Bonner, 1938; Roberts and Struckmeyer, 1939) the light flash has a temperature coefficient of approximately one (Snyder, 1940; Harder et al., 1944).

From these and other observations we must conclude that a continuous dark period of a certain minimum duration (the length depending on the species) is necessary for induction to occur. What is the nature of these light sensitive processes which take place during the dark period and which cause the SDP to redirect its whole activity from vegetative to reproductive development? The experiments described in this chapter have been directed to the study of the biochemical nature of those processes.

II. Auxin inhibition of the dark process.

1) Literature review. An interesting fact which makes the dark process susceptible to experimental study is that it is inhibited by applied auxin. Prior to 1947 there were many attempts to discover any possible role of auxin in flowering but because of the conflicting results of different authors it was impossible to assign auxin any definitive place in the flowering process. In spite of the inconsistent results the general idea developed, however, that a low auxin level in SDP was correlated with floral initiation. Cholodny (1939) appears to have been the first to postulate a definite participation of auxin in the flowering physiology.

> "While postulating the possible participation of auxin in the processes preparatory to flowering, we do not by any means discard the possibility that some other phytohormones may in one way or another affect these processes. At the present time it becomes more and more probable that the regulation of growth processes and development, that is, various changes in their rate and trend is a function not of a single substance, but of a complex of substances...

> "Involuntarily the question arises whether the 'flower-forming substance' is a complex of several phytohormones, for example auxin and vitamins of group B, which at a definite quantitative ratio acquire the faculty of diverting conspicuously the trend of development of embryonic tissues of the growing points toward the initiation of reproductive organs... If further investigations ultimately show that the substance stimulating flowering is of a hormonal nature, it will first be necessary to investigate its relation with auxin."

Although this idea of auxin involvement in the flowering process was not supported by any convincing data it did appear attractive. Galston (1943) following up Cholodny's suggestions, attempted to correlate the auxin level with flowering in soybean but like workers both before and after him, the attempts were fraught with many difficulties and were generally inconclusive. Galston tried to lower the auxin level artificially by the use of TIBA, a substance which he found to be an auxin antagonist in the Avena test. So far as floral initation was concerned his experiments were unsuccessful. He did find, however, that when induced plants were treated with this substance it resulted in an increase in the number of flowers formed, a fact already described by Zimmerman and Hitchcock (1942) for a photoperiodically indeterminate plant, the tomato.

The next attempt to affect the flowering behavior of SDP by auxin was reported by Thurlow and Bonner (1947). In this and in subsequent reports (Thurlow, 1948; Bonner and Thurlow, 1949) it was shown that auxin applied before or during the dark period inhibited floral initiation in Xanthium. The effectiveness of the auxin in inhibiting flowering was related to the method of application. Thus if auxin were applied to the leaves continuously during the dark period by immersing them in a solution of the auxin, 1 mg/l was enough to insure almost complete inhibition. As little as 10 mg/l of NAA was still sufficient to cause complete inhibition when supplied as a nutrient to the base of cuttings, but 500 mg/l were needed when the auxin was sprayed on the leaves. Thus Thurlow has shown for the first time that if auxin is applied continuously at the site of perception of the stimulus, physiological concentrations are quite effective in preventing floral initiation.

One is led to inquire whether this auxin inhibition may be reversed by an anti-auxin. Bonner and Thurlow (1949) showed that this is indeed the case. Thus when cocklebur plants were given 4 photoinductive cycles, 10 of 12 control plants flowered.

Application of NAA at 10 or 30 mg/l gave almost complete inhibition, whereas application of DCA (10 mg/l) or TIBA (10 or 30 mg/l) gave no inhibition. When 10 mg/l of NAA and 10 mg/l of DCA were applied simultaneously 8 of 9 plants flowered but when the concentration of NAA in the solution was raised to 30 mg/l only 4 of 10 plants flowered. These results show that the auxin induced inhibition of flowering can be reversed by simultaneous application of an auxin antagonist. These experiments show in addition that the reversal of inhibition itself can be reversed by application of more auxin.

The auxin inhibition experiments of Thurlow and Bonner have since been confirmed in many laboratories and on several different species of SDP (Harder et al., 1949; Lona, 1950; von Denffer et al., 1950; Leopold, 1949; Leopold and Thimann, 1949; van Senden, 1951). Li (1950) in this laboratory has shown also that the specificity of the inhibitory reaction is quite similar to that of other auxin responses. Thus he reports that IAA, NAA, 2,4-D, and 2,4,5-T are highly active, while 2,4-dimethylphenoxyacetic acid, 2,4-dichlorophenylglycine, 4-fluorophenoxyacetic and 2,4,5-triiodophenoxyacetic acids are only slightly active.

2) Confirmation and extension of the experiments of auxin inhibition of flowering. As a prelude to further investigations of the nature of the dark process the early experiments on auxin inhibition of photoperiodic induction of SDP were repeated and extended.

a) Experiments with Xanthium. Approximately six weeks old plants of Xanthium which had been grown under 20 hour days were moved to SD and given five photoinductive cycles each consisting of 8 hours of sunlight and 16 hours of darkness. Immediately prior to the beginning of the dark period the leaves of the plants were immersed for 10 minutes in solutions of varying concentrations of NAA. At the end of the SD treatment the plants were moved back to LD where they remained for 16 additional days. The results as shown by dissection are given in Table 14. These results are in keeping with those found earlier although apparently this method of application is more effective than the spray method.

b) Experiments with Chenopodium. Since another SDP, Chenopodium Amaranticolor, was to be used for studies related to the dark period it was desirable to determine its response to the application of auxin. Plants for this experiment were started

TABLE 14

Inhibition of the dark process in Xanthium with applied auxin. (X-45). Ten plants per treatment.

Concentration of NAA mg./liter	Plants Vegetative	Plants Flowering	% Flowering
1000	10	0	0
300	9	1	10
100	10	0	0
30	6	4	40
10	4	6	60
3	1	9	90

in the Phytotron and were then transferred to Dolk Greenhouse where they were grown under LD conditions until they were approximately 6 weeks old. The plants were given from one to five photoinductive cycles consisting of 12 hours of light and 12 hours of dark daily and were sprayed immediately before and immediately after each dark period with water solutions containing different concentrations of NAA. Following the SD treatment the plants were returned to LD for an additional two weeks and then dissected. The results of this experiment are shown in Table 15.

The data in Table 15 confirm the earlier experiments on auxin inhibition and extend the results in detail to another SDP. This experiment shows in addition that auxin has a specific effect upon floral initiation, that the auxin treated plants not only do not flower but also continue to grow vegetatively.

Among the commonly used criteria of growth are the increase in height, or the increase in dry weight per increment of time, but both of these measures are dependent upon rate of development. The best estimate of growth for work in photoperiodism

TABLE 15

Response of Chenopodium Amaranticolor to NAA application. C-2 *.

Treatment	No. of Plants	No. Flr.	% Flr.	No. of Lvs.
Long Day	5	0	0	34.8
1 Short Day	5	0	0	34.8
2 Short Days	5	4	80	37.2
3 Short Days	5	5	100	31.2
4 Short Days	5	5	100	30.0
5 Short Days	9	9	100	30.2
5 Short Days (NAA 1 ppm)	9	9	100	30.0
5 Short Days (NAA 3 ppm)	9	9	100	29.7
5 Short Days (NAA 10 ppm)	8	7	88	32.4
5 Short Days (NAA 30 ppm)	9	9	100	38.8
5 Short Days (NAA 100 ppm)	9	2	22	37.9
5 Short Days (NAA 300 ppm)	7	1	14	29.8

* Since these experiments were completed a paper by Lona (1950) has become available in which he demonstrated auxin inhibition in this plant.

appears to be the increase in the number of leaves on the plant. The advantages of this method lie in that it is entirely independent of the rate of development and in that it is strictly quantitative. The last column of data in Table 15 shows that the non-flowering Chenopodium plants have a geater number of leaves than do the plants which flowered, i.e., they continued to grow as well or better than non-treated controls. It may therefore be concluded that auxin (except at the highest concentrations used) does not interfere with growth but rather that its inhibitory effect is of a more specific nature.

3) The kinetics of auxin action.

a) Introduction to the problem. An important point to be considered in studies of the auxin inhibition of the dark process of SDP is concerned with the time at which auxin must be applied to the plant in order to bring about inhibition. The data available in the literature indicate that the auxin must be applied either immediately prior to, or during, the dark period. Thus when Galston (1943) applied auxin to soybean plants after six photoinductive cycles he found an acceleration rather than an inhibition

of flowering. Li (1950) reports a similar experiment with Xanthium in which auxin was applied either during induction or after four photoinductive cycles. Auxin applied during the photoinductive treatment gave complete inhibition, whereas application after the photoinductive treatment had no effect on flowering. Van Senden (1951) found only slight inhibition by auxin in Kalanchoe treated after 9 SD. After 10 SD auxin was no longer inhibitory. These results indicate that auxin affects the dark process rather than subsequent aspects of the photoperiodic response.

It was desirable, however, to repeat the above experiments under conditions in which photoperiodic induction could be achieved in response to a single dark period. When more than one dark period is used the effect of the auxin upon the light period following the dark period cannot be ruled out. If the effect is only upon the dark period then it should be possible to apply auxin before the dark period and secure inhibition whereas application after the dark period should give no inhibition.

b. Experiments with Xanthium. Because of this deficiency in the earlier experiments new experiments were designed to overcome the principal objections which have been raised

to previous work. Six weeks old Xanthium plants were defoliated except for 2 or 3 recently mature leaves in addition to the young expanding leaves. The plants were then moved to the dark room (23° C.) for either one or two 14.5 hour nights. The leaves of the plants were immersed for one minute in a water solution of IAA containing 0.05% Dreft either just before or just at the end of each of the two dark periods. Following the short-day treatment, the plants were moved back to the greenhouse for an additional two weeks of LD treatment before being dissected. Table 16 summarizes the data for this experiment.

These experiments open up some interesting possibilities not previously suspected. The results imply that the effect of auxin is not strictly limited to the dark period since 30 and 100 ppm of IAA applied during the terminal 30 minutes of the first dark period (and well past the 8.5 hour critical night length) show strong inhibition. At a concentration of 100 ppm, which of course is many times the physiological concentration, the inhibition was marked even when the auxin was applied 10

TABLE 16

The effect of auxin, applied before or after the dark period, upon floral initiation of Xanthium. X-113.

Treatment	Time of treatment	No. of plants Veg.	Int.	Flr.	%Flr.	Lf. No.
1 SD	---	0	1	14	93	14.8
IAA 100 ppm	Before 1st Dk pd	15	0	0	0	14.1
IAA 100 ppm	After 1st Dk pd **	12	3	0	0	14.1
IAA 30 ppm	Before 1st Dk pd	14	1	0	0	15.7
IAA 30 ppm	After 1st Drk pd	8	1	6	40	14.8 14.1 *
2 SD	---	0	0	15	100	14.5
IAA 100 ppm	Before 2nd Dk pd	3	2	10	67	14.3 14.6 *
IAA 100 ppm	After 2nd Dk pd	0	0	15	100	14.4
IAA 30 ppm	Before 2nd Dk pd	0	0	15	100	14.4
IAA 30 ppm	After 2nd Dk pd	0	0	15	100	14.3

* Leaf numbers of vegetative plants. Upper number is leaf numbers for flowering plants.

** Plants for after dark period treatment were treated about 30 minutes before removal to the greenhouse with the aid of light of less than 0.5 FC at the leaf level.

hours after the plants had been moved to the greenhouse following the first inductive cycle; 30 ppm had no effect when applied at this time. This cannot be a simple inhibition of growth since if plants were not treated until after the second dark period there was no inhibition by 100 ppm IAA. These data imply that there must be a period of fixation of whatever is made during the dark period before it is stable to such high concentrations of auxin.

The results of the above experiments made it obvious that there was a need to follow the time course of fixation of the substance which is formed during the dark period and which is unstable to high auxin concentrations. For this experiment six week old plants of Xanthium were given a single inductive cycle and dipped in auxin either before the dark period, at the end of the dark period but before removal to the greenhouse or at different periods of time after the plants had been removed to high intensity light in the greenhouse. The plants remained on LD conditions for 22 days after being removed to the greenhouse and were then dissected to determine the flowering response. The results of this experiment are shown in Table 17.

TABLE 17

Further data on the kinetics of auxin action. One 14.5 hrs. LN. X-119.

Time treated	Substance	Number of plants Treated	Flr.	% Flr.
Before LN	H_2O	15	15	100
Before LN	IAA 30 ppm	15	8	53
After 1 LN but 30 min. before removal to Gr.*	IAA 30 ppm	15	12	80
After 1 LN but 1 hr. after removal to Gr.	IAA 30 ppm	15	14	93
After 1 LN but 3 hrs. after removal to Gr.	IAA 30 ppm	15	12	80
After 1 LN but 7 hrs. after removal to Gr.	IAA 30 ppm	13	10	77
After 1 LN but 30 hrs. after removal to Gr.	IAA 30 ppm	15	15	100
Before 1 LN	IAA 100 ppm	15	0	0
After 1 LN but 30 min. before removal to Gr.	IAA 100 ppm	15	2	13
After 1 LN but 1 hr. after removal to Gr.	IAA 100 ppm	14	11	79
After 1 LN but 3 hrs. after removal to Gr.	IAA 100 ppm	15	10	67
After 1 LN but 7 hrs. after removal to Gr.	IAA 100 ppm	15	14	93
After 1 LN but 30 hrs. after removal to Gr.	IAA 100 ppm	14	14	100

* Gr. = greenhouse.

The plants in this experiment were slightly more sensitive to induction than was the case in the previous experiment. It is quite clear, nevertheless, that high concentrations of auxin (100 ppm) are able to inhibit flowering even after 14.5 hours of darkness if the application is made immediately before the plants are moved to high intensity light. After one hour of high intensity light this inhibitory effect of auxin treatment has decreased significantly and after 7 hours the effect has essentially completely decayed. We may conclude that the substance made in the dark is not stable to high auxin concentrations until after exposure to several hours of high intensity light. There is a necessity for experiments to determine more precisely the concentration range of auxin which must be applied to achieve this inhibition. It would also be of interest to determine in more detail the properties of this process which takes place during the early hours after return of the plant from dark to high intensity light.

A total of 5 experiments have been run with Xanthium to find if there is a point during the dark period after which physiological concentrations of auxin cease to inhibit the flowering res-

ponse. Unfortunately in all of these experiments only 10 to 20% of the controls have flowered so that it is impossible to interpret the results which have been obtained.

c. Experiments with Chenopodium. The experiments described above for Xanthium were carried out simultaneously with Chenopodium.

Eight weeks old Chenopodium plants were given different numbers of SD and treated with auxin either before or after the dark period. The results are shown in Table 18.

The results agree with those obtained with Xanthium in that they show that high concentrations of auxin applied at the end of the dark period still exert some inhibitory effect on flowering. They show also that this ability of auxin to inhibit decays with time.

In further experiments, only one concentration of auxin was used but this was applied before, immediately after, and 10 hours after the second and third dark periods. Thus all plants were given one long night (14 hours) and then were divided into two groups, those which received one additional night and those which received two additional nights. These two groups were further subdivided according to the time the plants were dipped.

TABLE 18

The effect of auxin upon the process of induction in Chenopodium when applied to the leaves before and after the dark period. 14 hour night. 10 plants per treatment. C-19.

Treatment	Time of treatment	% Flowering
1 LN	- - -	30
2 LN	- - -	40
3 LN	- - -	100
I LN and 30 mg/l IAA	Before dark period	0
1 LN and 30 mg/l IAA	After * dark period	10
2 LN and 30 mg/l IAA	Before dark period	0
2 LN and 30 mg/l IAA	After dark period	40
3 LN and 30 mg/l IAA	Before dark period	100
3 LN and 30 mg/l IAA	After dark period	90
1 LN and 100 mg/l IAA	Before dark period	0
1 LN and 100 mg/l IAA	After dark period	0
2 LN and 100 mg/l IAA	Before dark period	10
2 LN and 100 mg/l IAA	After dark period	60
3 LN and 100 mg/l IAA	Before dark period	60
3 LN and 100 mg/l IAA	After dark period	100

* Immediately before removal to greenhouse.

In this experiment, of plants treated with 100 mg/l of IAA before the second LN, 3 of 24 plants failed to flower. Of those dipped just at the end of the second LN, only one failed to flower. All plants which received three long nights flowered irrespective of the treatment. The results were thus similar to, although not so clear cut as, those obtained with Xanthium.

The results obtained in these experiments on the kinetics of auxin action give us a new insight into the mechanism of the photoperiodic response and support the general view that auxin is intimately associated with flowering response of SDP.

d. Literature concerning estimation of auxin. The data presented on the effects of applied auxins can be interpreted on the basis that floral initiation in SDP is controlled by auxin level in the leaves. According to this hypothesis flowering of SDP should be associated with low levels of leaf auxin and the photoperiodic induction which normally occurs during long nights would result from a decrease in auxin level during the dark period. Keeping the auxin level artificially high by auxin application or by a flash of light given during the dark period would prevent the level from falling below the required critical concentration and

induction would not occur. Conversely, influences which act to lower the leaf auxin level would be expected to promote flowering. Presumably TIBA and auxin antagonists in general would produce their effects by causing a decrease in the effective, if not the absolute concentration of auxin. In principle, the relation of endogenous auxin level to flowering might be studied directly by determination of auxin levels in leaves during the dark process and during the interplay of light and dark. That the auxin level of leaves does undergo diurnal fluctuations has been known since the work of Yin (1941) with the day neutral plant, papaya. In the papaya leaf as well as in the leaves of the day neutral Phaseolus coccineus (von Guttenberg et al, 1947) the auxin content of the leaf as a whole is lower during the night. Went's data (1944) for tomato also indicate that there is a diurnal fluctuation of the auxin level in the leaves. The leaves of day neutral plants contain a higher level of auxin when grown under LD than when they are grown on SD as has been shown for Coleus by Leopold (1949). Direct measurements of auxin levels in the leaves of SDP have proved exceedingly difficult since such leaves

have invariably proved to contain substances which interfere with the determination of the native auxin. The only data available, those of Zhdanova (1945) for Perilla and those of v. Witsch (1941) for Kalanchoe, indicate that leaves of vegetative plants (grown on LD) contain much more auxin than leaves of induced (grown on SD) plants. Extensive attempts of Bonner and Thurlow (1949), Thurlow (1948), Li (1950), the author, and others to obtain reproducible auxin determinations with leaves of Xanthium have thus far met with no success. Similar difficulties have beset work with SD soybean (Galston, 1943) and Chenopodium (the author), and with LDP, including Hyoscyamus (the author). The difficulties encountered have been primarily due to the presence in leaves of substances which either (a) inhibit the Avena test, or (b) destroy auxin during the extraction, or both. Because of the presence of Avena test inhibitors, it is necessary to carry out extensive dilution series in the assay of plant extracts and to ascertain and use only that part of the range in which auxin is the actual limiting factor. This has not been done in many of the less critical investigations of leaf auxin content.

Interpretations of the dark process as one in which the auxin level of the leaves is decreased are strengthened by the experiments of Khudairi and Hamner (1951), in which it is shown that flowering may be induced in Xanthium even on LD by application of ethylenechlorohydrin. This substance has previously been shown by Michener (1942) to bring about a rapid and considerable decrease in the auxin level of the treated plant tissue.

Naturally occurring inhibitors of the Avena test have long been suspected of being in plant tissue and recently Linser, (1951), Luckwill (1952), and Bennet-Clark et al. (1952) have all been able to separate the active auxins from the inhibitors by chromatographic techniques. These methods must be applied to Xanthium and to Hyoscyamus if the kinetics of auxin fluctuations are to be followed in any further attempts to correlate these fluctuations with known photoperiodic responses.

III. Inhibition of the dark process by substances other than auxin.

1) The problem. If one assumes as a working hypothesis that the flowering of SDP is controlled by the auxin level in the leaves.

one might next inquire whether the plant possesses a system capable of bringing about rapid changes in this level. As a direct result of work carried out in this laboratory (Tang and Bonner, 1947, 1948) one may say with considerable assurance that such an enzyme, an enzyme capable of rapid destruction of auxin, is of rather general occurrence in plants. This enzyme, indoleacetic acid oxidase, has been found in cabbage, spinach roots, Avena coleoptiles, Avena roots, etiolated pea seedlings (Tang and Bonner, 1948), bean roots (Wagenknecht and Burris, 1950) and in green pea plants (Galston and Baker, 1951). These and other workers (Goldacre, 1949, 1951) have confirmed and extended these earlier results and have characterized the system in detail. Galston and Baker (1951) have suggested that the enzyme may be a flavoprotein-peroxidase complex wherein the flavoprotein furnishes the peroxide necessary for oxidation of the IAA by the peroxidase component. It has further been shown that dark inhibition by manganous, cupric, cuprous, cobaltous or ferrous ions is light reversible (Galston et al., 1951). These workers suggest that the inhibitors

destroy the peroxide before it can be utilized in the oxidation of IAA. In light, the flavoprotein would produce sufficient peroxide to reverse this inhibition. This interpretation is strengthened by the experiments of Goldacre (1951) which show that catalase also inhibits the enzymatic oxidation of IAA. Goldacre (1951) also found that many peroxidase substrates such as guaiacol, pyrogallol and o-cresol are oxidized by the system in the presence of 0.05 M hydrogen peroxide. Guaiacol at a concentration of 5×10^{-6}M acted as a competitive inhibitor to the oxidation of IAA. These results taken together provide good evidence that the IAA oxidase system does function through a peroxidase.

Suppose now that the postulated decrease in leaf auxin level during photoperiodic induction were somehow related to destruction of auxin by IAA oxidase. Goldacre's (1951) results suggest that a profitable way to approach this question would be to treat Xanthium plants simultaneously with SD and with peroxidase substrates. In principle, if the oxidation of IAA by the above described system is in any way involved in the flowering process, the peroxidase substrates fed under SD conditions should

prevent flowering. That this is indeed the case is shown by the following experiment.

Xanthium plants, six weeks old, were made into cuttings and the bases of the cuttings placed in brown jars containing various peroxidase substrates dissolved in distilled water. The solutions were renewed daily during the four day induction treatment. Each inductive cycle consisted of 12 hour days and 12 hour nights. Following the inductive treatment the plants were returned to the long day bench where the solutions were poured off, the bases of the plants and the jars rinsed and refilled with Hoagland's solution. The plants remained under LD conditions for 15 additional days before the tips were dissected. The results of this experiment are shown in Table 19.

Manganous, cupric, cuprous, and cobaltous ions and ascorbic acid gave essentially no inhibition under these experimental conditions. Ten additional experiments have been completed and the results of all of them show that these typical peroxidase substrates give some inhibition of flowering, especially at the higher concentrations. These substances are not so effective in preventing flowering as are active auxins such as 2,4-D and IAA.

This is, however, most likely a technical problem related to the rapid auto-oxidation and slow penetration of the substrates as compared with the auxins. It appears possible that better results might be obtained with the inhibitors if they were applied directly to the leaves during the inductive treatments or at threshold conditions.

TABLE 19

The effect of various peroxidase substrates upon the dark process in Xanthium. Control plants treated with water gave 100% flowering.

Substance	Concentration in ppm.	No. of plants Veg.	Flr.	% Flr.
Hydroquninone	300	8	2	20
	100	8	2	20
	30	6	4	40
	10	4	6	60
Catechol	300	8	2	20
	100	4	6	60
	30	1	8	89
	10	5	5	50
Pyrogallol	300	7	3	30
	100	4	5	55
	30	2	9	82
	10	4	7	64
Resorcinol	300	4	7	64
	100	4	7	64
	30	1	11	92
	10	1	11	92
Benzidine	300	3	9	75
	100	3	7	70
	30	6	5	46
	10	5	6	55
p-phenylene diamine * (Expt. X-57)	300	3	6	67
	100	3	11	79
	30	4	13	76
	10	5	10	67
	3	3	12	80

* 67% of controls flowered in this experiment.

IV. The Threshold Condition - - A Tool for Studying Simultaneously the Induction and the Inhibition of Flowering.

The concept of using threshold conditions to study the biochemical nature of the flowering process is just becoming appreciated by investigators in the field of photoperiodism. It is quite true that there are isolated instances in which threshold conditions have been used but the idea is not generally familiar to the majority of workers. In those cases in which the threshold condition has been used for biochemical studies, important results have often been obtained. Thus Melchers and Lang (1942) and Melchers and Claes (1942) found that only by growing Hyoscyamus very close to the critical day length, in fact so close that an occasional control flowered, were they able to achieve flowering by sugar feeding or by maintaining the plants in an oxygen free atmosphere. Other workers (Parker, et al., 1945, 1946, 1950; Borthwick et al., 1948) have approached the threshold for flowering by giving plants a single flash of light during the dark period in order to determine the quality of light which inhibits flowering of SDP and insures flowering in LDP. Bonner (1949) made use of threshold conditions consisting of an 8 hour period of high intensity

light plus constant light of just sufficient intensity to insure that the control plants would remain vegetative. Treatment of the plants under normal LD conditions with TIBA or DCA gave no flowering whereas treatment of plants with these substances under the threshold conditions outlined above gave consistent flowering of about 30 to 40 % of the treated plants.

As a result of these experiments by Bonner and the experiments to be discussed in Chapter III, it became quite clear that in order to gain any insight into the biochemical nature of the flowering process it was essential to bring the plants as close as possible to the threshold for flowering. Previous experiments in which attempts were made to determine the effect of various substances on the flowering response (with the exception of those instances mentioned above) have been done under strictly long-or short-day conditions. These biochemical studies which have been carried out far removed from the threshold have yielded few useful results.

At the threshold flowering can be elicited or suppressed by relatively slight influences. Thus studies carried out at the threshold promise to reveal more about the biochemical nature of

the dark process than have those studies carried out far removed from the threshold, i.e., under strictly LD or SD conditions.

Several methods may be used to approach the threshold for flowering. One method which has been widely used is one in which the plants are grown under photoperiods just above or just below the critical day length (Melchers and Lang, 1942; Melchers and Claes, 1942). A second method is that in which the plants are given a basic period of high intensity light and a flash of light during long dark periods. This method has proven quite useful for determining the quantitative aspects of the action spectrum of the low intensity light reaction of both LDP and SDP. A third method makes use of a basic period of high intensity light followed by continuous light of low intensity for the remainder of the 24 hour cycle. This type of threshold experiment has been used extensively for determining the minimum intensity of light which will just inhibit or just promote flowering of SDP and LDP respectively.

It is apparent that each type of threshold condition has its own merits for particular experiments, but the last type has

many advantages for studying the biochemical nature of the processes which take place during the dark period in both LDP and SDP. The principal advantage of this method is that a graded series of intensities close to the threshold can be included in a single experiment and the effect of a given substance can be determined under these varied conditions. It is possible to do this comprehensive type of experiment because of a gradation of light intensities which can be obtained simply by arranging several stations at different distances from a single light source. The source is selected so that with SDP the control plants located at stations near the light will not flower, the control plants most removed from the light will all flower. Between these two extremes there will be stations at which the slightest promotive or inhibitive effect of applied substances on flowering can be detected. A second advantage of this method of approaching the threshold is that it is applicable to both SDP and LDP. Figure 10 illustrates the method in detail.

This method has been used by the author principally for studies on the auxin relations of flowering in LDP (see Part III). The experiments reported below (and an additional experiment re-

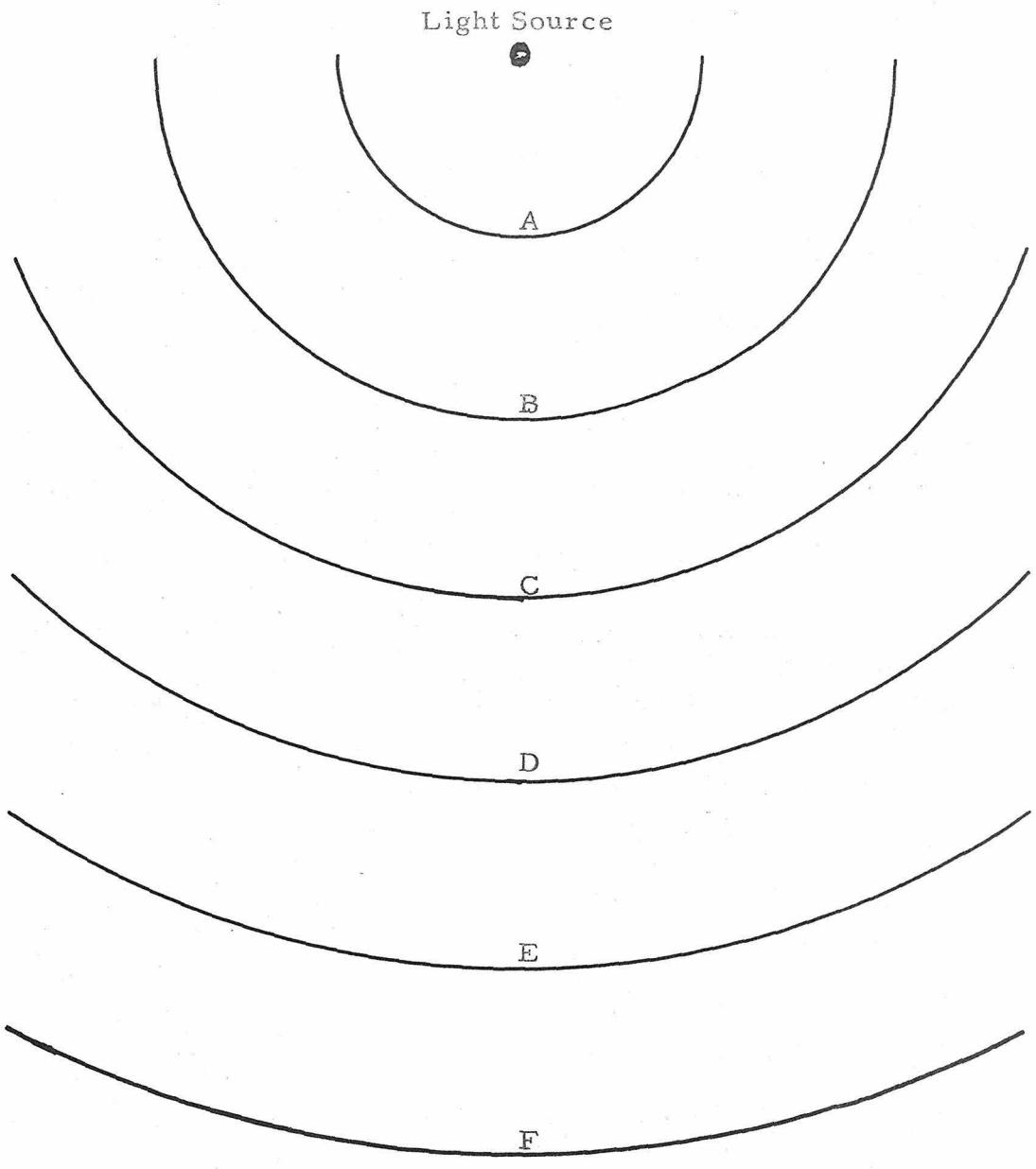


Figure 10. Threshold conditions used to study dark process in SDP. The light source is placed approximately 4 feet above the top of the table on which plants are placed to prevent shading effects from one intensity to another, i.e., plants at all stations received light directly from the source.

ported in Part II, Chapter III) have, however, been run with SDP. The following experiment is an illustration of the effectiveness of the threshold method for studying the dark process of SDP.

Six weeks old Xanthium plants were trimmed to three recently mature leaves, made into cuttings and placed in individual test tubes containing a solution of the substances to be tested. The plants were put in the solution at 1500 hours and at 1800 hours were moved to the darkroom where they were placed under the threshold conditions outlined above. The experimental treatment consisted of 8 hours of high intensity light daily plus an additional 16 hours of low intensity light from a 15 watt bulb. Three groups of plants treated with different concentrations of IAA and a group of control plants were placed at each intensity station. After 4 days under these threshold conditions the plants were moved to the LD bench. After the IAA solution was washed off the bases of the plants they were placed in Hoagland's solution to remain for an additional 10 days before dissection of the tips. Table 20 presents the results of this experiment.

As discussed earlier, Bonner and Thurlow found that from 30 to 100 ppm of IAA were necessary to achieve 90% inhibition of

TABLE 20

Response of Xanthium to IAA applied under threshold conditions.

Intensity Station	Approx. Intensity	Concentration of IAA in ppm	% of plants flowering
A *	1.8 FC	-	0
B *	0.7	-	0
C *	0.25	-	0
D	0.15	0	20
		1	0
		3	0
		10	0
E	0.05	0	100
		1	100
		3	60
		10	20
F	0.01	0	100
		1	100
		3	100
		10	100

* All treatments and control were vegetative.

flowering when cuttings were used under normal SD conditions. In this experiment carried out at the threshold it is seen that at intensity station D where 20% of the control plants flowered, none of the treated plants flowered. At E where 100% of the controls flowered, only 60% of the plants receiving 3 ppm, and only 20% of the plants receiving 10 ppm IAA flowered. At the lowest intensity, F, all plants flowered regardless of treatment. Thus in the experiment above the response at station F is consistent with the results of Bonner and Thurlow. The response at E, however, shows that at the threshold the plant is approximately 3 to 10 times as sensitive to applied auxin as it is under normal SD conditions such as have been in general use.

The importance of the threshold condition for studying the biochemical nature of the dark process in both SDP and LDP will become more apparent as experiments in the remaining portions of this thesis are presented.

CHAPTER III. THE LOW INTENSITY LIGHT PROCESS

Contents.

I. Introduction.

1) General. Experiments mentioned in the introduction to this part of the thesis and in the previous chapter have shown that floral induction in SDP may be inhibited by a flash of low intensity light given during an otherwise inductive dark period. Data presented in Chapter I have shown light to have another important role in the flowering process of SDP - - that of the production of photosynthate. These two light controlled processes, i.e., the one which interrupts the dark period to prevent flowering and the other which must precede an inductive dark period to insure flowering, differ in two important respects. In the first place there is a thousand-fold difference in the light energy required to consummate the two processes. In the second place the action spectra for the two processes are quite different.

2) Comparison of the energetics of the high and low intensity light processes. The data of Table 10, page 57, show that an average of 3.3×10^4 FCM of light daily are required for the high intensity light process. With soybean, about 10 FCM of light given as a flash near the middle of the dark period seems to be sufficient for complete suppression of the dark reaction (Borthwick and Parker, 1950). Although other plants vary somewhat their requirements are of this same order of magnitude. Table 21 summarizes some of the data

from the literature on the light energies required to inhibit the flowering of SDP and to promote the flowering of LDP.

The data in the table reveal then that the amount of energy required to consummate the low intensity light reaction in Xanthium or soybean is of the order of one thousand times less than is required for carrying out the high intensity light reaction with the same species. Another interesting fact evident in the table is that light energy given as a flash of relatively high intensity is the more effective in bringing about photoperiodic effects than is continuous low intensity light. This apparent discrepancy may, however, be related to the length of treatments involved since in those instances where continuous supplementary light treatments have been given for extended periods of time flowering is achieved by amounts of light which normally are effective only if given as flashes. Thus 54 FCM as continuous light (Claes, 1947) or 24-94 FCM given as a flash (Parker et al., 1950) were both effective in causing flowering in Hyoscyamus.

TABLE 21

Energetics of the low intensity light process in LDP and SDP.

Total Energy in FCM	Method of Application	Name of Plant	Source
SHORT-DAY PLANTS			
420-900	Flash	Kalanchoe	Harder and Bode, 1943
16.8	Continuous	Soybean	Parker et al., 1946
24.0	Continuous	Soybean	Borthwick et al., 1938 b
10.0	Flash	Soybean	Borthwick et al., 1950
150 [a]	Flash (9 hr. night)	Xanthium	Hamner and Bonner, 1938
14.4	Continuous light	Xanthium	Parker et al., 1946
30.0	Flash	Poinsettia	Parker et al., undated
LONG-DAY PLANTS			
9.6 - 28.8	Continuous	Callistephus	Withrow et al., 1936
24-94	1 hr. Flash	Hyoscyamus	Parker et al., 1950
52	Continuous	Hyoscyamus	Claes, 1947
240	Continuous	Hyoscyamus	Parker et al., 1950
27×10^3 [b]	Flash	Phaseolus, Bismarck Pea	Katunskij, 1936
185-750 [c]	Continuous	Wintex Barley	Borthwick et al., 1948
12-50	Flash	Wintex Barley	Borthwick et al., 1948

a The first experiment of its kind with SDP; therefore no minimum determined.
b The first experiment of its kind with LDP; therefore no minimum determined.
c No sharp breaking point in response between flowering and non-flowering.

3) Comparison of action spectra of high and low intensity light processes. The low intensity light reaction differs from the high intensity light reaction in that the former does not appear to be directly related to photosynthesis. This has been established through investigations of the action spectrum of the process. Although numerous workers (cited by Borthwick et al., 1948, in Murneek and Whyte) have shown that red and blue light are both effective in the photoperiodic response it was not until the studies of Parker et al. (1945, 1946, 1950) and Borthwick et al. (1948) that the quantitative relationships of the action spectrum of SD and LD plants became known. They found that the wave lengths which are most effective in causing the low intensity light reaction are those in the red, while blue light is much less effective relative to red light than is the case in photosynthesis. That chlorophyll does not participate in the low intensity light process has also been shown in recent experiments with (Parker et al., 1949, and Borthwick et al., 1951) etiolated and albino plants. With these chlorophyll-free tissues internode elongation(in barley) as well as leaf elongation (in

pea) was inhibited by light with the same action spectrum as that for the low intensity light process of photoperiodism.

From their studies on the photoperiodic action spectrum the Beltsville workers (Borthwick et al., 1948, 1951; Parker et al., 1945, 1946, 1949, and 1950) conclude that the pigment responsible for the absorption of the light energy needed to consummate the low intensity light process may be an open-chain tetrapyrolle. The only pigments whose absorption spectra closely correspond to the photoperiodic action spectrum are the phycocyanins which occur in blue-green algae, but attempts to isolate such pigments from higher plants have failed. Although phycocyanins appear to be the only naturally occurring pigments with the necessary absorption spectrum, the possibility still exists that the pigment may be an as yet unknown porphyrin. Further research is needed to clarify this point.

4) Relation of auxin to the low intensity light process. A knowledge of the nature of the pigment responsible for perception of the photoperiodically active light is essential to a total understanding of the biochemistry of the flowering process. This general ap-

proach is, however, now blocked by the difficulties inherent in determination of the chemical identity of the pigment. A more profitable approach at present appears to be that utilized by Bonner (1949). In these studies Xanthium plants were treated with auxin antagonists under conditions close to the threshold for flowering (continuous supplementary light of 1 FC for 16 hours daily). Thirty-five percent of the plants treated with either TIBA or DCA flowered whereas the controls remained vegetative. When the supplementary light was raised to 100 FC these substances were no longer effective in causing flowering. From these studies Bonner concluded that the low intensity light inhibits flowering by preventing a decrease in the auxin level during the dark period.

II. Reversal of flash inhibition of the dark reaction by anti-auxin application.

1) The problem. One question which arises about the low intensity light process is whether or not the flash of light and continuous light affect the same reaction step in the flowering process. The end effect of the two types of treatment is equivalent, i.e., in both cases flowering is suppressed. There have been, however,

no experiments designed specifically to discover whether the light flash as well as low intensity continuous light may be overcome by application of anti-auxins. The experiments to be described are ones which indicate the identity of the physiological stimuli of the two types of low intensity illumination. They also furnish further evidence that the inhibitory action of the low intensity light reaction is concerned with maintenance of the leaf auxin level.

2) Orientation experiments with Xanthium. A series of orientation experiments were run to find the optimal night length as well as a suitable flash length to use in studies of the low intensity light process. In all cases the plants were seeded at Orlando Road Greenhouse transplanted to 4 inch clay pots and then grown under 20 hours of light daily until the beginning of the experimental period. At this time the plants were, in general, about 24-30 inches high and had from 1 to 3 recently mature leaves. Whenever it was possible the plants were selected for uniformity as to height and leaf number. The experimental treatment invariably consisted of a basic eight hour period of high intensity illumination

plus the amount of supplementary light needed to give the desired day length. The flash of light was given just after the beginning of the second half of the dark period. Following the experimental treatment the plants were removed to LD conditions for an additional 10 days before dissection of the growing points. Table 22 shows the results of several experiments on the effectiveness of various flash lengths on induction. The results are expressed as a ratio of the number of plants flowering to the number of plants treated.

During the time these experiments were run the damage to the plants by smog was often very great and there was always some lack of uniformity in plants from experiment to experiment. This lack of uniformity may account for part of the variability in these experiments (12 hours).

The apparent discrepancy in FCM required for inhibition of the dark reaction between these experiments and those of Parker et al., (1945, 1946) is probably not real. Parker used a single leaf placed perpendicularly to the incident light whereas in the experiments presented above 2-3 mature

TABLE 22

The relationship between the length of flash required to achieve inhibition and the length of the dark period.

Expt. No.	No. of SD	Hours in Dk. pd.	Length of Flash of Light in Minutes 0	5	10	15	20	30
FCM of Light in Flash			0	250	500	750	1000	1500
94	4	8	0/8	0/8	0/8	0/8	0/8	-
96	4	8.5	5/9	0/9	0/9	0/9	0/9	-
95	5	9.5	8/8	6/8	0/7	0/7	0/7	-
90	4	10.0	8/8	-	0/8	0/8	0/8	0/8
91	4	11.0	8/8	-	5/8	5/8	1/8	0/8
92	4	12.0	8/8	-	5/8	4/8	3/8	4/8
93	6	13.0	8/8	-	6/8	5/8	6/8	0/9

leaves were used. It is readily apparent that additional intensity would be required to overcome the shading effects produced by these extra leaves.

The important fact apparent from the data in the table is that there is a positive correlation between the length of the dark period and the intensity of light required for inhibition. Thus with longer night lengths more light energy is needed to secure inhibition.

The data indicate that under these experimental conditions a night length of 9.5-10.0 hours interrupted by a 10 minute flash of light lies very near the threshold for flowering. Unless otherwise stated this combination has been used for the studies on the physiological nature of the light flash.

3) Floral induction in Xanthium with anti-auxins. The most obvious approach to use in the study of the biochemical nature of the light flash reaction is to discover whether the inhibition of flowering caused by the flash can be reversed by the application of various anti-auxins. This approach has been utilized in the following experiments.

Xanthium plants were grown at Orlando Road Greenhouse until they were 4-6 weeks old. All experimental treatments were carried out in Dolk Laboratory in the specially constructed cabinets described in Part I of this thesis. In most cases the anti-auxins were applied to the leaves twice daily as a spray once immediately before removal to the cabinets and again the following morning immediately before removal to the greenhouse. After an experimental treatment of 7 to 14 days the plants were put under LD conditions in the greenhouse for an additional one to two weeks. At the end of this 21 day period the growing points were dissected to determine the stage of floral development. Table 23 summarizes the data from all experiments.

The left half of this table shows the results of a typical experiment while the right half gives a summary of all experiments which have been run. These results show quite conclusively that an anti-auxin is able to reverse the inhibition produced by a flash of light given during an otherwise inductive dark period. These experiments together with those of Bonner (1949)

TABLE 23

Reversal of flash inhibition of the dark process by applied anti-auxins.

Treatment	EXPERIMENT 49				ALL EXPERIMENTS		
		Number of Plants			Number of		% of Plants
	% Flowering	Vegetative	Intermediate	Flowering	Plants	Experiments	Flowering
Flash control	0	10	0	0	62	8	0
Dark control	100	0	0	6	16	3	37
TIBA 300 ppm	40	2	4	4	10	1	40
TIBA 100 ppm	65	6	1	13	75	4	32
TIBA 30 ppm	30	3	4	3	62	2	37
TIBA 10 ppm	50	2	3	5	35	4	26
TIBA 3 ppm	20	5	3	2	20	2	15

* In one experiment in which 3 ppm of 2,4-dichlorophenoxyisobutyric acid was used, 2 of 10 treated plants flowered while all controls remained vegetative.

show also that the effects of the flash of light and of the continuous low intensity light are similar in that they can both be antidoted by anti-auxin. It may be noted that Khudairi and Hamner (1951) have recently found that Xanthium can be caused to flower by treatment (under threshold conditions) with ethylene-chlorohydrin, an agent known to cause destruction of auxin (Michener, 1942).

The results reported here are all consistent with the notion that the important thing governing flowering in SDP is a normal decrease in the auxin level at night. It appears to make little difference whether the effective auxin level is lowered by endogenous enzymatic processes, by auxin antagonists or by straight forward destruction as long as it is lowered.

4) Minimal induction and anti-auxins. Subsequent to the experiments outlined above an attempt was made to confirm the findings of Galston (1943, 1947 with soybean) and Esteves-de-Sousa (1950 with Kalanchoe) that TIBA application to minimally induce SDP causes an increase in the number of flowers formed. The same experimental setup was used as for the above experi-

ments except that the flash of light was reduced to an intensity which permitted some of the control plants to flower. Table 24 summarizes the data of one of seven such experiments.

These results show that in Xanthium anti-auxins given in addition to minimal induction cause an increase in the percent of plants flowering. The greatest difference between control and treated plants is observed at the lower concentrations of anti-auxin. These results are to be contrasted with those presented in the preceding table which shows that about 100 ppm of TIBA is optimal for reversing the light effect and actually causing floral induction. Table 24 shows that several other known anti-auxins are as effective as TIBA in promoting flowering under conditions of minimal induction. Unfortunately there was an insufficient supply of these materials and their effect on flowering in non-induced plants could not be tried.

It is interesting to speculate at this point about the nature of the response demonstrated in the last experiment.

TABLE 24

Anti-auxins plus minimal induction in Xanthium. X-74.

Treatment	No. Veg.	No. Int.	No. Flr.	% Flr.
Flash control	7	1	2	20
TIBA 100 ppm	6	1	3	30
TIBA 30 ppm	7	0	3	30
TIBA 10 ppm	5	0	5	50
TIBA 3 ppm	4	1	5	50
TIBA 1 ppm	1	2	7	70
2-NIBA 31.5 ppm	4	2	4	40
1-NIBA 30 ppm	3	2	5	50
PIBA 33 ppm	1	0	8	89
2,4-DPIBA 100 ppm	5	1	4	40
2,4-DPIBA 30 ppm	4	0	6	60
2,4-DPIBA 10 ppm	4	2	4	40

Why should minimal induction plus an anti-auxin be effective in increasing the number of flowers in soybean (Galston, 1943, 1947) and in increasing the percent of plants flowering in Xanthium? Tables 16 and 17 of Chapter II show that auxin application in the terminal portion of an inductive dark period causes some inhibition of flowering. The interpretation given these data was that the substance which is formed during the dark period must be fixed in some manner by high intensity light before it is stable to high auxin levels. If this is true then a possible role of anti-auxin in minimal induction might lie in preventing destruction or inactivation of the dark formed substance by auxin until it has been fixed by the high intensity light. Thus in the control group 80% of the plants remained vegetative. According to the present hypothesis this would be because the dark produced, auxin-labile substance was destroyed or inactivated in the presence of auxin and light before it could be fixed. In the treated plants, on the other hand, auxin, although present, would have been prevented from acting by the anti-auxin and more of the plants would then be able to initiate floral primordia. This is but a working hypothesis but it does appear to be in accord with the data presently available.

III. Induction of flowering in Chenopodium Amaranticolor with TIBA applied under threshold conditions.

Induction of flowering in SDP by applied anti-auxins has previously been demonstrated only in Xanthium. It was therefore desirable to determine if the phenomenon might be of more general occurrence. Chenopodium Amaranticolor was used to investigate this problem.

Six weeks old Chenopodium plants which had been grown under LD were made into cuttings and placed in individual test tubes containing different concentrations of TIBA. The plants were grown for two additional days under LD conditions to permit absorption of part of the TIBA solution. At the end of the two day treatment the plants were given five photoinductive cycles consisting of daily periods of 8 hours of high intensity light and 16 hours under the threshold conditions outlined in Section 4 of Chapter II, i.e., continuous low intensity light. The plants were moved back to LD conditions for 20 days and then dissected. The results are shown in Table 25.

TABLE 25

Floral induction in Chenopodium Amaranticolor by TIBA. C-20. Results shown as percent of plants flowering. 10 plants at first 4 intensities; 5 plants at each of last two intensities.

Chemical Treatment	Approximate Light Energies in FCM					
	1728	480	192	96	48	9.6
Water	0	0	0	30	60	100
TIBA 3 mg./l	20	30	30	60	80	80
TIBA 10 mg./l	40	60	70	50	100	80
TIBA 30 mg./l *	20	0	0	10	40	60

* All plants still living at this concentration had flowered but most of plants were dead.

It is clear that TIBA causes flowering of Chenopodium plants under conditions in which the control plants remain vegetative as well as under conditions in which the plants are minimally induced. Thus at the three highest intensities where none of the controls flowered from 20 to 70 percent of the treated plants flowered. At the next two intensities where part of the controls flowered, more of the treated plants flowered. At the lowest intensity where all the control plants flowered there does not appear to be a significant difference between treated and non-treated plants. The results of this experiment with Chenopodium point out again the value of this particular type of threshold experiment for studying the biochemical changes which take place during inductive treatment.

CHAPTER IV. TRANSLOCATION OF THE FLORAL STIMULUS

Contents.

I. Introduction.

The previous chapters in this portion of the thesis have been concerned primarily with a discussion of the physiology and biochemistry of the production of the flowering stimulus in the leaves of SDP. At present the final production of the floral stimulus in the leaf as a result of the interplay of light and dark in a photoperiodically sensitive plant such as cocklebur can be studied only by the effectivenss of such an induced leaf in bringing about floral initiation in a bud. The production and the nature of the stimulus can be studied then only after the translocation of the stimulus from the leaves to the buds.

The characteristics of this transport have been investigated in detail by numerous workers. Perhaps the most important fact which these investigations reveal is that the flowering stimulus travels only in living tissue and usually along the pathways which

would be expected of a substance moving with the photosynthetic stream from supplying to receiving organs. The stimulus can pass readily from an intact leaf to a bud. It passes less readily from a leaf in which the vascular bundles have been cut, and in which the transport is then restricted to the petiolar parenchyma (Chailahyan, 1940 a). All of the commonly used methods of interrupting transport in the phloem greatly retard or completely prevent flowering. Thus flowering can be prevented by girdling the stem by the removal of a ring of bark (Chailahyan, 1936; Withrow et al., 1943), girdling the petiole by steaming (Withrow et al., 1943), scalding (Galston, 1943), or by localized cooling (Chailahyan, 1941; Borthwick et al., 1941). Inactivation of the cells of the stem by ether or chloroform also prevents transport of the stimulus (Chailahyan, 1941).

Further indication that living cells are involved in transport has been obtained from grafting experiments. The stimulus is readily transmitted across grafts provided only that a tissue union of even the most tenuous nature is made but interposition of impervious lens paper or other membranes (Melchers et al., 1948; Withrow

et al., 1943) or of water gaps (Galston, 1943) completely stops the transport. The stimulus moves quite readily from a leafy shoot to a non-leafy or darkened shoot (Hamner and Bonner, 1938; Stout, 1945), but it moves much less readily from a leafy shoot into another leafy shoot. Thus these results indicate that any treatment which disturbs the normal transport of photosynthate also interferes with transport of the flowering stimulus.

An interesting fact which has been noted many times in the literature is one related to a naturally occurring inhibition of the transport of the stimulus past leaves maintained under photoperiods unfavorable to photoinduction. Garner and Allard (1923, 1925) appear to have been the first to observe this localized effect of photoperiodic induction. Thus they found that when one branch of a two-branched Cosmos plant was induced to flower by SD, the LD branch remained vegetative for many months. It was not until after the hormonal theory of floral initiation had been developed that the effect of LD leaves on the flowering of SD plants came to be considered as an inhibition. An inhibitory effect of LD leaves on the flowering of SD plants has since been observed in many

species. In experiments in which each of the branches of two branched Xanthium plants were kept in different photoperiods, Hamner and Bonner (1938) found that the flowering stimulus would not move from the SD branch (donor) to the LD branch (receptor) unless the receptor was a) left intact, b) completely defoliated, or c) stripped of its mature leaves. Flowering did not occur on the receptor when only mature leaves and the growing point were allowed to remain. Borthwick and Parker (1938 b) confirmed these findings with two-branched soybeans. Harder and coworkers (1948 a, b) and van Senden (1951) confirmed these results in single branched plants of Kalanchoe blossfeldiana. In these experiments they found an almost direct relationship between the number of LD leaves located directly above (on the same orthostichy) the SD leaves and the number of days to visible buds. Thus when the ratio of LD/SD leaves was as great as 14/1 the plants did not flower during the experimental period, whereas with a ratio of 10/1 flowering occurred after 48 days. When the ratio was reduced to 7/1 it took 40 days, at 4/1 only 29 days were required, while the controls with but a single SD leaf flowered af-

ter 21 days. It was further shown that the light intensity given the LD leaves during the 14 hour supplementary light period was an important consideration. Thus if the intensity fell to as little as 1-2 FC there was very little inhibition. LD leaves located on a different orthostichy from the SD leaves gave essentially no inhibition so that an inflorescence on the same plant could be half flowering and half vegetative.

Chailahyan (1946 a, b) reported similar results in experiments with Perilla. Thus whenever a LD leaf was placed between the perceiving leaf and the responding bud there was a delay in flowering. This delay was reduced by shading the LD leaf (Chailahyan, 1947) with layers of paper which reduced the light intensity. When three layers were placed above the LD leaf this leaf no longer gave an inhibition.

More direct experiments have been carried out with single leaf blades. When the base of the leaf is placed in LD and the tip in SD flowering is delayed, (Chailahyan, 1945; Harder et al., 1949; van Senden, 1951) when the SD portion is at the base there is on the contrary no delay. When darkness is given to one-half the leaf in any combination with SD or when the leaf is divided

longitudinally and one half given LD, the other half SD, there is no inhibition of flowering.

II. Experiment with Chenopodium Amaranticolor.

All of the above data seem to be in accord with the view that the transport of florigen takes place in the phloem together with the photosynthate, and that any condition which interrupts the natural flow of the photosynthate from perceiving leaf to responding bud inhibits or markedly delays flowering. The experiment cited below gives more evidence to support this view and extends the data to include another SD plant.

Chenopodium plants were grown in the Phytotron in 4 x 4 x 8 inch plastic containers under a 16 hour day until the beginning of the experimental period. Special boxes were constructed in order that one branch could be given SD while the other was maintained on LD. All plants were given a basic 8 hour period of high intensity light at 26°C. In each case the donor (SD branch) was covered for the remaining 16 hours whereas the receptor (LD branch) received light of 600-800 FC. The plants were divided into two principal groups on the basis of

the treatment of the receptor branch. All axillaries were removed from the plants every third or fourth day during the experimental period or until the plants had flowered. The experiment was terminated after 39 days and all plants which had not flowered by that time were dissected to determine their flowering status. Table 26 presents the results of this experiment.

The results in the table show once again that there is a direct relationship between the number of mature leaves on the receptor and the degree of delay in flowering. The table also shows an additional fact, namely that removal of the growth centers on the SD branch causes a faster translocation of the stimulus to the LD branch. Thus when the donor is left intact and three mature leaves are left on the receptor 35 days are required for the receptor to flower, if the growing points are removed from the donor only 28 days are required. These results confirm those of Chailahyan (1936 a, b, c; 1946, a, b) and those of Hamner and Bonner (1938).

TABLE 26

Translocation of the flowering stimulus in Chenopodium Amaranticolor. C-10. All donor plants flowered by the 11th day.

Treatment of Donor	Treatment of Receptor	Response of Receptor Number of plants		Days to Bud
		Flowering	Vegetative	
Left intact	Left intact [a]	3	1	39 *
Left intact	Defoliated to 5 mature leaves	0	4	--
Left intact	Defoliated to 3 mature leaves	4	0	35.2
Left intact	Defoliated to 1 mature leaf	4	0	28.0
Left intact	Defoliated to terminal bud	4	0	23.5
Growing areas removed, then defoliated to 5-8 mature leaves	Left intact [b]	4	0	39.0 *
" "	Defoliated to 5 mature leaves	1	3	39.0 *
" "	Defoliated to 3 mature leaves	2	2 tips dead	28.0
" "	Defoliated to 1 mature leaf	4	0	24.5
" "	Defoliated to terminal bud	4	0	21.0

* Determined by dissection at end of experiment.

a Ratio of LD/SD leaves was 3.2. Mature leaves only considered.

b Ratio of LD/SD leaves was 8.25. Mature leaves only considered.

Figures 11 a, b, c, and d show visually the effect of removing the growth centers on the donor branch and on the flowering of the receptor. Figures 11 a and b show the method of treatment to secure LD and SD on the two branches, while Figures 11 c and d show typical plants from the principal experimental groups.

Figure 11. Effect of defoliation of receptor and donor on translocation of the floral stimulus.

Figure 11 a. Chenopodium plants showing setup for giving SD treatment to donor branch. Growing points on donors removed. Receptor on left with 3 mature leaves. Receptor on right defoliated.

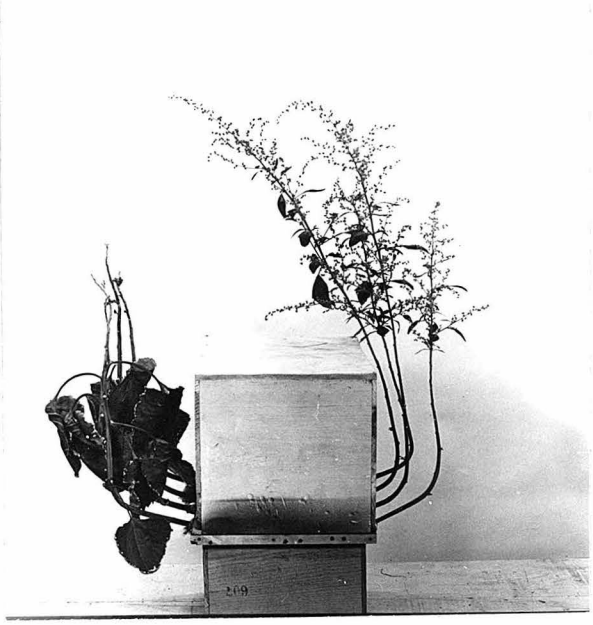


Figure 11 b. Chenopodium plants showing setup for giving high intensity light to donor branch. Receptors both intact. Donor on left with growing points removed. Donor on right intact.

Figure 11 c.
Donor intact.

Figure 11 d.
Growth centers of donor removed.

Figures 11 c, d. The difference between c and d lies only in that the donors in c have been left intact, whereas those in d have had all growing points removed. The receptors from left to right in C have been trimmed to 1) 3 leaves, 2) 1 leaf, 3) completely defoliated. The receptors from left to right have been 1) completely defoliated, 2) trimmed to 1 eaf or 3) trimmed to 3 leaves. Note the difference in development of flowering shoots on the receptors first as related to treatment of donors and then as related to foliation of the receptor.

III. Suggestions Concerning the Mechanism of Transport.

The experiment reported above has shown that the inhibitory effect of LD leaves on the flowering of the receptor branch of SDP is directly related to the number of leaves on the LD branch. All of these experiments on transport can without exception be interpreted in terms of the hypothesis that the inhibition produced by LD leaves on the flowering of SD plants is directly related to an interference with the transport of sugars between SD leaves and the buds. The experiments of Chailahyan (1947) suggest this as the correct interpretation. In these experiments Perilla plants were defoliated to two mature leaves and these were treated in several ways. If a single leaf was exposed to SD and the other removed the plant flowered in 24 days. If a LD leaf were placed opposite this SD leaf 33 days were required for flowering. If the blade of this LD leaf was removed and sugar fed through its petiole, 31 days were required for flowering. When a LD leaf was placed above the SD leaf, 51 days were required and if this leaf were removed and sugar fed through the petiole, 49 days were required. Since applied sugar is able to inhibit flowering as well as mature leaves it is quite clear that sugars produced as a result of photosynthesis in the LD leaf

are able to cause a delay in flowering. Although Thurlow (1948) was not able to repeat the experiment of Chailahyan by sugar application to Xanthium, there is little basis for suspecting that it would not be possible to show such an inhibition by a suitable technique.

Chailahyan's conclusion was:

> "There exist no special substances formed in the plants for inhibiting flowering. The inhibitory action of LD leaves in SD plants is based on the action of assimilates, the sugars, produced in these leaves and transported to the stem buds."

Even though this statement is moderately clear, Chailahyan's view that LD leaves only acted by interrupting the transport stream from the SD leaves is still not generally accepted or appreciated, (Harder, 1948; van Senden, 1951). At least part of this lack of appreciation of the concept is probably caused by the absence of any clear idea as to the kinetics of transport which would be expected on this basis.

Let us consider Chailahyan's experiment in more detail. In every instance one leaf was given SD. With this leaf alone on the plants flowering occurred in 24 days, with a LD leaf

opposite or above the SD leaf there was respectively a 9 or a 27 day delay.

The interpretation of these experiments according to the hypothesis outlined above would be as follows. It may be assumed that the growing point demands only a given amount of assimilate regardless of the source. When the SD leaf is the only one present, all of this assimilate comes from it and the flowering hormone is carried passively in the stream flowing to the growing tip. In the case where one of the leaves is on an 8 hour day (SD) and the other on a 16 hour day (LD) the demand for assimilate by the tip remains the same but since the LD leaf can make more photosynthate than the SD leaf, more of the requirement of the tip will be satisfied by the LD and less by the SD leaf.

This reduced transport from the SD leaf reduces the amount of hormone flowing from this leaf and consequently there is a delay in flowering. The case in which the LD leaf is located immediately above the SD leaf is even clearer in this respect. Here not only is the LD leaf synthesizing more of the material

needed for the growth of the tip, but it is closer to the growing point. Assimilate flowing from the LD leaf will satisfy the requirements of the bud so nearly completely that materials from the SD leaf would reach the tip slowly if at all. The experiment of Harder et al. (1949) which shows that a shoot located at the base of the plant will flower under the above conditions whereas the growing tip will not flower support this interpretation. The experiments of Hamner and Bonner (1938) with two-branched plants also fit this scheme. When receptors were left intact the demand for assimilates by young expanding leaves which were not photosynthesizing sufficiently to supply their own needs added to the needs of the growing tip caused a greater demand for sugars than could be supplied by the mature LD leaves on that branch. An assimilate flow was therefore set up between the SD branch and the tip on the receptor. Flowering hormone could then travel with this flow. When the young expanding leaves were removed, the buds by themselves did not exert such a large demand and during the two weeks period of the experiment none of the assimilate flowed from the SD branch to the tip

and as a result transport of the flowering hormone did not occur. In recent experiments with Xanthium (experiments which extended for much longer periods than did those of Hamner and Bonner) Lincoln and Raven (1951) demonstrated a quantitative relationship between the number of LD leaves on the receptor branch and the degree of inhibition of flowering. These results clarify the transport question in Xanthium.

The one additional fact which must be established before the interpretation outlined above can be assumed to be entirely correct is to show that the flow of assimilates from the SD leaf to the growing point is correlated with the flowering behavior of the receptor. The best method of approach to this problem would appear to be by the use of indicators which would travel with the assimilate stream. The most desirable indicator would of course be the flowering hormone itself, tagged with some radioactive element to make it easily identifiable. Since the hormone is not yet chemically known

a more practical approach appears to consist of following the transport of other materials in the phloem. Thus sucrose transport might be followed with radioactive sucrose. The author has completed a preliminary experiment in which 2,4-D was used as an indicator but this experiment was unsuccessful presumably because of metabolism of the 2,4-D prior to its entry into the receptor branch.

CHAPTER V. GENERAL SUMMARY AND PROPOSALS CONCERNING THE NATURE OF THE PHOTOPERIODIC MECHANISM OF SHORT-DAY PLANTS, PARTICULARLY THE DARK PROCESS.

Contents:

I. Brief review of basic facts.

In the previous sections the flowering of SDP has been discussed on the basis of the individual partial processes which make up the photoperiodic response of this group of plants. The author's contributions to a better understanding of the flowering response of SDP have been discussed in the chapters related to the individual partial processes. Let us review briefly the facts as they appear now and try to present an integrated picture of the photoperiodic mechanism of SDP.

a) The high intensity light process. It has been shown in Chapter I that SDP have a high intensity light requirement which can be satisfied in most cases by approximately 10^4 FCM of light. This light, however, must be given in the presence of CO_2 if induction is to occur as the result of a subsequent dark period. Thus the data imply that the high intensity light process is directly concerned with the photosynthetic production of a respiratory substrate. That this may actually be the case is shown by the author's experiments in which the high intensity light process in Xanthium has been replaced by the application of sugars and Krebs cycle acids to the leaves. It would be of interest to know whether these substrates

are utilized solely for the generation of high energy phosphate or whether the carbon skeletons of the sugars and acids are themselves involved directly in a synthesis of flowering hormone. We do not at present have enough data to choose between these two alternatives.

b) The dark process. Chapter II has been concerned with the processes which take place during the dark period. Evidence in the literature had indicated that the feature controlling the dark process might be the auxin level. Thus, auxin given before the dark period inhibited floral initiation and this inhibition could be reversed by simultaneous applications of anti-auxins. The author's work has confirmed the auxin inhibition experiments in Xanthium and has extended them to another SDP, Chenopodium. This work has also shown that certain other substances have the ability to prevent floral initiation when properly applied to the plant. These substances are all known to act as substrates for the peroxidase enzymes. They may conceivably act as substrates for the peroxidase component of IAA oxidase to create a sparing action on IAA. It has further been shown in both Xanthium and Chenopodium

that the inductive substance (or substances) formed during the dark reaction is destroyed or inactivated by auxin application even after dark periods longer than the critical, but that after a suitable period of high intensity light the substance (or substances) becomes stable to auxin.

c) The low intensity light process. The literature reveals that the inhibitory nature of the continuous low intensity light in Xanthium is related to maintenance of the auxin level at a high value since the plants may be made to flower by anti-auxin application. Experiments of the author extend this result to another plant, Chenopodium. Other experiments have indicated the similarity of the two basic types of low intensity light treatments (continuous and flash) since the effects of both are reversed by anti-auxin. The list of anti-auxins known to be effective in floral induction has been extended to include a group of isobutyric acid analogs of the auxins. Other experiments presented in this thesis have confirmed the reported effect of anti-auxins in increasing the intensity of flowering when given in addition to minimal induction.

II. Previous hypotheses to explain the photoperiodic mechanism in SDP.

Several hypotheses have previously been advanced to explain the processes involved in the floral initiation of SDP. These have in common one serious drawback - - namely, all have postulated a series of formal step-wise progressions or reactions in the photoperiodic response but none has made definite suggestions as to the chemical nature of the various substances which have been supposed to participate in these reactions. This has not been the fault of the people who have made the hypotheses, for it is only in the last two to four years that the biochemistry of any one of the partial processes of photoperiodism has become well enough understood to permit an informed speculation as to the nature of the reactions which compose them.

Previous theories have all recognized the high intensity light process, the dark process and the low intensity light process, and each has been referred to with a symbol. Thus Hamner (1940, 1942, 1948) has used A to represent the substances formed in the high intensity light reaction, B for the substances formed in the dark and C, for the summation or the resultant of these two changes.

Harder and Bode (1943) postulate a different scheme. According to these authors, two factors, L and X, must be present in the leaf for induction to occur. L is a light produced substance, X is produced in both light and dark but is sensitive to high intensity light prior to its transformation to a light stable substance Z. This transformation is mediated by photosynthesis. Since X is light sensitive, it is only after a long dark period that sufficient X is present to allow some fixation into Z by photosynthate before all available X is destroyed in a subsequent light period. Z reacts with L to form H, the flower hormone or a precursor of it. H is also light sensitive but less so than X. The scheme of Harder and Bode and of Hamner have been synthesized into still another scheme by Gregory (1948). Gregory uses Hamner's symbols but proposes that the reaction A to B is reversed by light if B is still in the leaf. Thus Gregory proposes that B is unstable only in the leaf but is stable outside the leaf where it is transformed to C, a light stable flowering hormone. According to his hypothesis the critical day length is directly governed by the time required for B to be translocated out of the leaf. Gregory's

proposal concerning the lability of B in the leaf seems unlikely since Lona (1949 c) has shown that a single leaf of Perilla, which has been induced by SD while detached from the plant, will cause flowering when grafted to a non-induced plant kept in LD. The substance which is produced in the dark, then must be stable to light while still in the leaf. As will be pointed out later in the discussion, other factors such as a high auxin level may alter this supposition. For these reasons the critical day length must be related to some factor other than the time for translocation of florigen out of the leaf.

The most comprehensive scheme yet proposed is that of van de Sande Bakhuyzen (1952). His is a synthesis of the schemes of Harder and Bode (1943), Hamner (1940, 1942, 1948) and Gregory (1948). The essence of his scheme is that A is produced only in light, B is produced all the time but is light sensitive. A and B combine stoichiometrically to form AB which is also light sensitive. One mole of AB combines with one more mole of B to form BAB which is light stable and is identical with florigen. In unfavorable photoperiods B, which arises from P by a series of

reactions, is diverted to LFS, a leaf forming substance, and no flowering occurs.

III. Proposals concerning the nature of the dark process.

1) Introduction. The photoperiodic mechanisms which have been suggested have been useful in stimulating thought on the part of workers in the field. All have, however, the disadvantage that they cannot be readily tested. More concrete proposals are needed if a better analysis of the photoperiodic response is to be achieved. The proposals below are intended only as supplements to the more general schemes; supplements expressed in more specific terms. These proposals must of necessity still be quite tentative and are admittedly not always based on all the evidence which might be desired. They are however of such a nature as to permit experimental test.

The experiments of Bonner and of the author have shown that the substance (or substances) formed in the high intensity light process can be completely replaced by sugars or by Krebs cycle acids. In this process substrates necessary for the maintenance of normal leaf activity during the subsequent dark

period appear to be produced. There seems to be no reason then, at the present time, for assuming that the A of Hamner, Gregory, or van de Sande Bakhuyzen, or Harder and Bode's L is anything different from photosynthate produced in the high intensity light process.

2) Statement of hypothesis. What postulates may be made about the dark process? Data have been presented which make it appear that both the dark process and the low intensity light inhibition of the dark process are intimately associated with the auxin metabolism of the leaf. The dark process is inhibited by applied auxin. Both this inhibition and the inhibition caused by the low intensity light process are prevented by applied anti-auxin. These two facts put together suggest at once the possibility that the feature controlling photoperiodic induction may be a decrease of auxin level in the leaf during a long uninterrupted dark period. Thus the normal decrease of auxin during a long uninterrupted dark period would be retarded or negated by applied auxin. Similarly we might suspect that the low intensity light reaction may have to do with

the inhibition of the process by which auxin level in the leaf is normally caused to fall during the dark period. According to this hypothesis the action of an auxin antagonist on flowering would be due to a lowering of the effective auxin level in the leaf to such a point that flowering might be achieved even in the presence of amounts of light which normally would inhibit the dark process.

3) Kinetics of the dark process in continuous dark. If this working hypothesis is correct, how may the kinetics of the processes which take place during the dark period be envisaged? How can the known facts be fitted into a coherent picture?

In the first place it is known that a high intensity light process must precede the dark period. Although the principal need for this light process appears to be related to requirements for the assimilates which are formed, undoubtedly many other things take place which are not necessarily beneficial to flowering. Among these is probably an increase in the auxin level in the leaves. Thus when a dark period begins, at least two things are

present in relatively great amounts in the leaf - - sugar, or its storage form, starch, and auxin. According to this hypothesis we should expect a decrease in the effective leaf auxin level as the dark period progresses. There are at least two possible kinds of enzymatic mechanisms which might mediate such a decrease, 1) an inhibition or destruction of the auxin producing system, or 2) an auxin destroying or inactivating system.

According to the first hypothesis it might be, for example, that some enzyme in the chain of reactions responsible for auxin production is inhibited by dark and/or activated by light. Thus when the dark period begins, auxin production would stop and after a finite time the normal utilization of auxin would result in a decrease of auxin to a level which would permit synthesis of the flowering hormone or a precursor of it.

According to the second hypothesis there would be an actual enzyme controlled destruction or inactivation of auxin. Destruction of IAA could be mediated, for example, by IAA oxidase, which, as we have seen in Chapter II, is present in several plants. If a destruction or inactivation of auxin were concerned,

there are at least two possible kinds of enzyme systems which could be involved: 1) The system could be insensitive to light and there would be a constant rate of destruction in light or dark. The rate of auxin decrease would then be primarily a function of the auxin producing system mentioned above. 2) The system might, on the other hand, be inactivated in some way by light so that auxin would be made ineffective or destroyed only during the dark period. This system could operate a) by the destruction of auxin which would be precluded or lessened in the light, b) by the production of an auxin antagonist, c) or by the production of a substance which combines with auxin.

It is quite possible that both the auxin destroying and the auxin producing systems are involved in the total picture, but it is much less likely that both continue full activity during the dark period. Purely as a working hypothesis the author would like to suggest that the auxin destroying or inactivating mechanism may be the more important and that it is the one with which we are concerned in the reactions involved in the dark process. The reasons for this choice will become more apparent later in the discussion (see "Flashing Light").

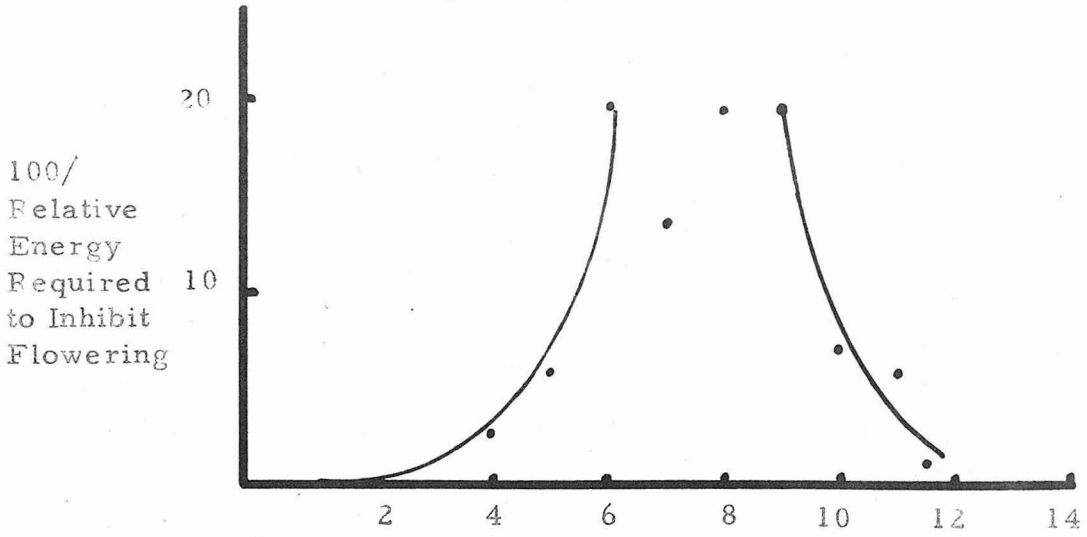
If an auxin destroying mechanism is the effective agent during the dark period, what kind of properties would we expect of this enzyme system? The assumption which must first be made is that the auxin destroying or inactivating system (either some part of the enzyme or its reaction product) is, in SDP, destroyed or inactivated *in vivo* by red light. On this basis the processes which take place during an uninterrupted dark period may be visualized as shown in Figure 12.

At the beginning of the dark period the auxin level is high and no functional IAA destroying or inactivating enzyme or its reaction product are present in the leaf because of inactivation of the system during the previous light period. As the dark period progresses a synthesis of reaction product or reactivation of the enzyme takes place, slowly at first, but much more rapidly as the dark period progresses. Finally, after an appropriate interval this synthesis or reactivation levels off or ceases completely. With this increase in enzyme there must be a concurrent decrease in the effective IAA concentration. The concentration of IAA falls slowly at first and then very rapidly so that at some time before the critical night length is reached the effective auxin has fallen to or below its

critical level. Once the auxin has reached this critical level synthesis of florigen or precursors to florigen begins. This synthesis continues in the dark until the necessary assimilates or precursors have been depleted. It is possible of course that synthesis of florigen may continue in the light for sometime since such a possibility is not excluded. If the dark period is brought to an end and the plants are moved into the light the florigen or florigen precursors formed during the dark period are changed in some way during the first few hours of light so that they become stable to high concentrations of auxin.

4) Kinetics in flashing light.

a) Single flash. Under the present hypothesis what would be the expected sequence of events when the dark period is interrupted by a flash or flashes of light. It must be assumed that the same conditions prevail at the beginning of the dark period in this case as in the former case. The sequence of events, then, would be the same up to the time of the light flash, i.e., activity of the IAA destroying or inactivating system would increase and the effective auxin level would decrease. By the time of the flash the enzyme or its product would have reached some finite level.



Data taken from Parker, et al., 1946 for inhibition of flowering in Soybean given 14 hour night.

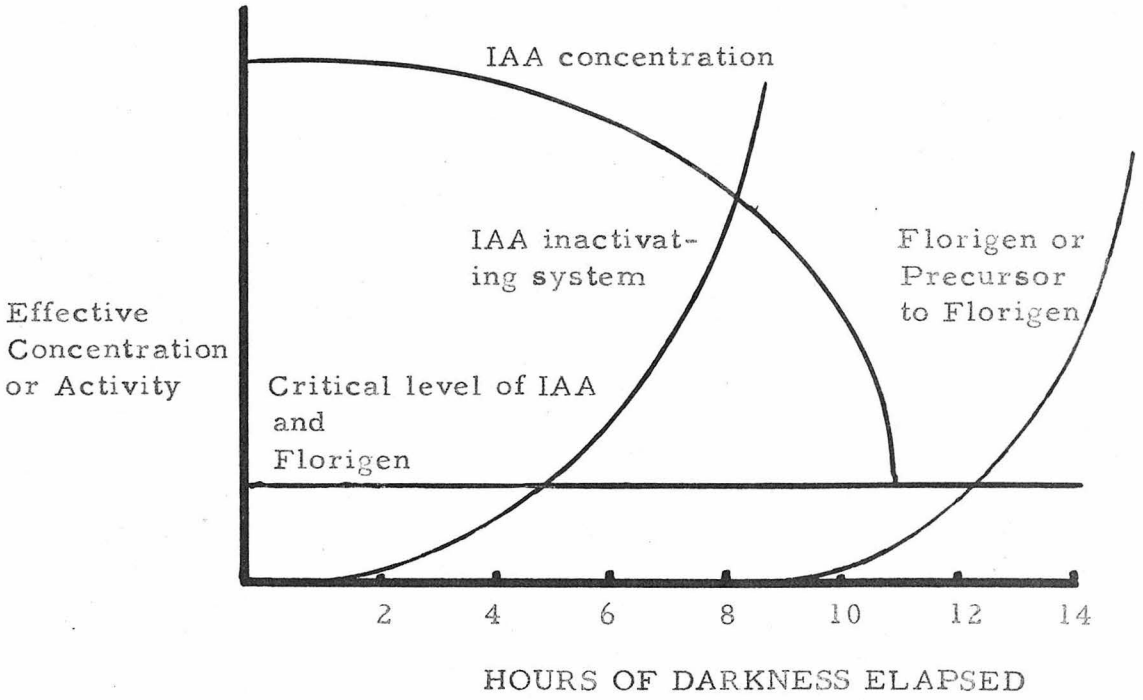


Figure 12. Kinetics of the Dark Process.

In the arbitrary example of Figure 12 it is shown as attaining 5 per cent of its final level after five hours of darkness. If a flash of red light of sufficient intensity were given at this instant the functional level of the system would fall to zero, the level which it had at the beginning of the dark period. When the light would go off a resynthesis or reactivation would begin and again this synthesis would proceed slowly at first and then progress more rapidly. What would have happened to the auxin level in the meantime? At the end of five hours of darkness the level would have dropped, say, 20 per cent. We assume that before any significant synthesis of florigen can occur the auxin level must reach some minimum threshold value, say 25 per cent of the level which is present at the end of a high intensity light period. If the light flash caused a destruction or inactivation of the IAA destroying system, the auxin level would remain essentially at this 80 per cent level until reactivation of the system had occurred. If it is assumed that another 20 per cent of the auxin would be destroyed in the next 5 hours, this would still leave the level some 30 or 40 per cent above the critical and thus no significant synthesis of florigen would occur. The experiments of Parker

et al., (1945, 1946) and those of Harder et al., (1943, 1944) show that if the flash is given near the middle of the dark period it is effective at lower energy levels than when given either near the beginning or near the end of the dark period. These two observations can be neatly fitted into the present scheme. Thus if the flash were given near the beginning of the dark period the IAA destroying system would be inactivated, but since a relatively long dark period would still remain there would be sufficient time for a significant reactivation of the systems. The IAA level might still fall to or even slightly below the critical and the plants would be minimally induced. One may ask, however, why greater amounts of light should be required when given during the early part of the dark period than when given near the middle? If the light acts only on the enzyme then less light should be required to destroy the small amount present after only two to three hours. In this case one should expect a linear relationship between the amount of light required to inhibit flowering and the amount of darkness which has elapsed before the flash of light. The facts will not support this view. It seems therefore necessary to postulate the existence of precursors to the IAA destroying system, precursors which are also light sensitive.

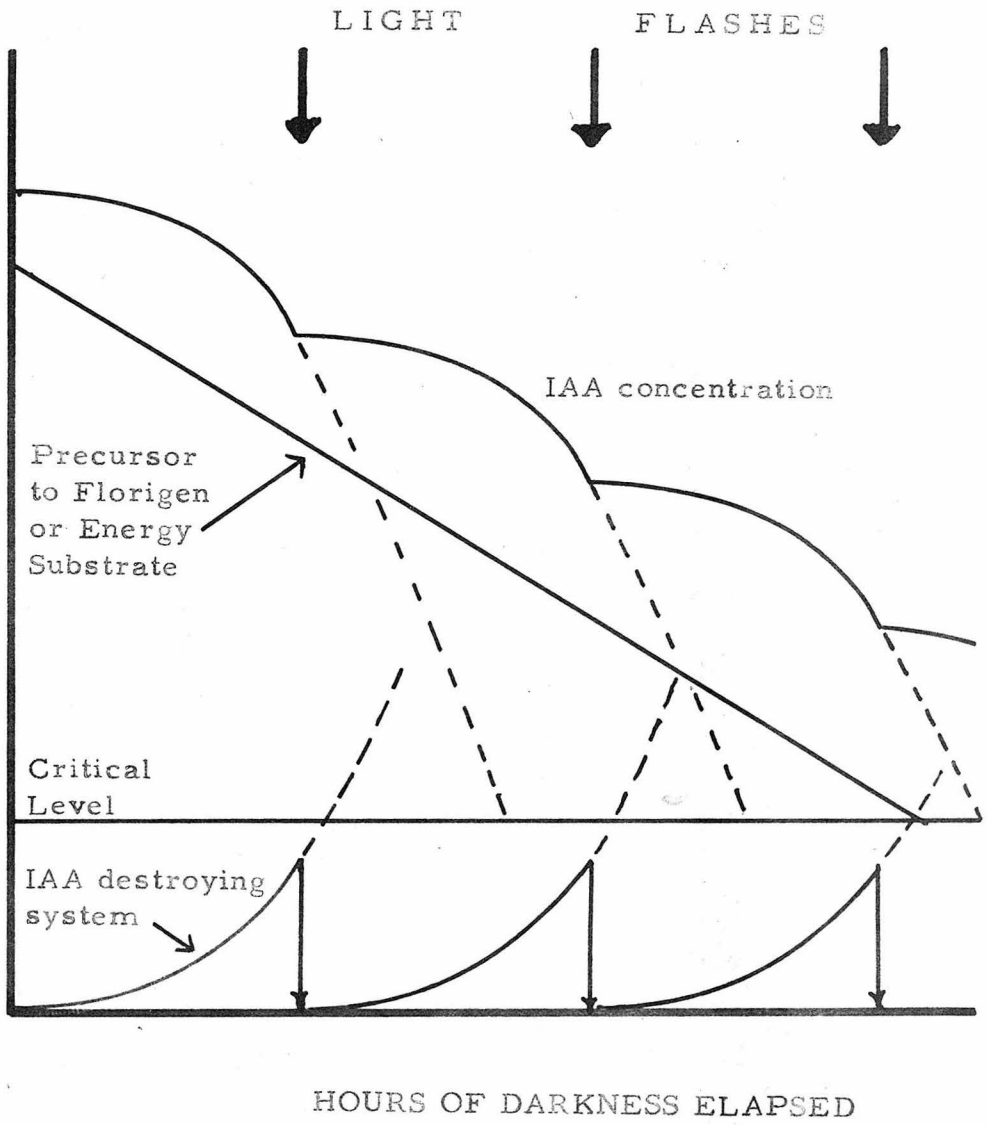
We also need to assume these precursors to be less light sensitive than the enzyme itself. These assumptions would explain the need for more light during the early part of the dark period.

If the flash were given near the end of the dark period when the concentration of enzyme is relatively high, more light energy would be required for its total destruction or inactivation. Since the IAA level would have fallen to very near the critical before this flash of light it would be necessary to destroy all of the enzyme; otherwise enough additional IAA would be inactivated to permit some synthesis of florigen or its precursors.

b) Several flashes. The experiments with several flashes of light separated by intervals of darkness which were used for studying the high intensity light process could also be explained by the present hypothesis. In this instance there would be a destruction of the enzyme at each light flash followed by resynthesis or reactivation of the system. Since it has been shown by Hamner (1940) that Xanthium will not flower after three of these cycles even if given a night longer than the critical, it may be assumed that some precursor of the IAA destroying enzyme or of florigen, or energy needed for synthesis of this enzyme or of florigen becomes limiting with time. That one of these alternatives

may be the correct one has been shown by the sugar feeding experiments discussed in connection with the high intensity light process. It was found that the plants could be made to form flowers following this sequence of flashes of light and periods of darkness if sugar were supplied to the leaves. In either of the cases mentioned above the processes which would take place as a result of one or several flashes of light may be visualized as shown in Figure 13. This figure shows that if a single flash of light were given the enzyme level or activity would approach zero and then begin to rise again after the flash has ended. Concurrently with this rise the auxin level would begin to fall and if the dark period following the flash were sufficiently long, the auxin level would fall below the critical and the plants would become induced. With each succeeding flash this process would be repeated until after 3 flashes the plants would not become induced even if given a night longer than the critical. Some factor, therefore, must have become limiting. This factor could be energy substrate, or it could be a precursor to the enzyme or to florigen itself. If the factor were a precursor to the enzyme responsible for the destruction

Figure 13. Interruption of the dark period with flashes of white light. Solid lines represent what would happen in flashing light system. Dotted extensions of these lines represent what would normally occur if the flashes were not given.



of IAA, then TIBA application should be able to cause flowering. If energy substrate or florigen precursor were the limiting factor then sugar or other like substance would be able to remove the limitation and allow the process to continue to the point where induction would occur. Although TIBA has never been tried in this system, it is known from experiments reported earlier that sugar and Krebs cycle acids are effective. It is likely therefore that the condition which prevents flowering after 3 flashes of light is not high leaf auxin but a lack of florigen precursor or the absence of substrate to produce the necessary energy for florigen synthesis. Unfortunately sucrose addition could also be supplying energy or carbon chain for enzyme or cofactor synthesis, hence the TIBA experiment is necessary.

In Chapter III it was shown that anti-auxins were able to reverse the inhibitory nature of the low intensity light reaction. The mechanism of anti-auxin action in the proposed scheme could be through its effectiveness in blocking the action of IAA destruction or inactivation of florigen or florigen precursor by lowering the effective auxin level. Of course, it is possible that the anti-auxins act to protect the IAA inactivating system from being destroyed or

inactivated by light. This is unlikely, however, since in this event the anti-auxin would presumably not be in its usual role of preventing auxin action.

The view that the anti-auxin is acting to protect florigen is borne out by the experiments of Bonner and Thurlow (1949) which show that anti-auxins are able to reverse the inhibition of auxins applied during an otherwise inductive dark period. The experiments described in Chapter II in which peroxidase substrates were shown to cause inhibition of flowering might be explained on the basis that they keep auxin level artificially high by competing with IAA for a site on the auxin destroying enzyme.

This present hypothesis as to the nature of the dark process in SDP rests on two basic assumptions which have not yet been clearly established. In the first place we have no data to show that the auxin level decreases in the general way outlined above. Attempts to study the kinetics of auxin disappearance have met with many difficulties, concerned mainly with analysis of the auxin. These difficulties have been caused by the presence in leaves of substances which interfere in the Avena test. It now

appears, however, that it may be possible to separate growth hormones from inhibitors by paper chromatography. The amount of growth substance may then be determined by simply eluting the paper and determining the concentration by means of the Avena test. Experiments designed to follow the auxin level in leaves of Xanthium and Chenopodium by such methods can be expected to put the problem of auxin fluctuation in leaves to the critical test.

A further important aspect of the hypothesis is based on the assumption that the effects of light are on the IAA inactivating system. At present the only system known for carrying out the inactivation of IAA is IAA oxidase, which is thought to be a peroxidative enzyme. Is there any reason to assume that this enzyme is affected differently by red and blue light, both of which are effective in the photoperiodic action spectrum? Galston et al., (1951) has shown that in in vitro systems, IAA oxidase is activated by blue light. Thus the maximum activation of the enzyme from etiolated tissue occurs at 4400 Å. The relation of the enzyme to red light is however not known in any detail. In Xanthium the energy of blue light required to inhibit the floral

process is roughly 600-700 times as great as is the energy required in the red region of the spectrum. Unfortunately the action spectrum for the destruction of IAA in green tissues has not yet been studied, so we do not know whether red light is effective in inhibiting this destruction. There are in the literature indications that peroxidative enzymes may be subject to light destruction. Thus Eyster (1950) found that the catalase activity of albino corn grown in light was only 10 to 20 per cent of that of similar plants grown in darkness. In genetically green plants catalase activity is also decreased 10 to 20 per cent in light grown as compared with dark grown plants. Extension of work in this direction holds obvious promise for the further elucidation of the nature of the dark reaction.

Despite these uncertainties which cloud the issue as to the nature of the dark process, it is quite clear that auxin plays a vital and most probably a controlling role in the flowering response of short-day plants. Further work in the direction of elucidating this presently obscure role of auxin appears to be the most promising point of entry into the complex biochemistry of the flowering process.

PART III. THE FLOWERING OF SOME LONG-DAY PLANTS AS INFLUENCED BY TEMPERATURE AND APPLIED AUXINS

Introduction.

It has been pointed out earlier that the long night is the determinative element in the photoperiodic induction of SDP provided only that the plants have sufficient photosynthate present in the leaves during the dark period. The flowering of SDP then is promoted by a suitable alternation of light and dark periods but this is not the case with LDP. Thus it has been shown by numerous workers (Hamner and Naylor, 1939; Moshkov, 1940; Naylor, 1941; Lang and Liverman, 1951; Lang and Melchers, 1943; and others) that the flowering of LDP occurs best in continuous light.

The concept of the critical day length so far as LDP are concerned has to do then with a minimum required duration of the daily light period. Near this critical length, very small changes in day length have relatively great effects on flowering whereas further from this critical length differences in day length

seem to have little effect upon floral initiation (Moshkov, 1940; Lang and Melchers, 1943; Lang and Liverman, 1951). With cycles longer than 24 hours, the critical day length may decrease significantly. In Hyoscyamus, the critical day length under 24 hr. cycles, is 10-11 hours, whereas under 48 hr. cycles, it is less than 9 hrs. (Claes and Lang, 1947). This shows then that it is the length of light period rather than shortness of the dark period which determines the critical day length for LDP. It is also possible, however, to induce flowering in LDP grown under SD conditions by interrupting the dark period with low intensity light. The amount of light energy required to induce flowering is, in fact, much less when this energy is given as a flash or flashes during the dark period than when it is given in the form of an uninterrupted light period (Bunning, 1944; Claes and Lang, 1947) (Naylor, 1941; Razumov, 1941; Borthwick et al., 1948; Parker et al., 1950). Hyoscyamus which requires a regime with 10-11 hours of uninterrupted light will flower if given a 5 hour high intensity light period plus one hour of light during the dark period.

Thus interruption of the dark period by light is sufficinet to insure flowering in LDP and to inhibit flowering in SDP. In both

cases, relatively small amounts of energy are required, approximately 100 foot candle minutes in LDP and 10 foot candle minutes in SDP (see Table 21, page 109).

It should ultimately be possible to separate the photoperiodic response of LDP into partial processes just as has been done above for the SDP. In spite of the great amount of effort that has been put into work with LDP, however, the individual processes are not yet so well defined nor so clearly separable as is the case with SDP. Nevertheless, an attempt will be made to point out the similarities between the two photoperiodic types and the relationships of the partial processes in the photoperiodic response of SDP to those of LDP.

The High Intensity Light Process.

In LDP, the high intensity light process has not been clearly separated from the requirement for a minimum duration of the light period. There are, however, experiments which show quite clearly that a basic light period of high intensity extended by supplementary light of low intensity causes a stronger and faster flowering response than occurs when the whole light period is of low intensity (Claes, 1947; Fabian, 1938; Harder et al., 1937; Naylor, 1941; Withrow and Benedict, 1936). Naylor's

experiments with dill present the essentials of the method required to show this necessity for high intensity light in LDP. Naylor grew dill under light of 4-12 FC for 3 SD, then moved the plants to continuous light of different intensities. Unfortunately his measurements only record the amount of elongation which occurred under such conditions. Since the process of elongation and the process of initiation could be two different reactions and therefore show a different dependence on light, his data are of little value. The data with dill indicate, however, that as little as 50 FC of continuous illumination is sufficient for elongation and that there is a linear increase in elongation with intensities up to 300 FC. Naylor's (1941) experiments with annual beet are better in some respects, since they show that when beet is moved from SD to continuous light of different intensities, no elongation occurs if the plants are given less than 700 FC. Here again, however, no data are available on flowering. It has been shown recently by Klein and Leopold (1951) that application of malic acid to the leaves of barley (a LDP) during photoperiodic induction increases the rate of development of floral primordia. This result is roughly parallel to the sugar experiments of the author with SDP and it may indi-

cate a need for high intensity light. With LDP, then, there is an indication that high intensity light processes are involved in the flowering response but the clear-cut demonstration of this fact is still lacking.

The Dark Process.

In contrast to the state of affairs with SDP, a dark period is not critical to induction in LDP, and there are indications, moreover, that dark may play more than a mere passive role. Thus, Hyoscyamus grown under SD will remain vegetative indefinitely. If, however, all the leaves are removed, the plant will flower in SD or even in continuous darkness if defoliated (Lang, Melchers, 1943). Hyoscyamus is then no longer photoperiodically sensitive when defoliated. It should be noted that this result has been obtained only with Hyoscyamus, a plant which has a relatively large root-stock and thus a large assimilate reserve. All efforts to extend this result to include other LDP have thus far failed.

The leaves of Hyoscyamus must then do something during long nights which inhibits the flowering process. It is possible that this inhibition may be related to respiratory or closely linked metabolic processes which proceed in the leaves

in the dark. The inhibitory effect of long night leaves may be decreased by (a) applying sugars to the leaves (Melchers and Lang, 1942), (b) by maintaining the plants in a nitrogen atmosphere during part of the dark period (Melchers and Claes, 1942), or (c) by lowering the temperature, i.e., the critical day length is decreased by decreasing temperature (Lang and Melchers, 1943; Melchers and Lang, 1948). Another, and equally logical, possibility is that the leaves of Hyoscyamus produce an active inhibitor during the dark. The defoliation experiments mentioned above would support this view. Experiments with several other species of LDP appear to show, however, that SD leaves are not in general inhibitory to flowering (Chailahyan, 1946 a, 1946 b, 1947; Withrow et al., 1943). These experiments present the same qualitative picture as is found with LD leaves on SDP, i.e., these SD leaves on LDP interfere with transport of the stimulus in some way. It is possible however that in this instance the SD leaves act as a sink for the substances produced by the LD leaves. At the present time it is not possible to choose between these two alternatives. With the possible exception of Hyoscyamus, then, there

is no positive proof to support the view that an active inhibitor is formed by SD leaves on LDP. In any case, however, it is clear that the dark period is not completely passive, but that its action may be principally concerned with the frittering away of substances necessary for formation of the flowering stimulus.

It is the low intensity light process which appears to be the controlling one in the flowering of LDP. It has been shown above that, if a period of high intensity light (below the critical in length) is given to the plant to provide photosynthates; then a small amount of daily supplementary light properly supplied will result in flowering. The energy given need total only of the order of 100 foot candle minutes per day whether given continuously or as a flash in the middle of the dark period. The action spectrum for this process is similar to the action spectrum for the inhibition of flowering of short-day plants (Borthwick et al., 1948; Parker et al., 1946, 1950). In contrast to the state of affairs with SDP, there have been no reports of concerted attempts to discover what the light flash does in the case of LDP nor indeed to determine the nature of the response evoked by low intensity supplementary light.

That the flowering hormone of LDP is similar to that of SDP has been shown by use of the appropriate interspecific grafts. Thus a LD scion induces flowering in a SD stock provided only that the leaves of the LD scion have been photoinduced. In like manner it can be shown that a SD scion is able to cause the formation of flowers in a LD stock provided the SD scion has been photoinduced (Melchers and Lang, 1941, 1948). It has also been indicated that the hormone of SDP may be identical with that of indeterminate or DNP (Thurlow, 1948).

The author's investigations on LDP have been restricted to experiments designed to explore the questions of 1) the effect of temperature upon the flowering response and 2) the relation of applied auxins and anti-auxins to flowering of LDP treated under threshold conditions. The literature on each of these topics will be discussed in the individual experimental sections.

CHAPTER I. THE EFFECT OF TEMPERATURE ON THE FLOWERING OF SILENE ARMERIA

Contents.

I. Review of literature on the relationship between temperature and flowering in long-day plants.

Temperature, which has only been mentioned in the introduction, is an important factor in the flowering of LDP. It was pointed out that the inhibition exerted by the dark process in Hyoscyamus

could be overcome by application of sugar, by maintaining the plants in a nitrogen atmosphere, or by lowering the temperature. The principal effect of temperature on the flowering of Hyoscyamus is probably exerted through an effect upon the critical day length. The critical day length at 28°C., for example, is approximately 11.5 hours, whereas at 15° C. it is less than 9 hours. Temperature, then, would appear to be important in controlling the rate of the processes which occur during the dark period. Studies on the relation of temperature to the flowering of LDP, therefore, are potentially capable of yielding information about the relative importance of the light and dark processes in the induction of LDP.

The relation of temperature to the flowering response of annual LDP has been under investigation for many years but most of these experiments have been directed toward solving practical agricultural problems rather than toward gaining an insight into the basic physiological mechanism of the interaction between temperature and flowering. Such problems, for example, as prevention of bolting of spinach, radish, celery, lettuce, sugar

beet, and other important food plants have been investigated extensively since the economic value of these crops decreases markedly once bolting has occurred. Many of the data which have been gathered concerning these matters are not presented in a quantitative manner. All that can be gained from surveys of the published work (given in brief form below) is a qualitative indication of the general effects of temperature.

Steinberg and Garner (1936) used sugar beet and Rudbeckia in extensive experiments with photoperiods of 6, 9, 12, 13, 14, 15, 18 and 24 hours daily at 60, 65, and 73° F. Sugar beet flowered only when it received 18 or 24 hours of light. With an 18 hour photoperiod the plants flowered at the same time at 60 and 65° F., but did not flower during the course of the experiment (56 days) at 73°F. With continuous light there was a marked acceleration by high temperature. Thus at 73° the plants flowered after 39 days, while at 65° and 60° there was a delay of 9 and 26 days respectively. Rudbeckia responded in a different manner to high and low temperature. Under 13 hour photoperiods plants grown at 83°F. flowered in 56.5 days whereas those at 77° F only

elongated. The effect is less marked with longer daily light periods but there still appears to be a significant acceleration by the higher temperature. The delay in flowering at 77° at photoperiods of 14, 15, 18, and 24 hours daily was 2, 14.5, 9, and 11 days respectively. The qualitative result of these experiments is that near the critical day length bolting and flowering of sugar beet is promoted by low temperature whereas the development of Rudbeckia is promoted by high temperature.

Roberts and Struckmeyer (1938, 1939) studied the interaction of temperature and photoperiod on the flowering of over 100 plant species. Some plants responded as LDP or SDP at one temperature but responded as DN, SDP, or LDP respectively at another temperature. In these experiments the plants were grown under photoperiods of approximately 16 hours (LD) in one case and of 9-11 hours (SD) in the other case. The temperatures were 55, 63-65, and 70-75° F. in one experiment and 55 and 70° F. in other experiments. A brief summary of the data of all experiments is given in Table 26. It should be pointed out that the designations LD, SD, DN as used here are the author's.

The basis for designation was as follows: If a plant flowered under a 16 hour but not a 9-11 hour day it was designated LD. If it flowered only under a 9-11 hour day it was designated SD, or if flowering occurred under both day lengths then it was arbitrarily classified as DN. If no flowering occurred they were designated vegetative (V).

TABLE 26

General types of responses of several plants to temperature and photoperiod. Data after Roberts and Struckmeyer (1938, 1939).

Temperature	Type of Response to Photoperiod (at particular temperature)						
Low (55)	LD	DN	DN	V	LD	V	LD
Medium (63-65)	LD	LD	LD	LD	-	-	-
High (70-75)	V	V	LD	DN	LD	LD	DN
Number of plant species in class	6	7	1	1	1	1	1

In these experiments only 2 day lengths, the shorter still quite long, were used. One might suspect, then, that the critical day length had been lowered by temperature so that plants which appeared to be LD at one temperature and DN at the other actually are LDP which have their critical day length greatly decreased by either high or low temperature.

Knott (1939) observed that spinach plants kept for 4 week periods at low and then moved to higher temperatures flowered much sooner than plants kept constantly either at low or at high temperature. Since dissections were not made it is impossible to determine whether or not flowers were initiated at the time the plants were moved, but in any event there appears to be an acceleration of flowering by low temperature.

Sivori and Went (1944) found that Baeria chrysostoma remains vegetative at 28° C. even in 24 hours of light whereas if the temperature is reduced to 22° C. the plants flower rapidly and eventually flower under SD. Went (1945) found that of 8 photoperiodically sensitive desert annuals, the flowering of 3 was promoted by low temperature at night, the flowering of 1 was promoted by high temperature at night, whereas the other 4 flowered more or less independently of the temperature.

Murneek (1940) reports that Rudbeckia bicolor responds as a DN plant at high temperatures since it flowers under a 7 hour day, whereas at low temperature it responds as a strict LDP. Unfortunately only two day lengths and two temperatures were used so only a qualitative conclusion may be drawn.

The literature already reviewed indicates that, in LDP, the principal interrelation between photoperiod and temperature is that flowering is favored by low temperatures. Of the approximately 35 LDP described above only 10 flowered when maintained at high temperature; in 7 of these cases the responses have been strictly LD ones whereas in the other 3 cases the plants were able to flower in SD as well as LD at the higher temperature. In only one case (Steinberg and Garner), however, was any attempt made to correlate critical day length with temperature and no general conclusion can be made concerning these two factors.

Lang and Melchers (1943) appear to have been the only investigators who have related the critical day length to temperature in a specific way. They grew annual Hyoscyamus niger under several different day lengths and at temperatures of approximately

15, 18, 22, and 28° C. It was observed that not only did the plants elongate faster at lower temperature, but that the critical day length varied linearly with temperature. Thus at 15° C. the critical day length was 9 hours, at 18-22° it was 10 hours and at 28° it was 12 hours. The time until appearance of buds, however, was promoted by high temperature under the longer photoperiods. These workers also found that although the temperature during the light period was of some importance, the effect was most pronounced during the dark period. That temperature has a specific effect upon floral initiation is shown by comparing the actual numbers of leaves formed by the plants at day lengths near the critical. Under an 11 hour photoperiod the number of leaves produced before floral buds at 28, 22, 18, and 15° C. were 28, 12, 12, and 10 leaves respectively. With a 10 hour photoperiod the numbers of leaves were 34, 21 and 19 respectively for the three lowest temperatures.

The above facts taken as a whole indicate that the flowering of LDP is usually favored by low temperatures. There are one or two noteworthy exceptions but as will be pointed out later, these exceptions may be more apparent than real. In the generalizations which have been made concerning the photoperiodic mechanism

of LDP it has usually been assumed, and not without justification, that the flowering of LDP is favored by low temperature. Since only a single plant, Hyoscyamus niger, has been studied in detail, the experiments on its response have served almost entirely as a basis for these theories.

It becomes quite apparent that more detailed knowledge of the temperature response of several additional LDP is needed before a comprehensive scheme can be proposed for the temperature response of LDP. With this deficiency of information in mind an extensive series of experiments were designed to study the effect of temperature on the flowering behavior of a new, but quite responsive, LDP, Silene Armeria.

II. Temperature Experiments with Silene.

1) The problem. The experiments described below were carried out in the controlled temperature greenhouses of the Phytotron. They were designed specifically to determine the effect of temperature upon the critical day length of Silene and to determine the portion of the inductive cycle, i.e., the light or the dark period, which is more affected by temperature.

2) Growth conditions prior to actual experiment. About 2 weeks after germination, four Silene seedlings were planted into 4 x 4 x 2.5 inch plastic containers which were filled with a sand-vermiculite-gravel mixture for growing media. During the growing period prior to the beginning of the experiment the plants were moved to the darkroom each afternoon at 1600 hours where they received 16 hours of darkness. The following morning at 0800 hours they were moved back to the greenhouse for 8 hours of high intensity light. The plants were given 23°, 20° or 18° C. during the day and 14° or 17° during the night. To eliminate differences in growth rate which might arise, the plants were moved from one temperature condition to another in accordance with a regular schedule. After the plants had grown under these conditions for approximately 2 1/2 months, they were prepared for the actual experiment. This preparation involved removal of all senescent leaves, randomizing the containers to secure uniformity of plants in the different treatments, and dissecting one lot of 8 plants to determine the initial number of leaves.

The actual photoperiodic treatments are shown in Table 27. For the light period the plants were placed in artificial light

of approximately 700-800 FC and were then moved to the darkroom and covered with light-tight boxes. All manipulations in the darkroom were carried out in absolute darkness, or with the aid of a small 2-celled pen light covered with green celluloid. The green shield was necessary to eliminate light of spectral qualities which promote flowering of LDP. As shown at the bottom of Table 27 all plants were moved either to light or dark at 0800 hours and were then moved again from dark to light or light to dark respectively between 1600 and 2000 hours depending upon the length of photoperiod which the plants received. The plants were transferred from dark to light and light to dark on a regular schedule starting 30 minutes before the hour. To eliminate any systematic error in day length the transfers began with a different room on each successive day.

On July 27, 1951, the day before the experimental treatment began, the individual experimental lots were set up and at 1600 hours the plants went either to 14° or 17° C. and remained there until the beginning of their light period on the regular treatment schedule. Thus the plants which began their light period at 0800 hours received a dark period of 16 hours

TABLE 27

Plan of experimental treatments for temperature study with Silene. LL No. 2. 1951. Blanks indicate photoperiod not included.

Temperature in C.°		Length of Photoperiod in Hours									
Day	Night	8	9	10	11	12	13	14	15	16	24
30	30	*	*	X	X	X	X	X	X	X	X
23	23			X	X	X	X	X	X	X	X
17	17		X	X	X	X	X	X	X	X	X
10	10	X	X	X	X	X	X	X	X	X	X
23	30			X				X			
23	17			X				X			
23	10			X				X			
30	23			X				X			
17	23			X				X			
10	23			X				X			
Time of movement to											
	Dark	1600	1700	1800	1900	2000	0800	0800	0800	0800	
	Light	0800	0800	0800	0800	0800	1900	1800	1700	1600	

* These two treatments were started after it became evident that the 10 hour plants were elongating.

before the first light period, whereas those whose light period began at 1600 had 24 hours of darkness, etc. It is felt that this slight difference is unimportant insofar as the results are concerned.

A record was kept of the date on which axis elongation occurred and the date and height of the plants at the time of appearance of flower buds. All plants which had not produced visible flowers by the end of the experimental period were dissected and the stage of floral development (Part I) was recorded. At regular intervals during the course of the experiment the senescent leaves were removed and their numbers recorded. These leaves plus those remaining on the plant at the termination of the experiment made up the total number of leaves as recorded in the table.

4) Results obtained with constant temperature. Let us first consider the flowering response of Silene under constant temperature conditions. Table 28 records the data on elongation, flowering, height of plants at the time of flowering or at the end of the experiment, and the number of leaves formed before flowers were initiated. The results are shown in graphic form in Figures 14, 15, and 16.

TABLE 28

Days to elongation, to visible buds; height at time buds were visible, leaf numbers before flowers. See following page for footnotes.

Length of Photoperiod in Hours	Days to elongation Temperature at which plants were grown 30		23		17		10	
24	8.9	0.3*	9.4	0.2	10.6	0.3	17.7	0.5
16	11.3	0.5	11.0	0.6	12.1	0.4	26.0	0.0
15	11.9	1.3	13.8	0.5	13.1	0.5	27.0	0.4
14	9.3	0.2	18.4	1.4	17.3	0.5	32.0	0.9
13	10.6	1.0	19.4	0.7	17.8	0.5	47.7	1.7
12	11.2	0.8	16.0	a	26.1	1.4	-	-
11	13.5	1.3	-	-	27.0	a	-	-
10	16.9	0.4	-	-	-	-	-	-
9	Died							
8	Died							
	Days to visible buds							
24	14.8	0.3	15.0	0.3	17.4	0.8	30.6	0.3
16	23.1	0.6	22.3	0.3	24.0	0.0	37.3	0.3
15	24.6	1.1	26.1	0.03	26.4	0.2	45.6	0.6
14	23.9	0.2	32.9	1.0	32.8	0.6	55.9	1.3
13	28.3	1.1	39.9	1.9	36.9	0.4	78.5	b
12	29.0	1.1	79.0	a	68.7	3.3	-	-
11	28.8	2.1	-	-	83.0	b	-	-
10	37.5	6.5	-	-	-	-	-	-
	Height in cm. at visible buds							
24	16.9	0.9	14.9	0.3	16.2	0.8	20.3	0.1
16	14.1	0.7	13.4	0.5	18.1	0.4	13.4	0.2
15	13.3	1.0	14.1	0.7	19.4	0.6	14.9	0.6
14	15.2	1.1	13.7	0.7	19.3	0.8	16.5	0.5
13	15.9	1.1	11.9	0.7	19.1	0.8	14.4	0.7
12	12.3	0.3	21.0	a	16.1	0.6	3.2	0.1
11	13.3	2.1	4.0	0.2	8.2	a	2.7	0.4
10	13.0	2.5	4.6	0.6	4.4	1.6	2.7	0.1
9	-	-	-	-	3.6	0.2	3.2	0.2
	Leaf numbers before flowers c							
24	50.3	0.3	50.3	0.7	50.9	0.9	51.5	1.7
16	56.3	0.5	51.0	1.4	53.8	0.8	56.0	0.8
15	53.2	1.4	52.1	0.9	55.4	0.8	56.9	0.6
14	53.4	0.1	58.0	1.1	57.3	1.6	61.0	0.8
13	57.3	2.1	61.8	1.4	59.0	0.5	66.8	1.7
12	54.0	1.2	88.4	1.1	79.4	2.1	77.9	2.1
11	52.2	1.1	86.5	1.7	82.4	1.5	74.3	1.5
10	57.5	2.2	77.9	1.7	83.2	0.6	70.8	1.5
9	-	-	-	-	83.5	1.8	74.8	1.2
8	-	-	-	-	-	-	75.6	1.6

The important conclusion from these data is that a temperature of 30° favors faster elongation and earlier flowering at any given day length. At the two intermediate temperatures of 23° and 17° there is little difference in response according to either of these criteria. Both of these temperatures favor elongation and flowering less than does 30° but more than does 10°. At 10° there is a marked retardation of elongation and flowering, but this is principally a reflection of the slower growth rate. It is noteworthy that the curves relating photoperiod to elongation or flowering are quite flat for the longer photoperiods but near the critical day length the slope changes rapidly and the curve appears to approach asymptotically to some particular day length.

* Standard error of the mean.

a 1 plant flowered.

b Extrapolation according to stage of development at end of experiment.

c Beginning leaf No. was 48.8.

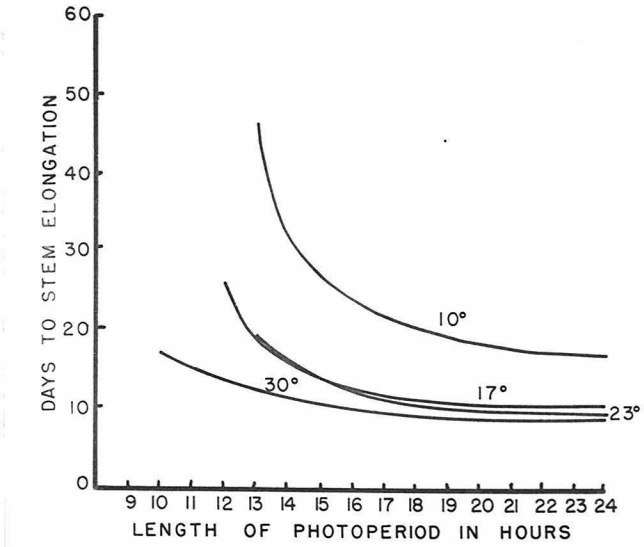


Figure 14. Number of days to elongation as affected by photoperiod and temperature.

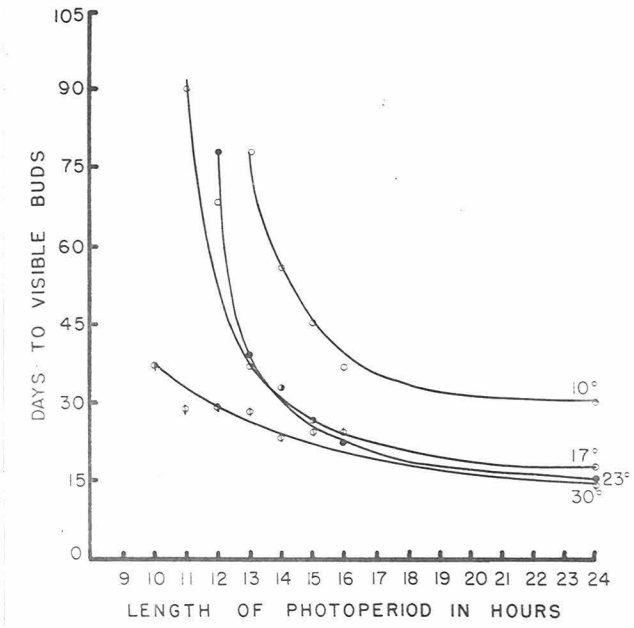


Figure 15. Number of days to visible buds as affected by photoperiod and temperature.

The most meaningful measure of the effect of temperature upon flowering is the absolute amount of growth which takes place from the time the experimental treatment begins until flowers have been initiated. This is best measured in terms of the numbers of leaves formed before flowers are initiated. The last group of data in Table 28 present the leaf numbers and Figure 16 presents it graphically.

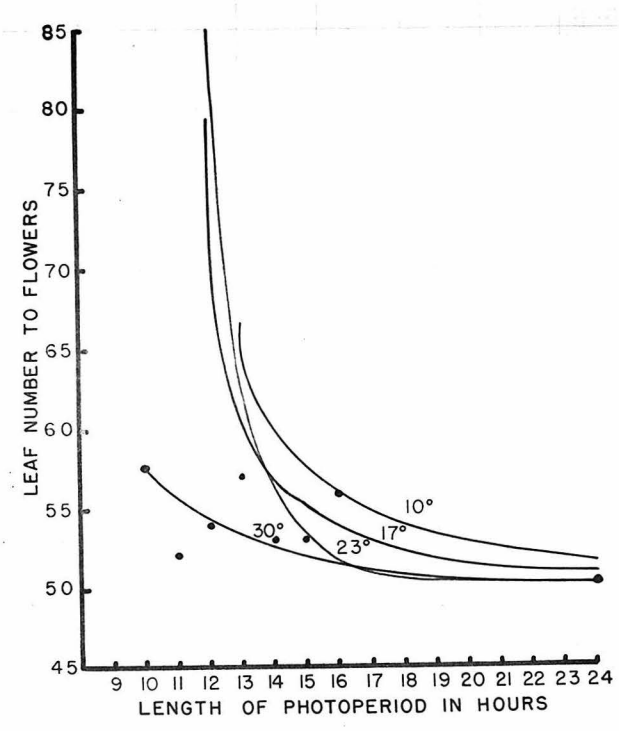


Figure 16. Number of leaves formed before floral initiation as affected by photoperiod and temperature.

The leaf number data bear out the general trends observed for the number of days to elongation and number of days to appearance of visible buds. Thus there is a slight delay in flower initiation at 10° as compared with the other temperatures. The points shown are for the 30° curve only.

A striking conclusion to be drawn from this data is that one of the two optimal temperatures for flowering of Silene appears to lie at 30° or above. That there is also a second temperature optimum in the neighborhood of 17° is evident from the data of Figure 17 in which the days to visible buds are plotted against temperature.

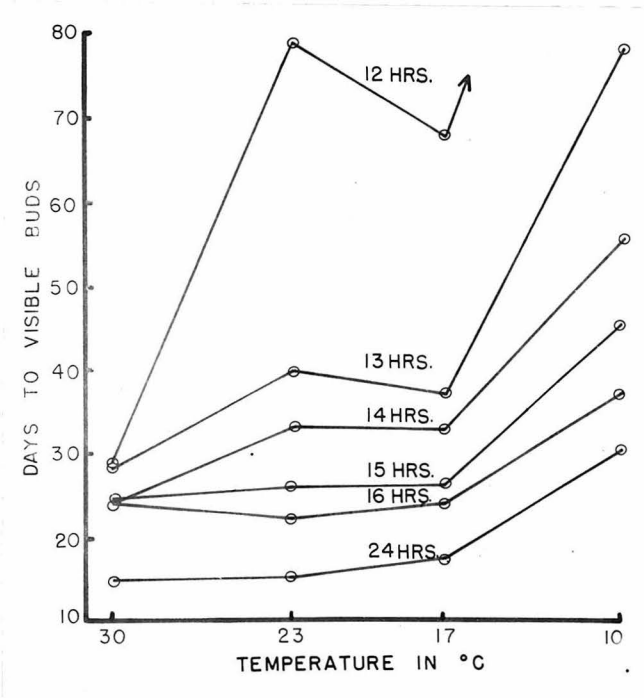


Figure 17. Temperature optimum for flowering of Silene. Note apparent optima at 30° and at 17° C.

This 17° optimum is not so marked as that at 30° and it appears only near the critical day length. Table 28 shows that this effect is also evident in the leaf number data. With a 12 hour photoperiod the number of leaves produced before flowering at 30°, 23°, and 17° was 54, 88, and 79 representing respectively an absolute increase of 5, 39, and 30 leaves at these temperatures. The plants did not flower at 10° under this day length but by extrapolation it may be estimated that had the plants flowered at all, the number of leaves formed before flowering would have been greater than 100.

5) Results obtained with different day and night temperatures. One is led to inquire whether the promotive effect of high temperature found above is a reflection of the effect of temperature on the light or on the dark process of the photoinductive cycle. As pointed out earlier the experimental design was such as to give some insight into this matter. Unfortunately neither space nor plants were available to run a complete series at all temperatures so a compromise temperature (23° C.) was chosen as a constant day or night temperature. The plants which received day or night temperature of 23° C. received the remaining portion of their photoinductive cycle at 30°, 17°, or 10° C. Only photoperiods

of 14 and 10 hours were used. The data for the 14 hour photoperiod at a day temperature of 23° and different night temperatures is shown in Table 29 and is pictured graphically in Figure 18.

TABLE 29

The relation between constant day temperature and different night temperatures upon the flowering of Silene.

Day Temp.	23		23		23	
Night Temp.	30		17		10	
Photoperiod	14		14		14	
Days to Elong.	11.0	0.0*	24.8	0.7	25.6	1.6
Days to Bud	23.1	0.7	45.6	1.2	52.8	1.2
No. of Leaves	43.5	0.6	58.1	1.2	64.4	0.7
Height (cm.)	14.3	0.3	15.5	0.8	22.0	1.1

* Standard error of the mean.

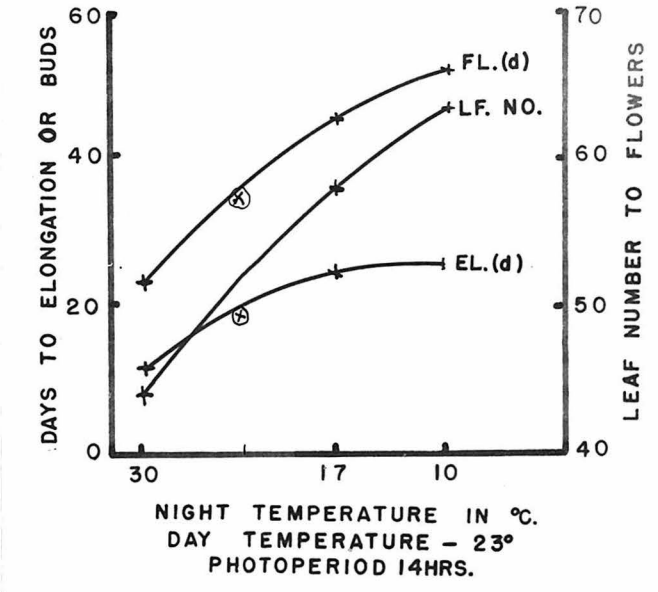


Figure 18. The effect of constant day and different night temperatures upon flowering of Silene. Fl (d) - days to flowering. Lf. No. - leaf number. El (d) - days to elongation.

The facts which appear here show once again the effect of 30° C. in promoting flowering. The effect is quite apparent irrespective of the criterion used. If the points for constant 23° day and night temperature are now put into the figure it is seen that the curve for the other 3 points passes directly through these added points. The important fact, illustrated by the curve, is that the higher night temperatures favor flowering. What now is the effect, if any, of the day temperature? Table 30 and Figure 19 summarize the data for 30, 17, 10° day temperature and a 23° C. night temperature.

TABLE 30

The effect of constant night and different day temperatures upon the flowering of Silene.

Day Temp.	30		17		10	
Night Temp.	23		23		23	
Photoperiod	14		14		14	
Days to Elong.	19.9	1.2 *	18.3	0.7	26.1	0.3
Days to Bud	44.4	4.5	33.5	0.2	33.4	0.2
No. of Leaves	54.7	2.5	50.0	1.1	44.5	0.6
Height (cm.)	18.8	1.7	16.9	0.7	9.4	0.2

* Standard error of the mean.

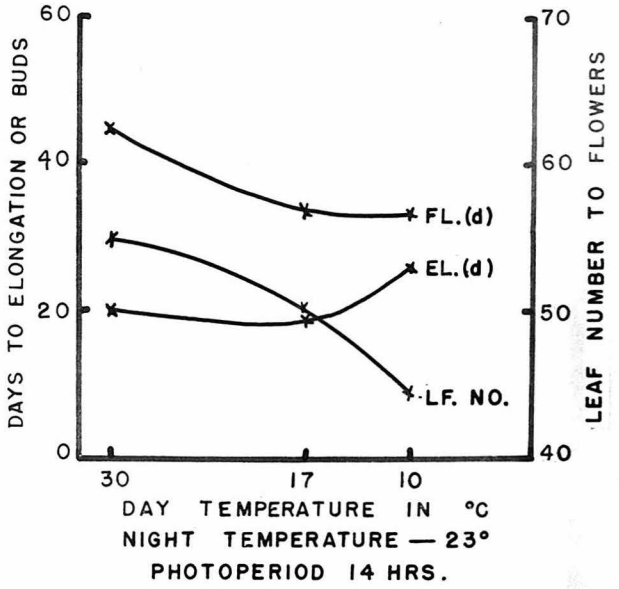


Figure 19. The relation between constant night and different day temperatures upon flowering of Silene. Abbreviations same as for Figure. 18.

The striking fact which the graph and table reveal is that the day temperature has a different effect upon the beginning of elongation and upon further development of the plant. The beginning of elongation is much faster at 30^{o} and at 17^{o} than at 10^{0}, but the time lapse before buds become visible shows the reverse response. The number of days, then, between the beginning of elongation and the appearance of buds at 30^{o}, 17^{o}, and 10^{o} is 25, 15, and 7 days respectively. When the specific effect of the temperature is considered, i.e., the number of leaves formed before floral initiation, the leaf number is much greater at 30^{o} and 17^{o}

than at 10°. The implication is that flowering is inhibited in a specific way by high day temperature.

These results are to be contrasted with those of the previous experiment in which a constant day temperature and different night temperatures were used. In this instance increasing night temperature was positively correlated with faster flowering, not only in time but in a specific way, i.e., the number of leaves formed at the higher temperatures was smaller. It appears then that irrespective of the temperature considered, when the night temperature is higher than the day temperature flowering is accelerated. Thus Figure 20 shows that with a constant night temperature of 23° C. flowering is faster at day temperatures of 10° and 17° than at 30°. With a 23° day temperature, flowering is much faster at a 30° night than at either a 17° or a 10° night.

The conclusion that the night temperature is the controlling one is confirmed by experiments similar to those described above but using a 10 hour instead of a 14 hour photoperiod. With this 10 hour photoperiod only those plants grown under conditions of 23° day and 30° night temperature flowered. The number of leaves produced by the flowering plants was only 46.6 whereas the

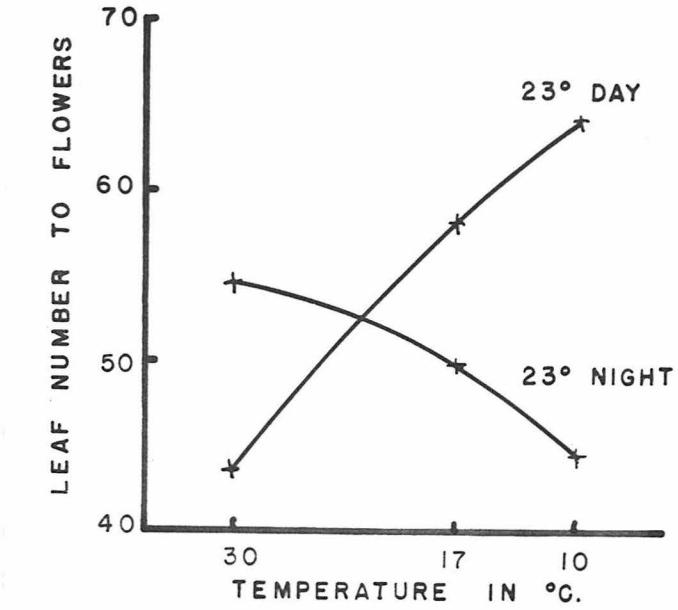


Figure 20. Composite plot of number of leaves formed before floral initiation with either the night or the day temperature held constant and the temperature of the other part of the cycle varied.

the plants in all other conditions were vegetative and averaged approximately 68 leaves. It may be concluded, therefore, that the night temperature plays the more important role in the flowering of Silene, but that the day temperature is also of some significance. It is indicated, moreover, that high day temperatures may be inhibitory. Further experiments are needed to elucidate more clearly this response to high day and night temperature.

6) Discussion of results. These studies with Silene have shown that the flowering response of this LDP is promoted by high night temperature, especially when this temperature is higher than the day temperature. Now let us compare the response of this plant with that of Hyoscyamus niger, the only other LDP which has been analyzed in such detail. Figure 21 shows the relation between the critical day length and temperature of both Silene and Hyoscyamus. The curves show that with Hyoscyamus the lower the temperature the shorter the critical day length. The curve for Silene shows the opposite general tendency, but this does not hold over the whole temperature range for we see that at 23° the critical day length is 12 hours, whereas at 17° it has again fallen to 11 hours. Thus that part of the Silene curve between 17° and 23° indicates that flowering is favored by low temperature and in this respect almost exactly parallels the Hyoscyamus curve. That this change in the curve for the critical day length is real is indicated by the fact that at 23° C. all plants flowered at 13 hours, only one flowered at a 12 hour photoperiod. At 17° all plants flowered at 12 hours, but only one flow-

ered under the 11 hour photoperiod. The reduced critical day length at 17°, then appears to be real.

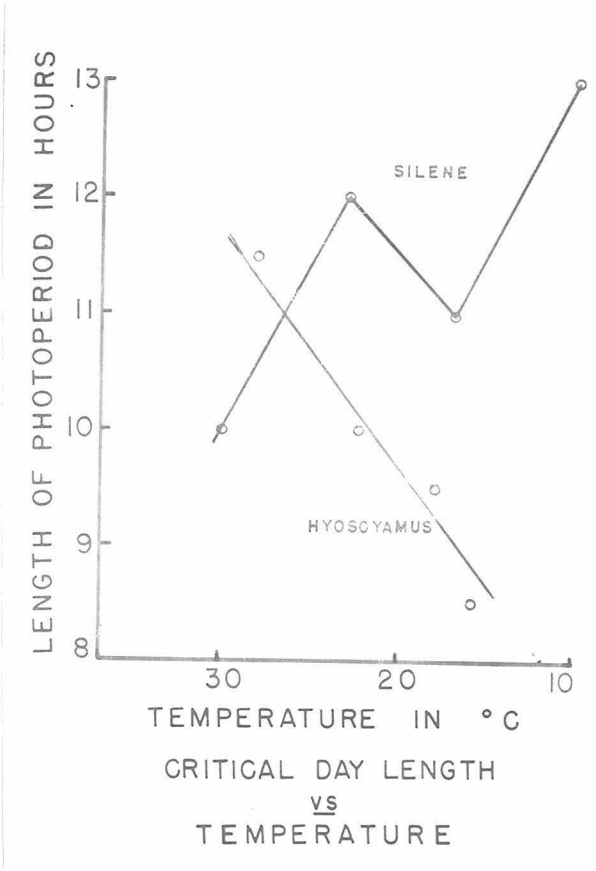


Figure 21. Comparison between the response to temperature of Silene and Hyoscyamus.

This curve for Silene appears to be the only case on record where the flowering of a single LDP has two temperature optima. It is possible, however, that all LDP would show such a response if they were tested over an extended temperature range. Thus if Hyoscyamus could be grown above 28° (the highest temperature used by Lang and Melchers, 1943) there might be a decrease in the critical day length. It is also possible that temperatures below 15° would have caused an increase in the critical day length.

The temperature experiments with Silene have pointed out two important facts, 1) that critical day length is a relative thing, and 2) that the range of temperatures and the length of photoperiods near the critical day length must be made more inclusive for future studies. The critical day length, then, is not an absolute day length which is independent of all other factors; on the contrary, it is affected very greatly by the temperature given during inductive treatment and is quite dependent upon the length of cycle used. It is necessary, then, to report not only the critical day length but also the length of cycle and the specific temperature used during the experimental period.

The second point mentioned above, i.e., the need for using a wider range of day lengths and temperatures is quite apparent from the curve for the response of Silene. If only 23° and 17° had been used in the experiments it would have been concluded that flowering was promoted by low temperature. Conversely if either 17° or 23° had been omitted from the experiment, the conclusion would have been that flowering was always accelerated by high temperature. Neither of these conclusions would have been correct. In view of these results one is led to inquire about earlier investigations in which two or at most three temperatures and two or three day lengths were used. Let us consider the case of Rudbeckia whose flowering is reported to be promoted by high temperature. In these experiments only 7 and 14 hour photoperiods were used. Flowering occurred at either high or low temperature at 14 hours but only at high temperature at the 7 hour photoperiod. The results of this experiment and others like it, although interesting, perhaps do not contribute greatly to a better understanding of the photoperiodic mechanism in LDP. It is quite probably that this plant may show a response similar to that of Silene. Only further research can reveal

this fact. At the present time generalizations are not justified concerning the relation between flowering and temperature because of the very limited amount of definitive data which are available.

CHAPTER II. AUXIN RELATIONSHIPS IN THE FLOWERING OF LONG-DAY PLANTS

Contents.

I. Review of literature.

The action spectrum of the low intensity light process is similar in LD and SDP. The flowering hormones of the two reaction types are apparently identical. What, then, might one conjecture as to the similarities and differences between LDP and SDP as to the auxin relations of flowering?

Logically, and by analogy with SDP plants, we might expect that the application of auxin to a LDP would either a) inhibit flowering when applied to plants during photoinduction or b) replace low intensity light and hence actually cause flowering of plants kept in a SD regime. The hypothesis under a suggests that the flowering of LD and SD forms would be similar in that high auxin levels would inhibit flowering in both cases. The hypothesis under b suggests that LD and SD forms resemble one another in the nature of the dark and low intensity light processes. Which of these hypotheses is correct?

The role of auxin in the flowering of LDP is more obscure than is the case for the SDP. This is true even from a qualitative standpoint. With but few exceptions the experiments recorded in the literature reveal that auxin applied to LDP grown under LD conditions has inhibitory effects on flowering. The extent of this inhibition appears to vary greatly from species to species. Thus von Denffer and Grundler (1950) found that of 14 species of LDP treated with auxins (NAA or IAA), 6 showed essentially no response, 7 showed only nonspecific growth inhibition while one showed specific floral inhibition as measured by the num-

ber of leaves produced prior to floral initiation. High concentrations of auxin have also been observed to give inhibition of flowering in Wintex barley, a LDP, (Leopold and Thimann, 1949; Li, 1950) whereas low concentrations give an increase in the number of flowers formed. Other experiments (Leopold and Thimann, 1948, 1949), however, show that when barley is grown on LD and given three consecutive weekly X-ray treatments (25 r units) there is an increase in the number of flower primordia formed. It is not at once obvious that these two observations are compatible since it has been shown (Skoog, 1935; Gordon and Weber, 1950) that a principal effect of X-rays is to decrease the auxin level in the plant by interference with the auxin synthetic mechanism. Application of coumarin, an inhibitor of auxin action (Veldstra and Havinga, 1943) was also found by Leopold and Thimann to cause an increase in number of flowers formed by barley. Recently, Claes (1952) has reported that neither IAA or TIBA have any promotive effect upon the flowering of Hyoscyamus, either in LD or SD.

II. Auxin and photoperiodic induction of long-day plants.

1) The problem. A review of the literature on the role of auxin in the flowering of LDP has shown that as a whole the picture is quite obscure. In some instances auxin applications have been reported to cause strong inhibition of flowering while in other instances they have had either no effect or have caused a slight promotion of flowering. Because of this confused picture of the role of auxins in the flowering of LDP experiments were set up to test whether auxin application to a LDP would a) actually inhibit flowering, or b) replace low intensity light and thus cause flowering under conditions in which flowering would usually not occur.

2) Experiments with Silene.

a. Under LD conditions. In the first of a series of experiments Silene Armeria was treated with auxins and anti-auxins under LD conditions to determine the gross effects of these substances and also to determine the concentration range suitable for use with this plant.

Silene plants which had grown under SD conditions for 3 1/2 months were removed to LD conditions consisting of approxi-

mately 12 hours of natural light plus 12 hours of supplementary light from 200 watt bulbs suspended 3 to 4 feet above the plants. The auxins, IAA and NAA and the anti-auxin, TIBA, were used at three different concentrations. These substances were applied twice daily, once at 0800 and again at 1800, by spraying the leaves. A detergent, Dreft, was used at a concentration of 0.005 per cent by weight. One half of the plants were moved back to SD after 7 LD and the remainder after 16 LD. The data are summarized in Table 31.

One fact which stands out is that the plants which received 16 LD gave a faster and better response in every criterion considered. The important fact which emerges from these data is that IAA and NAA promote flowering in Silene. Thus the time to beginning of elongation is reduced below that in the controls by as much as one half. NAA is equally effective at all concentrations used, whereas IAA is less effective at the lower concentrations until at 3 ppm there is only a very slight acceleration. This pronounced effectiveness of NAA is probably related to its greater stability in the plant since 300 and 30 ppm NAA killed the

TABLE 31

Promotion of flowering in Silene by auxin application under LD conditions.

Data Recorded	Air	H_2O	Triiodobenzoic Acid			Naphthaleneacetic Acid			Indoleacetic Acid		
			300	30	3	300	30	3	300	30	3
* Days to Elong.	14.5	15.7	12.0	14.8	14.0	7.8	7.2	7.5	8.0	10.7	13.8
Days to Elong.	11.0	11.0	11.0	10.2	11.0	7.2	7.0	8.0	8.5	9.5	10.0
* Days to Visible buds	23.0	24.0	24.5	24.2	23.2	a	a	21.2	21.8	24.5	23.0
Days to Visible buds	18.2	18.7	18.0	18.5	18.2	a	a	19.0	18.2	18.0	18.0
*Days to Open Flowers	39.7	39.0	41.0	37.5	38.0	-	-	36.8	36.5	40.0	38.5
Days to Open Flowers	30.3	31.0	29.8	30.2	30.8	-	-	35.0	33.3	31.5	32.0
*Height at Buds	9.1	10.9	10.2	11.0	10.0	-	-	13.2	13.6	14.1	11.8
Height at Buds	17.9	19.5	12.9	16.6	17.0	-	-	13.4	16.1	16.4	18.0
*Height at Open Flower	26.0	29.0	22.0	26.0	26.8	-	-	28.8	32.5	30.0	28.4
Height at Open Flower	28.6	42.4	28.3	38.2	41.2	-	-	28.5	40.1	39.8	42.0

* These plants received 7 LD and were then returned to SD. The other one half received 16 LD.

a These plants died before the appearance of visible buds. Plants treated with 30 ppm were dissected after 26 days and these showed the presence of flower primordia in stages III and IV.

plants after a few days whereas these concentrations of IAA gave no detectable inhibition. This promotive effect of auxins is apparently significant only as regards elongation of the stem although in the plants treated with 300 ppm of IAA buds became visible one or two days earlier than in the controls. TIBA was essentially without effect at any concentration used.

b. Under threshold conditions. Since the results of this experiment showed that auxin applied under LD conditions actually caused an acceleration in the flowering response, other experiments were set up immediately in the Phytotron where temperature and light quality and quantity could be controlled. During the experimental treatment the plants were maintained at near threshold conditions by the use of the special equipment described on page 44.

Prior to the experimental treatment the Silene plants were grown on a regime consisting of 8 hour days at 20° C and 16 hour nights at 14° C. When the plants were 2 1/2 months old they were randomized, a number of them taken for determining the initial number of leaves and the others were moved to the basement darkroom for treatment. The plants were grown for

2 additional 8 hour days at the new constant temperature of 23° and during this time they were sprayed once daily with various growth substances. At the end of this two day period a small 15 watt bulb was turned on and the plants were sprayed once daily for the duration of the experimental period. Four intensities of supplementary light (A, B, C, and D) were utilized. At each of these four intensities there were 9 different experimental treatments in which a set of plants was sprayed with one of the following solutions: water, 100, 30 or 3 mg/l IAA, 100, 30 or 3 mg/l TIBA or 10 mg/l of NAA. Plants which had not flowered by the time the experiment was terminated were dissected to determine the stage of floral development. The plants were photographed (Figure 22) at the end of the twenty-one day threshold treatment before they were moved to the greenhouse for additional growth under SD conditions.

The photograph reveals a general promotion of elongation by IAA application. The most spectacular response occurs at intensity B. At this intensity, at which the controls show no elongation and 3 mg/l of IAA show only slight elongation, the plants treated with 30 and 100 ppm of IAA average respectively 8.0 and

Figure 22. The appearance of Silene plants after 21 days treatment at the threshold. Intensities A, B, and C are shown in a vertical direction. The water controls and plants treated with 3, 30 and 100 mg/l IAA are shown in that order from left to right. (Highest intensity at A.)

10.9 cm. in height. Table 32 confirms these observations in a more quantitative way.

The first point to be noted is the effect of auxins upon time of elongation. The response at Intensity A is almost identical with that found in the previous experiment under LD conditions, i.e., that the elongation is markedly accelerated in time by auxin and the degree of this acceleration is dependent upon the concentration of auxin. In this experiment TIBA at intensity A caused a slight retardation of elongation especially at the higher concentrations whereas it was without any effect in the previous experiment. The effect of auxin is qualitatively the same at Intensity B. Thus the number of days to the beginning of elongation is reduced by about 370% at the highest concentration of IAA, whereas at the lowest concentration (3 ppm) there is essentially no effect. Again TIBA shows but little effect. The differences at intensities C and D are most pronounced for here where the controls or TIBA treated plants did not appear to elongate IAA at 100 and 30 ppm and NAA at 10 ppm were all quite effective in causing elongation. The interpretation here then is that auxins have a promotive effect upon elongation. This promotive

TABLE 32

The effect of auxins and anti-auxins upon the flowering of Silene grown at threshold conditions. S-11.

			IAA			TIBA			NAA
	INT.	H_2O	100	30	3	100	30	3	10
Days to Elongation	A	9.3	5.4	7.4	10.2	11.8	10.8	10.4	5.0
	B	22.5	6.0	14.5	19.3	20.7	22.0	17.5	5.4
	C*	-	9.0	13.0	-	-	-	-	5.0
	D*	-	6.4	9.4	-	-	-	-	5.8
Days to Visible Buds	A	28.6	30.3	28.0	29.8	31.4	28.0	died	died
	B	40-50	40.0	40-45	40-45	40-45	Veg.	40-50	died
No. of Plants Flr./ Treated	B	2/10	4/5	4/5	1/5	3/5	-	2/5	-
Increase in Lf. No.	B	17.4	17.9	21.4	17.4	17.4	21.4	23.4	-
Height	A[a]	13.1	12.0	10.1	15.3	13.1	11.2	12.4	-
	B[a]	5.8	15.0	13.0	5.7	7.7	3.8	9.1	-
	C	3.75	6.9	4.2	4.5	-	3.3	4.9	-
	D	3.4	6.0	3.7	3.6	2.0	3.3	-	-

* All plants at intensities C and D were vegetative. These were dissected 1-5-52.

a A and B dissected 12-4-51.

effect of auxin is confirmed by the data on the final heights of the plants.

What is the effect of auxins and anti-auxins applied under threshold conditions on flowering itself? The data from this experiment show only a quantitative difference in flowering behavior between treated and untreated plants. All plants flowered at about the same time at the highest intensity (A) irrespective of the treatment, except that the plants treated with NAA died before the LD treatment had ended. At intensity B only the plants which had received 100 ppm of IAA had produced visible buds at the end of 40 days, however, two of the 10 control plants had produced buds which were detectable on dissection. The increased percentage of flowering of the treated plants appears significantly greater than the controls but further experiments were set up to study more closely the possible effects of auxins on flowering.

Plants for this experiment were grown under the same conditions as those for the previous experiment. They were moved to the basement darkroom on 1/5/52 and sprayed for 3

days under SD before being put under threshold conditions for treatment. The 15 watt bulb was turned on, 1/8/52, and the plants sprayed daily at 1600 hours during the experimental treatment. Three intensities and several different auxins were used in this experiment. No data on dates of elongation or numbers of leaves were taken since it was desired to get only a qualitative measure of the flowering response. One half of the plants were returned to SD after 7 days under the threshold conditions, the other group remained for 14 days and was then also moved to SD to grow. The plants were dissected after 30 days from the beginning of the threshold treatment and the appearance of the terminal buds noted. Table 33 presents the essential data from this experiment. It may be seen in Table 33 that at intensity A there is only a slight difference between treated and untreated plants with the exception that those which received 3 ppm of either 2,4-D or 2,4,5-T were somewhat inhibited. IAA at 300, 100 and 30 ppm showed a slight increase in intensity of flowering above the controls after only 7 days of treatment. These differences at A are only quantitative but at intensity B

there is both a qualitative and a quantitative difference between treated and untreated plants. The qualitative difference occurs in the series which received only 7 days at the threshold. Here where none of the controls flowered, one plant sprayed with 100 ppm of IAA and 3 plants sprayed with 30 ppm of IAA flowered. After 14 days at the threshold 2 controls flowered whereas five each at 100 and 30 ppm of IAA flowered. Thus after 14 days treatment the qualitative difference as found in the previous experiment was maintained but by using shorter treatment times in this experiment it has also been possible to show that auxin is able to cause the initiation of flowers under conditions in which the controls remained vegetative.

Experiments were set up to discover whether this promotion of flowering by auxins is specific in nature, i.e., to determine if there are differences in the number of leaves produced by treated and untreated plants before floral initiation begins.

Six weeks old Silene plants were moved to the basement darkroom where they were allowed to remain for two days

TABLE 33

Promotion of flowering in Silene by applied auxins. Data shown as number of plants flowering as a result of 7 or 14 days treatment at threshold. Six plants per treatment. S-13.

Treatment	17 Days at threshold Intensities A	B	C	14 Days at threshold Intensities A	B	C
H_2O	4*	0	0	6	2	0
IAA 300 ppm	5	-	0	-	-	0
IAA 100 ppm	6	1	0	-	5	0
IAA 30 ppm	6	3	-	-	5	-
2,4-D 1 ppm	4	-	-	-	-	-
2,4-D 3 ppm	2	0	0	-	1	0
2,4,5-T 3 ppm	0	0	0	-	-	-
2,4,5-T 1 ppm	3	-	-	-	-	-

* Numbers represent plants flowering after indicated number of days of treatment at threshold.

before treatment. In contrast to previous experiments the plants were not sprayed before threshold conditions were begun. The lighting conditions for this experiment were also changed slightly in that the high intensity light was increased from 450 FC to 700 FC. Of necessity the intensity of supplementary light was decreased. The plants were treated under threshold conditions for 10 or 23 days with either H_2O, IAA, TIBA, or 2,4-D at various concentrations. At the end of these experimental periods the plants were moved back to SD conditions until the experiment was terminated. Table 34 presents the results on the increase in the number of leaves formed by plants under the different treatments.

The data suggest that the plants treated with 30 mg./l of IAA at intensity A produced fewer leaves before floral initiation than did the controls. The data for days to elongation and for final height which are given in Table 35 confirm the earlier results with Silene, namely, they show that auxins produce a marked effect upon elongation. Thus it is observed that the controls elongate only at the highest intensity (A) of supplementary

TABLE 34

Effect of auxins and an anti-auxin applied at threshold on increase in leaf number and floral initiation in Silene. S-14.

Days at Threshold	Intensity	H_2O	IAA mg/l. 300	IAA mg/l. 100	IAA mg/l. 30	TIBA mg./l 100	TIBA mg./l 30	2,4-D mg./l 0.1	2,4-D mg./l 0.5
10	A*	16.1	-	16.3	13.1	-	-	-	-
10	B	26.6	-	29.7	25.4	-	-	-	-
10	C	28.2	-	30.7	30.6	-	-	-	-
10	D	30.0	31.1	31.0	-	-	-	-	26.0
23	A*	12.1	-	12.6	11.1	12.6	13.6	-	12.3
23	B	26.2	-	27.9	31.6	22.6	22.2	-	20.9
23	C	25.4	-	23.2	29.6	23.7	24.4	-	26.1
23	D	28.0	28.3	31.1	-	-	-	24.0	23.8

* Only those plants at intensity A flowered.

light whereas the auxin treated plants begin elongation at approximately the same time at all intensities. It appears that TIBA at 30 or 10 mg./l is without effect either on flowering or upon elongation. The decrease in numbers of leaves caused by TIBA at the lower intensities (Table 34) is probably related to a decrease in growth rate brought about by the anti-auxins, since at the highest intensities there is no apparent inhibitory effect of TIBA on elongation.

Let us now summarize briefly what the foregoing experiments have revealed about the general relations of auxins to the flowering response of Silene.

It appears relatively clear that auxin has both a qualitative and a quantitative effect upon the flowering of Silene. Data presented in Tables 32 and 33 show that near the threshold IAA causes a greater number of plants to flower than would normally flower. Data of Table 33 also show that when plants are grown for only 7 days at the threshold, IAA causes flowering whereas the untreated controls remain vegetative. The data of Table 34 reveal that plants treated with IAA under threshold conditions initiate flowers earlier, than do the untreated controls. Thus it is logical to conclude that auxin must have a positive and specific

TABLE 35

Final height and days to elongation of Silene treated under threshold conditions.

Days at Threshold	Intensity	Water Control	CONCENTRATIONS OF GROWTH SUBSTANCES EXPRESSED as mg./l IAA 300	IAA 100	IAA 30	TIBA 100	TIBA 30	2,4-D 0.1	2,4-D 0.5
			FINAL HEIGHT						
10	A	12.3	-	13.2	11.1	-	-	-	-
	B	1.2	-	3.3	3.6	-	-	-	-
	C	1.3	-	3.5	2.2	-	-	-	-
	D	0.9	3.0	2.0	-	-	-	-	0.9
23	A	20.3	-	13.6	17.7	17.6	17.0	-	20.4
	B	1.5	-	7.2	5.1	1.4	1.7	-	3.5
	C	1.3	-	4.2	3.4	1.6	1.8	-	4.2
	D	0.9	4.5	3.4	-	-	-	1.6	1.6
			DAYS TO ELONGATION						
10	A	7.5	-	7.2	6.8	-	-	-	-
	B	*	-	7.2	7.3	-	-	-	-
	C	*	-	8.5	7.5	-	-	-	-
	D	*	7.7	8.8	-	-	-	-	*
23	A	7.8	-	6.2	6.7	7.6	7.5	-	7.0
	B	*	-	7.5	8.2	*	*	-	10.1
	C	*	-	8.2	8.0	*	*	-	10.1
	D	*	7.7	8.8	-	-	-	*	*

* These plants never appeared to elongate during the course of the experiment.

effect on the process of floral initiation in Silene.

It is also clear from the data of Tables 31 and 32 and 35 that under either LD or threshold conditions application of auxin promotes the elongation of the central stem axis. This is evidenced in two ways. In the first place plants which have been sprayed with auxin begin to elongate before the control plants. This is particularly true of threshold treatment if the plants have been sprayed for two or three days prior to the beginning of the experimental period. The faster elongation is evident at all intensities but is most striking at intensity B which is apparently very near the threshold for flowering. Table 32 shows that plants treated with auxin at this intensity elongate from three to sixteen days sooner than the untreated plants. The promotive effect of auxin on elongation is also reflected in the final heights of the treated plants. Table 36 presents a summary of data from all experiments on the absolute increase in height caused by auxin at the different intensities.

These data together with those on time of elongation, leave little doubt that auxin plays some specific role in the stem elongation

TABLE 36

Effect of auxin treatment on elongation of Silene plants.

Height of treated minus height of control plants.

Auxin	Concentration mg./l	Intensity Station B	C	D
IAA	300	-	3.0 cm.	2.9 cm.
	100	5.7 cm.	2.7	2.1
	30	4.1	1.1	0.3
	3	0.0	0.7	0.2
2,4-D	3	7.4	2.3	-
	0.5	2.0	2.8	0.8
2,4,5-T	3	2.9	0.0	-

process of Silene. There appears to be some interrelation between the amount of supplementary light which the plants receive and the amount of auxin necessary to give marked elongation. Thus at intensity B the application of 100 ppm IAA causes an increase in elongation of 5.7 centimeters. per plant, while 30 parts gives increased elongation of 4.1 centimeters and 3 ppm is without effect. The same picture prevails at intensities C and D, i.e., there is a decrease in elongation with decreasing concentration of IAA. It is obvious too that at a given concentration of IAA there is decreased effectiveness as the plants are further removed from the light source.

The over-all view of auxin in the flowering response of Silene is, then, that auxin has a specific effect upon the flowering response of plants treated under threshold conditions. Furthermore, there appears to be some direct relationship between the low intensity light process and auxin since auxin is able to replace the effect of light especially insofar as elongation of the central axis is concerned.

3) Experiment with Hyoscyamus. In view of the promising results obtained with auxin application to Silene it became of interest to find out the response of annual Hyoscyamus niger to auxins and anti-auxins applied under threshold conditions.

Plants of Hyoscyamus were grown under an 8 hour photoperiod in the Phytotron at 23° or 20° C. day temperature and either 17° or 20° C. night temperature until the plants were approximately 9 weeks old. They were randomized into 40 groups and put under cycles of 8 hours of high intensity light daily plus 16 hours of supplementary light under threshold conditions. The threshold was approached by placing groups of plants at different distances from a 15 watt bulb suspended 4 feet above the surface on which the plants were sitting. Plants were sprayed daily at 1600-1630 hours with various concentrations of TIBA or IAA to which a wetting agent (Tween 80 or Tween 20) had been added. All plants at the highest intensity had begun to elongate by the 17th day of treatment when they were moved to SD in the greenhouse. All other plants were treated for 25 days at the threshold before

being moved to SD. Table 37 presents the results obtained with IAA. The data for TIBA are impossible to interpret at this time and have, therefore, been omitted.

The data show that IAA is able to cause the initiation of flower primordia under conditions in which the control plants remain vegetative. Thus at intensities C, D, and E where none of the controls flowered, from 16 to 100 per cent of the IAA treated plants flowered. A concentration of 10 mg./l. IAA appears to be optimum at most of the light intensities used. That this effect on flowering is somewhat specific can be seen by comparing the numbers of leaves produced by control and treated plants. At intensity station C the controls produced 28.4 leaves during the experimental treatment and were still vegetative whereas plants sprayed with 30, 10, 3, or 1 mg./l. of IAA had produced approximately 20, 17, 17, and 17 leaves respectively. This difference is more marked at intensity D where the controls had produced an average of 33.3 leaves and flowering plants treated with 10 mg/l. IAA had produced only 17.4 leaves.

TABLE 37

Induction of flowering in Hyoscyamus niger (annual) by IAA applied under threshold conditions. Plants dissected by 6 weeks from beginning of threshold treatment.

Light Intensity (FCM)		Data Recorded	H_2O Control	IAA in mg/l 30	IAA in mg/l 10	IAA in mg./l 3	IAA in mg./l 1
218	(A)	Leaf number increase	7.3	11.8	10.9	10.4	7.9
		Plants flowering/ Treated	6/6	6/6	6/6	6/6	6/6
71	(B)	Leaf number increase	13.9	15.4	12.9	13.9	13.6
		Plants Flowering/Treated	6/6	4/6	7/7	7/7	7/7
34	(C)	Leaf number increase	28.4	19.9	16.6	16.5	17.2
		Plants Flowering/Treated	0/4	3/5	7/7	7/7	5/7
20	(D)	Leaf number increase	33.3	23.8	17.4	23.9	33.0
		Plants Flowering/Treated	0/6	0/5	5/6	0/4	0/7
16	(E)	Leaf number increase	34.8	30.8	20.9 [a]	16.6 [b]	12.6 [c]
		Plants Flowering/Treated	0/4	0/6	3/7	1/7	1/7

a Veg plants averaged 26.6 leaves.
b Veg plants averaged 30.1 leaves.
c Veg plants averaged 32.4 leaves.

Another fact which is evident from the table is that at the highest intensity, A, 30, 10, and 3 ppm IAA were actually slightly inhibitory to flowering as measured by the number of leaves formed before flowers were initiated. At Intensity B only 30 ppm shows a retardation, and this concentration still shows a slight inhibitory effect at Intensity C where only 3 of 5 plants flowered. Ten ppm appears to lie very near the optimum concentration at all intensities except the highest.

III. Summary and Conclusions.

The experiments reported above have shown that the flowering of Silene and Hyoscyamus is promoted in a specific way by auxins applied under threshold conditions. Data have further shown that Silene sprayed with IAA elongates much sooner than untreated controls and also elongates under conditions in which the controls remain in a rosette. Data obtained with Hyoscyamus in experiments not reported here show that this LDP also elongates in response to applied auxin. With both plants there appears to be a relation between the amount of supplementary light which the plants receive and their responsiveness to applied auxin.

In view of this evidence one is led to speculate whether the low intensity light process in LDP and SDP may not be identical. In both types of plants the action spectrum for the process is the same. In SDP it has been demonstrated that this process is related to auxin in an apparently direct manner, thus it is logical to assume that auxin may be in some way related to the low intensity light process in LDP. It is possible then that the low intensity light in both cases serves to keep the auxin level high. If this were true then LDP and SDP would be dissimilar only in their response to auxin, i.e., LDP would require a minimum level of auxin to consummate the processes leading to induction, whereas SDP would be inhibited by auxin levels above a certain critical. It would be interesting to know whether the inhibitory level in SDP and the stimulatory level in LDP are approximately equal, but unfortunately data are not available at the present time to answer this question. This is indeed a very attractive thought for if it were true we would at last be able to gain a unified picture of part of the photoperiodic response of the two until now totally different reaction types.

Only much more detailed analyses of the response of LDP to auxin application and studies of the kinetics of auxin fluctuations in LDP will reveal the validity of this thought. In spite of all of the uncertainties which still confront us, however, it is quite clear that auxin has a promotive role in the flowering of at least two LDP, Hyoscyamus niger and Silene Armeria.

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