FLORAL INITIATION

IN CESTRUM NOCTURNUM

(THE NIGHT BLOOMING JASMINE)

Thesis by

Roy Monroe Sachs

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ABSTRACT

1. The requirement for floral initiation in Cestrum nocturnum is satisfied if plants receive long days followed by short days. Continuous short or long days or short days preceding long days are ineffective.

2. A minimum of 5-7 long days followed by two short days is required for the production of flowers.

3. The sequence of reactions necessary for floral initiation in Cestrum is as follows:

long days short days
---------- L ---------- S (floral stimulus)

a. "L" is a heat labile substance produced in leaves as a result of long-day induction and required for short-day induction. It is not translocated from the leaves.

b. "S" is a heat labile substance, possibly identical with the floral stimulus, formed as a result of short-day induction. It is formed only in leaves which have previously received long-day induction and is translocated to the growing points.

The production of "L" in other short day plants is controlled by daylength independent reactions, and the production of "S" is controlled by daylength independent reactions in other long day plants.

4. For Cestrum the critical day and night lengths for long and short-day induction, respectively, overlap in the 11.5 to 12.5-hour photoperiod range; the low efficiency of the two processes in this range precludes the possibility of an intermediate daylength satisfying both requirements. Other plants, flowering only in intermediate daylengths, may be related to Cestrum in requiring long followed by short-day induction. However, the critical photo- and nyctoperiods, in these plants, overlap sufficiently to permit simultaneous satisfaction of both requirements in an intermediate daylength.

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LIST OF ABBREVIATIONS

1. pl., pls. - plant, plants
2. LD, LDP, LDs - long day, long-day plant, long days
3. SD, SDP, SDs - short day, short-day plant, short days
4. # - number of
5. lvs. - leaves
6. vis. - visible
7. % fl. - per cent flowering
8. % term. fl. - per cent of plants with terminal flowers
9. # nodes fl./pl. - number of nodes flowering per plant (Total number of nodes with flowers divided by the number of plants)
10. # nodes fl/pl.fl. - number of nodes flowering per plant flowering
11. ^{o}C. - degrees Centigrade
12. art. - artificial
13. nat. - natural
14. ft. cand. - foot candles
15. hrs. - hours
16. pp. - photoperiod
17. MAF - most advanced flower
18. rem. - remaining
19. lgst.- longest

I. Introduction

Floral initiation is of general interest to biologists, regardless of their specialty, since the fundamental problem of tissue differentiation is concerned. Probably, because we are largely ignorant of the processes causing differentiation, there is no simple and all-inclusive definition. One generally refers to differentiation in contrast to growth, i.e., distinct from all the processes resulting in an increase in size of cell, tissue, or organism. Differentiation is the end result of processes leading to a change in cell (tissue or organism) composition and/or structure.

In all plants flowers are produced at apical and axillary meristems (the growing points), the same loci as are involved in the production of leaf and stem structures. A growing point can produce both leaves and flowers but not simultaneously. The onset of flowering must be preceded by a changeover from leaf production. This change, called floral initiation, is the differentiation of growing points from leaf to flower producing regions. Since the flower contains the reproductive apparatus and the leaves and stems are called the vegetative organs of plants, the differentiation processes causing floral initiation involve the transition from a vegetative to a reproductive mode of development.

Though the development of leaf and stem tissues is also an example of differentiation, it differs from that of floral initiation in that it is, as yet, not subject to adequate separation from the normal growth of the plant (tissue culture studies excepted). All plants, if they grow at all, produce roots, stems, and leaves. In contrast to this requirement for vegetative differentiation some plants may be prevented from producing

reproductive organs by creating environments which specifically inhibit floral initiation, though supporting excellent vegetative development.

In most cases in which tissue differentiation has been studied the greatest impetus for research has come from attempts to control the processes. The study of floral initiation has been no exception. Since the environmental controls which permit the separation of the vegetative and reproductive phases of development are simple and exact, floral initiation has been studied intensively.

In 1920 Garner and Allard (26) published the first report on the specific effect of the daily light regime in controlling floral initiation, and at this time the authors stated that the literature pertaining to the effects of light on plant growth was so large as to make a detailed review prohibitive. Since this time the literature on floral initiation, and the effects of light upon the process, has grown enormously, and were it not for the number of excellent reviews in this field the following literature survey would not be possible.

For much of his information the author has referred to reviews by Lang (41), Naylor (51, 52), Melchers (46), and Bonner and Liverman (10).

II. Literature Survey

A. Historical

Credit is generally given to Garner and Allard for first elucidating the principles of the effect of light in controlling floral initiation although both Klebs and Tournois (52), some years prior to 1920, found that flower production was greatly affected by the daily duration of light. Garner and Allard formulated many of the principles which form the basis for present-day studies of photoperiodism, a field

which now includes phenomena outside the scope of floral initiation. They defined photoperiodism as the effect of the duration of light on the growth and development of plants, specifying the word "duration" as distinct from intensity or total radiant energy (26).

In their 1920 paper, they were concerned mainly with the seasonal behavior of certain varieties of tobacco and soybean. Maryland Mammoth tobacco grown outdoors, in the latitude of Washington, D.C., maintained the vegetative state throughout the summer growing season but developed flowers during the winter months when brought into the greenhouse. Two of their soybean varieties, Peking and Biloxi, flowered at the end of summer regardless of the planting date or the amount of vegetative growth attained. It was discovered that both the tobacco and soybean plants could be forced to flower simply by shortening the natural summer day length by a few hours. This finding separated the effects of light on vegetative growth from that on flower production and established the first clear control of floral initiation. In further studies they found that reducing the number of consecutive hours of light by mid-day darkening, giving two short light periods daily, was ineffective for the induction of flowering in those varieties which would respond to a single daily exposure of light of the same total energy (25). They concluded that it was not the total quantity of radiant energy that was important in producing the formative effects of light but the time at which the light was given and the duration of single light periods.

These experiments created the field of photoperiodism and directed much of the future research on floral initiation, which, since 1920, has been mainly concerned with interpreting photoperiodic controls in terms of plant metabolism.

In addition to the effects of light on floral initiation it has been known since 1913, from the work of Klebs (52), that some plants require low temperature treatments, sometime during their vegetative phase of growth, before they will initiate flower primordia. Working with the storage roots of beets, Klebs found that these plants would not flower during the summer if they had not received a cold treatment the preceding winter. Gassner extended these observations to include many other plants, including spinach, cabbage, rutabaga, and carrot, and noted the stimulating effect of low temperature treatments on the flowering of spring and winter cereals.

The studies of these two men formed the basis for the field of vernalization which is defined as the effect of low temperature periods on the growth and development of plants (by low temperature no absolute value is implied, rather the effective temperatures are generally lower than the temperature optima for subsequent growth and differentiation.)

Floral initiation is one aspect of photoperiodism and vernalization. It is convenient to discuss the fields separately and then unite them in single schemes if possible.

B. Vernalization

Though vernalization is concerned with the general effects of low temperature treatments on plant growth and development, we are concerned only with the experiments which refer specifically to the initiation of flowers. Plants may be categorized on the basis of their response to low temperature treatments (41):

1) Biennial plants germinate and attain a certain amount of vegetative growth during their first growing season. They overwinter in the form of rosettes and flower during the following growing

season. These plants have a qualitative low temperature requirement for floral initiation, i.e., they will not flower without this treatment regardless of the conditions during the succeeding stages of growth. It has been found that it is the low temperatures and not the short day-lengths which are responsible for the overwintering requirement. (Beet, Hyoscyamus, celery, cabbage, etc., are examples of such biennials).

2) Winter annual plants are sown during the late fall and begin germination before winter sets in. They overwinter as seedlings and flower the following spring or summer. Plants in this group have a quantitative low-temperature requirement, i.e., the low temperature treatment reduces the time to flowering, but floral initiation will occur whether or not the cold treatment is given. Most winter varieties of cereals are examples of this class.

3) Summer annual plants have no low temperature requirement for floral initiation although other environmental factors may be important.

4) Perennial grasses, such as Lolium, require low temperature induction every winter to insure floral initiation every year.

The cold treatment for vernalization has an optimum temperature which varies from one plant to another. For example, winter rye is most effectively treated by temperatures between 1 and 4°C. Hyoscyamus shows a 10° optimum for 7 day treatments, 6° for 42 days, and as low as 3° for 105 days (41). As the treatments are extended the optimum curve is broadened and includes lower temperatures. It is apparent from this and previous discussions that low temperatures need not be continued throughout the life of a plant in order to exert their effect, and a plant is said to be induced after some effective treatment regardless of its duration.

One interpretation of these results is that low temperature is not effective in increasing the rate of a chemical reaction, rather it differentially decreases the rate of two reactions. It can be assumed that there is an inhibitory as well as a stimulatory reaction, present in both biennials and winter annual plants, affecting floral initiation. The inhibitory reaction has a higher temperature coefficient than the stimulatory and is, therefore, more reduced in activity. The net result of low temperature is to inhibit differentially the unfavorable reactions(41). After vernalization a plant may be de-vernalized by high temperature treatments which become more effective as the temperature is increased and as the time after vernalization is decreased. In winter annuals it becomes impossible to de-vernalize plants after extended low temperature treatments, and for Hyoscyamus de-vernalization is impossible if vernalized plants are kept for five days at room temperature after vernalization.

The fact that de-vernalization loses its effectiveness in time and that all vernalizing temperatures become equally effective if they are prolonged suggests that some substance is formed as a result of the cold treatment. Definite proof of an end product of limited stability came when it was discovered that the effect of a vernalization treatment could be transmitted through grafts from vernalized to non-vernalized biennials (46).

The locus for perception of the cold treatment is the growing point alone. In the case of cereals low temperature may be perceived by the embryo as well as the shoot tissue, but biennial Hyoscyamus is relatively unaffected by treatments to the germinating seed (41) and requires a certain age to reach maximum sensitivity.

Very little is known about the chemical reactions involved in the production of the stimulus other than the fact that oxygen is required for the low temperature treatment to be effective. Excised embryos of cereals may be vernalized but they require an external source of sugar. The first two weeks of low temperature treatments of embryos are without vernalizing effect; the lag period disappears if the embryos are left attached to the endosperm for the first few days but cannot be removed by any treatment at room temperatures. This suggests that there is some temperature independent process, such as the diffusion of precursors from the endosperm to the embryo, required in the synthesis of the vernalization stimulus.

Recently Highkin (personal communication) has succeeded in extracting from pea seeds a mixture of substances which, when applied to non-vernalized seeds of peas and winter rye, partially replaces the requirement for low temperature. The identification of the active constituents of this aqueous extract is in progress.

The fact that the effect of low temperature treatment of embryos of winter annuals persists throughout the life of the plant after many cell divisions suggests that the vernalization stimulus is self-reproducing or the mechanism involved in its synthesis is self-reproducing. Though no information has been obtained as to the chemical nature of the vernalization stimulus, the only materials which are known to be self-reproducing are nucleo-proteins (or nucleic acids). [It will become evident during the course of this literature survey that many substances involved in cellular differentiation are thought to be nucleic acid containing compounds and there is no reason to exclude the possibility that the vernalization stimulus may fall in this class of compounds.]

There is one fact which suggests that the vernalization stimulus may be common to many plants. One is the fact that summer annuals which flower without a cold treatment, can transmit the vernalization stimulus to non-vernalized biennials across a graft union.

Many biennials not only require a cold treatment for floral initiation but, in addition, long photoperiods following vernalization (41, 46). The sequence of vernalization followed by long photoperiods is absolutely necessary, and for this reason it has been suggested that the vernalization stimulus may be a precursor for photoperiodic reactions and not directly responsible for the transition of growing points from the vegetative to the floral state.

C. Photoperiodism

Since 1920 the emphasis that Garner and Allard placed on the duration of the light period has been reduced, and has been shifted to the associated dark periods. Photoperiodism is still defined as the study of the effects of duration and time of application of light on the growth and development of plants, but it is now thought that the special effects of light are more concerned with inhibiting dark, rather than promoting light, reactions. In general the whole field of photoperiodism, particularly as it pertains to floral initiation, has become a good deal more complex, and in some cases partial processes have been discovered which make simple definitions very difficult.

Garner and Allard recognized that plants could be categorized according to the effect of light duration on the initiation of reproductive structures, and they created three categories designed to include all plants (41).

1) Day-neutral, or indeterminate, plants are those which flower according to the age of the plant and are not specifically affected by daylength in the initiation of flowers.

2) Long-day plants (LDP) are promoted to flower by increasing the duration of the light period. However, it is known that LDP will flower if a long dark period is interrupted with a small amount of light (59), indicating that it is not the duration of the light, but that of the dark period which is effective in controlling their flowering. Probably the most satisfactory definition of long-day plants is one based upon the fact that they have no dark requirement. All long-day plants investigated to date flower most readily in continuous light, any amount of dark being inhibitory. Some plants will not flower at all if they do not receive long light or short dark periods, and are termed qualitative LDP. In others flowering is promoted by long days but will occur in any daylength; these plants are called quantitative LDP. In some cases there is a critical daylength below which there is no effect upon floral initiation, whereas in others there is a continuous stimulatory response of increasing daylength with no sharp break in the curve of flower response vs. photoperiod.

3) Short-day plants (SDP) are promoted to flower by short light periods although there is a lower limit to the amount of light which is necessary for floral initiation. As in the case of LDP it has been shown that it is the length of the accompanying dark period, and not the duration of light, that is specifically responsible for the effectiveness of short-days. There are both qualitative and quantitative SDP with and without critical daylengths (dark period length).

4) Intermediate-day plants

In 1938 Allard (3) described the flowering behavior of four plants all of which had one common characteristic. Flowering was either completely or partially inhibited by daylengths too short or too long, i.e., there was only a narrow range of daylengths within which these plants would produce flowers. The most spectacular example was sugar cane (Saccharum spontaneum) which flowered only when the daylength was 13 hours, above or below this figure the plants remained completely vegetative.

5) Long-short day plants

Recently Resende (60) has reported that three plant species will produce flowers only if they receive long-days followed by short-days. No indication is given as to whether or not the long and short-day requirements are true photoperiodic phenomena or as to the stage of flower development that is affected by the transition from long to short-days. It is possible that floral initiation occurs in long-days and floral development in short-days. The possible relationship between categories 4) and 5) will be discussed later in light of the present findings with Cestrum nocturnum.

6) There is one plant known, Madia, which is stimulated to flower in very long or very short photoperiods but not in intermediate daylengths (Went, personal communication, 76).

7) Short-long day plants

Wellensiek (21a, 69) working with Campanula Medium, has found that short days greatly accelerate attainment of the stage at which these plants will respond to long-days (required for floral initiation). The short-day requirement may be replaced by suitable

low-temperature treatment. This case may be closely related to the cases of short-day vernalization as reported in Lang's review.

In all cases of floral initiation controlled by photoperiodic phenomena, it has been found that the light regime required to cause initiation need not be continued up to the time of transition of the growing point. Since the photoperiodic treatments are not required continuously, it is assumed that the affected plants have undergone some permanent change, they are said to be in an induced state as a result of an act of induction (the photoperiodic treatment).

The following discussion is modeled after Lang's review in order of presentation of facts pertaining to the photoperiodic induction of floral initiation.

1. The product of photoperiodic induction

Floral initiation in both long and short-day plants is dependent upon the photoperiodic treatment of the leaves (27), and there is little or no effect of subjecting the growing points to the inductive treatments (41). Since it is the growing points of plants which produce flowers, one must postulate a substance to transmit the photoperiodic effects from the leaves to the meristems. This substance is termed the floral stimulus although it is not known that the substance transported from the leaves is the final product causing differentiation. It is possible to think of the floral stimulus as the absence of an inhibitor which is normally produced in the leaves under non-inductive conditions preventing floral initiation. There is evidence that floral initiation is caused by a promoting stimulus and not by the absence of an inhibitor.

In some cases exposure of a single leaf to the proper daylength may cause initiation of flowers throughout the whole plant even though the

rest of the leaves are kept under non-inductive conditions (12, 62). If an inhibitor is produced in non-inductive conditions one would expect that systemic flowering would be prevented in the above case, and for this reason it is not generally believed that flowering is caused by the disappearance of an inhibitor.

If an induced plant is grafted to a non-induced plant, the receptor will flower when kept in non-inductive conditions, showing that something necessary for flowering was transmitted across the graft union - further evidence that flowering is not due to the removal of an inhibitor.

Grafting experiments have shown that the floral stimulus may be transferred from LDP to SDP and vice versa (21b). Though one is not certain that the stimulus is identical for both photoperiodic types, these grafting experiments indicate that some stages in the formation of the floral stimulus are the same for both. Experiments to be reported in this thesis show that there is probably a sequence of reactions leading to the synthesis of the floral stimulus.

That there is a floral stimulus (or precursor) common to plants regardless of their photoperiodic requirements has been shown in grafts of day-neutral to short-day species. In these experiments the day-neutral plants were used as donors and the short-day species as receptors kept on non-inductive conditions. The receptors flowered if the donors flowered indicating that there was a transmission of some stimulus across the graft union which freed the receptors from any day-length requirements (41, 33).

Though Lona has reported that it has been possible to induce detached leaves of plants and then use these leaves as donors of the floral stimulus in grafts to receptor plants, Carr has recently re-opened the

question by some experiments with soybean and Xanthium plants (18, 19). He found that it was impossible to induce flowering in either of these two plants by inducing detached leaves even though the grafts were completely successful. In other experiments he de-budded and decapitated plants prior to giving them their inductive treatments and afterwards grafted terminal cuttings to serve as receptors. None of the plants flowered. Carr has interpreted these results as support of Gregory's contention that the precursors for the stimulus are made in the leaves and then stabilized in the meristematic region, and further that there is no storage of these precursors in the leaves.

However, these experiments are open to serious objection since no control grafts are reported, i.e., the controls in which flowering was caused by induction of graft leaves immediately after union, and some days afterwards, or the induction of terminal cuttings immediately after grafting. In short there was no indication given that the reason for failure of these experiments was the failure of transmission of substances across the graft union. It is well known that the stimulus has a limited stability and will decay in a relatively short time. Salisbury (62) has shown for Xanthium that if there are no actively growing buds present within six days after induction then no floral initiation is possible. Auxin applications will replace the growing-bud requirement if given within two days after the induction treatment, and are completely ineffective if given after six days. Perhaps Carr's results may be explained on the basis of lack of stimulus after the graft union is completed; in any case, until further experiments are done, there is no reason to discard Lona's experiments showing that the stimulus is synthesized in the leaf.

Carr summarized the paper of Zhdanova on experiments with Perilla and cited her results as indicating that the terminal meristematic regions are active in transforming the stimulus. She removed the youngest leaves of plants and subjected the remaining leaves to two inductive cycles. The leaves received the treatments either simultaneously or each leaf was treated in turn, beginning with the uppermost leaf and proceeding towards the base. Immediately after receiving the second inductive cycle the leaf was removed. Plants in which the leaves were treated simultaneously did not flower, whereas those that received successive leaf induction flowered. This implies that the stimulus is translocated from the leaves and summated at the apex, and that it is possible to saturate the summation apparatus (as in the case of simultaneous induction). Carr interpreted these experiments as indicating that the terminal apex is active in stabilizing the stimulus and that no storage is possible in other organs. There is another explanation that may deserve consideration. It is possible that there is more than one stimulus produced in the leaves and these stimuli must be supplied in successive steps coinciding with different phases of floral initiation.

Summary

It has been established that there is a transmissible stimulus responsible for floral initiation and that its production is controlled by daylength dependent reactions occurring within the leaves. There is some evidence to suggest that the growing point is engaged either in some final stages of its synthesis or summation.

2. Day-Neutral Plants

A plant must attain a certain age before it will develop flowers, at this age it is said to be ripe to flower. Day-neutral plants

may be thought of as having as their only requirement for floral initiation the attainment of the ripeness to flower stage. Consequently any discussion of day-neutral plants involves a discussion of "ripeness-to-flower", and the two subjects are probably closely related from a physiological viewpoint.

Photoperiodically sensitive plants, with respect to floral initiation, most clearly show this ripeness to flower, in that prior to some definite number of nodes some plants show no response to inductive treatments. Xanthium (62) and Baeria (66) will flower at very early stages and it has been shown that the cotyledons of both of these plants are sensitive to photoperiodic induction. The sensitivity to photoperiod increases with age of the plant up to a certain maximum value as shown by Borthwick and Parker (12) for Biloxi soybean. The third compound leaf formed was found to be maximally perceptive to SD, to the extent that exposure of this leaf was as effective in causing floral initiation as was exposure of the whole plant. Similarly when Xanthium has reached full sensitivity, induction of one leaf will cause the same level of initiation as will the whole plant (35, 62).

Naylor has summarized the literature pertaining to ripeness to flower (52) and one may conclude that there is no consistent criterion that can be applied to all plants; some plants lose their requirement for a specific daylength as they increase in age, e.g., winter rye will flower on SD after about 200 days though prior to this time it behaved as a LDP. Other plants retain their sensitivity for extremely long periods of time as in the case of Sedum, Xanthium, and Cestrum.

One strain of popcorn was found to have flower primordia in the seed (52), whereas in the case of the tangelo (22) as many as 20 years may pass before the trees will flower.

Ripeness-to-flower is, perhaps, the most mysterious phenomenon of photoperiodic induction, particularly since no controls have been found which influence this factor.

3. Long-Day Plants

Long-day plants are stimulated to flower by extending the duration of light in their environment.

There is some question as to what the function of the light period is in LDP. It is known that at least part of the light constituting a LD may be given as low-intensity light without losing the effectiveness of the LD; Spinach and aster (72) will flower if they receive one foot-candle of light continuously throughout a 15-hour "night", as a supplement to nine hours of high intensity light. Any further increase in light intensity during the 15-hour period does not increase the flowering response. Lang has summarized a large body of evidence to support the idea that the supplementary low-intensity light period is required to overcome the inhibitory influence of the leaves in darkness. If Hyoscyamus plants are defoliated they will flower in complete darkness, as if the plants had a store of the floral stimulus or a means of producing it in darkness. If this were true then the inhibitory role of the leaves in darkness could be one of destroying the stimulus or producing a substance inhibiting the action of the stimulus. Though no other long-day plants have shown the same response to defoliation as Hyoscyamus, it is possible that they fail to flower because they do not have an adequate food supply. (Only Hyoscyamus plants with thick storage roots will flower when defoliated, and it has been shown that spinach will flower in total darkness if raised on a sugar-containing medium). Leopold (42) has shown

that etiolated plants of Alaska pea, red kidney bean, and Irish potato (LD or day-neutral plants) will flower when raised in complete darkness, although green plants of the same species will not flower when transferred to darkness for extended periods of time. There are no general hypotheses which account for all of these facts, but if one were created it would necessarily say that the leaves are not required for the production of the floral stimulus in LDP (or that it can be stored in seeds, roots and tubers), and that the leaves must be exposed to light before they become inhibitory (the etiolated leaves of peas, beans, and potatoes did not inhibit whereas the green leaves did). As far as is known to the author, the pea-seed diffusate of Highkin represents the only substance extracted from storage organs which has an effect upon floral initiation. The diffusate substitutes for vernalization but not for photoperiodic induction in peas and winter rye. Highkin has shown, however, that the diffusate greatly stimulates the flowering of spring rye grown in SD. Since spring rye is a LDP, not requiring cold treatment, it appears that the action of the diffusate in this case is in substituting for photoperiodic induction.

There is other experimental evidence which indicates that supplementary light is effective in removing dark inhibition. Continuous light is the most favorable condition for flowering in most LDP, at least there is no dark requirement. If short light periods are combined with short dark periods then flowering will occur, whereas short light periods with long dark periods produce only vegetative plants. Evidently it is the length of the dark period which is important in determining whether or not LDP will flower. The Withrows (72) have determined, for spinach and aster, that 2-3 hours of uninterrupted darkness will suffice to

inhibit flowering. These experiments will be discussed in more detail when the nature of the low-intensity light reaction is discussed.

Though the action of low-intensity light may be to remove an inhibition of darkness, there is no general belief that it is only the absence of an inhibitor which permits flowering to occur. The fact that the stimulus to flower may be transferred across a graft union from induced to non-induced plants indicates that something positive is produced by plants held in inductive daylengths. The leaf inhibition is visualized as a side reaction which prevents the formation of the floral stimulus and its eventual translocation to receptor growing points.

The existence of a critical photoperiod implies that a certain amount of time with the proper light or dark conditions is required for the accumulation of a minimal amount of floral stimulus or its precursor. A critical number of inductive cycles implies that the accumulations from each inductive photoperiod must be summated before they will be effective in causing floral initiation. The fact that a number of minimal photoperiods can be summated indicates that intervening dark periods do not inhibit by destroying the products of induction, rather they prevent the synthesis of some essential substance. Further proof of this comes from the observation that inductive cycles can be separated by long dark periods without markedly inhibiting flowering.

The high intensity light requirement of plants without food supplies is probably for the purpose of synthesizing the precursors of the floral stimulus. The energies satisfying the high-intensity light process are in the range required for photosynthesis, and the fact that spinach plants flower in continuous darkness with the addition of sugar indicates

that the precursors are derived from normal photosynthates (41).

None of the above experiments prove that the floral stimulus itself is formed in the leaves. The possibility exists, and has been suggested by Gregory (19), that only the precursors are formed in the leaves, translocated to the growing points, and there transformed into the floral stimulus. However, it is probable that the transformation taking place in the leaves proceeds past the simple sugar stage, and evidence for this will be presented when experiments performed with Cestrum are discussed.

One of the experimental facts concerning LDP is that the inhibitory influence of a long night can be almost completely removed by light interruptions. There are many reports to the effect that the most effective time for a single light interruption is in the middle of a long night (59). If the light of a 24-hour cycle is not given in a single application, but is broken into two periods, then the total duration of light necessary for effectiveness can be reduced. It is possible to interrupt the long night with a series of low-intensity light applications more effectively than with a single high intensity light break in the middle of the night. Continuous low-intensity light is the most effective treatment. The Withrows (71) discovered these relations for spinach and aster by measuring the effectiveness of equal total light energies applied at different times and at different intervals during a 15-hour night. They concluded that the inhibitory influence of darkness was maximal after $1\frac{1}{2}$ to 3 hours and that the inhibiting substance activated by the low irradiances was effective in extremely low concentrations.

Garner and Allard (25) recognized that the effect of light on plant development could be divided into three categories; the effect of light

intensity, the effect of light duration, and the effect of light quality. The first two categories have already been discussed along with some mention of the effect of time of application.

The high-intensity light process, as previously mentioned, is apparently photosynthesis. However, low-intensity light shows maximum effectiveness in the red region with a small peak in the blue; distinctly different from the action spectrum for photosynthesis. This corresponds with the absorption spectrum one would expect for an open chain tetrapyrrole. No pigment system fitting this description has as yet been isolated from phctoperiodically sensitive plants, however the Withrow's experiments show that the pigment exists in very low equilibrium concentrations in spinach and aster, and it is to be expected that the identification of small quantities would be exceedingly difficult. The problem is further complicated by the fact that not all plants have been found to have the same action spectrum for the low-intensity light process. Wassink, et al. (69) have reported that Brassica napus oleifera responds maximally to light in the blue and violet regions and not at all to red light.

Summary

Long-day plants are promoted to flower by extending the length of the light period. The effect of light may be divided into two parts; the high intensity light requirement to satisfy the energy and/or food requirements for the formation of the floral stimulus, and the low-intensity light requirement to prevent the inhibitory influence of dark. The effectiveness of the dark period may be reduced by light interruptions during the night. The dark effect becomes maximal after 2-3 hours and is administered by a pigment existing in very low concentrations with the absorption

spectrum of an open chain pyrrole. It is probable that the dark period exerts its effect by preventing the synthesis of the floral stimulus and not by destruction of essential substances.

Flowering in total darkness and in completely defoliated plants is not accounted for unless it is assumed that the floral stimulus has been previously accumulated in storage organs.

4. Short-Day Plants

Short-day plants are promoted to flower by decreasing the duration of light periods in their environment. However, this definition must be modified if one questions the relative effectiveness of the light and accompanying dark periods in causing floral initiation in these plants.

The requirement of SDP is not for short daylengths, rather it is for long dark periods. This fact is particularly apparent when one works with a plant such as Xanthium, a qualitative SDP with a critical night-length, requiring only one inductive cycle. In this case it is found that any dark period longer than $8\frac{1}{2}$ hours is sufficient to cause floral initiation, regardless of the length of the preceding or succeeding photoperiod provided that the requirements for food supply are met. (If only a single inductive cycle is given then the critical dark period is nine hours, whereas continuous cycles permit initiation with $8\frac{1}{2}$-hour nights.)

Since separated inductive cycles are less effective than continuous cycles (41), it has been difficult to determine unequivocally, except for Xanthium, the importance of the photoperiods for the inductive process in SDP. It is possible, however, to show that it is not the

ratio of light to dark that determines the effectiveness of inductive cycles. Allard and Garner (2) have shown this in their experiments with Peking soybean using cycles ranging from 1 to 72 hours. They found that induction was possible with a 12-hour dark period on a 24-hour cycle, but not possible with 7-hour dark on a 14-hour cycle or with 18 hours on a 36-hour cycle. We now know that 7 hours dark is too short for induction of this variety, but it is not easily understood why the 18-hour dark period is non-inductive. Fortunately Allard and Garner include data on this point. If they gave a 27-hour dark period on a 36-hour cycle then the soybeans flowered, indicating that it was the extra light of the 18-hour treatment that inhibited flowering and not simply the elapsed time between inductive cycles. One may conclude from these experiments that it is not the 1/1 ratio of light to dark which is important for floral initiation in soybean, but the absolute length of the dark period.

It is also well established that extension of the dark periods beyond a certain limit results in a reduced flowering response, this is so even when the light periods are kept optimal (2, 68, 62). Referring back to Allard and Garner's soybean experiment with 36-hour cycles and comparing the relative effectiveness of the 18- and 27-hour dark periods, it is found that the 27-hour period is inductive whereas the 18-hour period is not. The 27-hour dark period is beyond the optimum for induction yet it is relatively more satisfactory than an extended light period; this experiment shows that there is probably a greater light than dark lability of the stimulus. At any rate the inhibitory effect of extension of either the light or the dark periods, when more than one cycle is required, is interpreted as reflecting the lability of

the stimulus in all theories of photoperiodism save one, that of Bünning. Bünning's theory will be discussed in detail at the end of this section.

Though increasing the length of the dark period reduces the effectiveness of the induction treatment, it has been possible to cause floral initiation with continuous dark periods. For example, Perilla will flower after 130 hours of darkness (41). The extent by which the effectiveness of a single long dark period is reduced is shown by the fact that Perilla will flower if given 36 hours of darkness applied in three inductive cycles of 12 hours per cycle.

One may conclude that it is the duration of the dark period that is important in the induction of SDP, and that there is some product formed after each inductive cycle having limited stability. The product formed may be accumulated and become effective after several cycles or a single cycle. The differences among SDP may be a reflection of different rates of production or destruction of the stimulus or of different threshold concentrations required for initiation.

It is possible to inhibit the inductive effect of a long dark period by a brief light interruption. For Xanthium the most effective time of application of the light break is about eight hours after the beginning of the dark period (for night lengths ranging from 10-20 hours) (62). There is a broad maximum inhibition for the shorter night-lengths reflecting the fact that the critical night-length cannot be reached if the interruption occurs within a few hours of the beginning or the end of the dark period. The fact that maximum inhibition occurs at the same time for longer night lengths has a different explanation; this explanation is dependent upon the concept of accumulation of a relatively light stable product past the critical dark period, at any

time prior to the critical dark period there is no stable stimulus and complete destruction (or diversion) of the immediate precursors may be caused by light interruption. Past the critical night length there is an accumulation of the stimulus which cannot be affected by light interruptions; therefore, one would expect that the time of maximum inhibition would occur at the critical night length no matter what the length of the dark period. As a result of the destruction there would be little or no precursor remaining to be converted into the floral stimulus during the remainder of the dark period.

Borthwick et al. (13), have found that red light is most effective in causing an inhibition of floral initiation in short-day plants. This inhibition may be reversed if far-red radiation is applied after the red light interruption. These facts have led the authors to postulate a comprehensive theory explaining photoperiodic effects in both long and short-day plants. Since light must be absorbed to be effective, the assumption that pigments exist which absorb the red and far-red radiations cannot be avoided; however, it is further assumed that it is the same pigment in two different forms which is responsible for the absorption in both regions. The pigment absorbing red light is converted into the far-red absorbing form, and vice-versa. The action of the dark period is to cause the conversion of the pigment from the far-red to the red absorbing form; the effect of the far-red is to speed up the dark reaction. SDP cannot form any floral stimulus when the pigment is in the far-red absorbing form, but can when in the red absorbing form.

In this same paper it is reported that the critical dark period may be lengthened to nine or shortened to seven hours by one-half hour pretreatment with red or far-red radiation, respectively. From this experiment one can deduce that the night-length required for complete conversion

of the pigment from one form to the other is two hours, and that the remainder of the dark period, comprising the critical night-length, is concerned with the production of some precursor or of the stimulus itself. This deduction agrees with that made by the Withrow's for spinach and soybean (72).

In the case of Xanthium the effect of the high light intensity period, preceding the inductive night, is to supply structural and/or energetic requisites for the synthesis of the stimulus. The fact that either high intensity light and CO_2 or the application of photosynthates is required preceding the dark period makes it almost a certainty that this is the explanation for the light requirement in SDP.

Recently Lockhart and Hamner (44) have found that a second high intensity light period following the inductive night is required to attain maximum effectiveness of the inductive treatment. Prior to this second high light intensity period the stimulus is very unstable to 40°C. temperatures, whereas following this treatment there is relatively no inhibition resulting from high temperatures.

If auxin, indole-acetic or naphthalene-acetic acid, is applied to SDP during the course of their inductive treatment, then a substantial inhibition in floral initiation is observed (9, 62). Though much has been written on the subject, the effects of auxin upon floral initiation are exceedingly complex and cannot be generalized with very much assurance. Salisbury (62) investigated the effects of auxin application upon floral initiation in Xanthium and came to the tentative conclusion that auxin is not involved in the primary mechanism controlling the critical night

length, but is inhibitory by virtue of its ability to cause the destruction or prevent the synthesis of the floral stimulus. Anti-auxins may reverse the effects of applied auxin.

One of the truly remarkable cases of floral initiation was that found by van Overbeek and Cruzado (55) for one variety of pineapple. Cabezona pineapples, placed in a horizontal position for at least three days, are stimulated to flower. Since it is known that this variety can be forced to flower by auxin applications, the authors believe that the geotropic induction is a result of an increased auxin concentration on the lower side of the terminal apex. This increase is sufficient to cause the lower cells to undergo the transition from vegetative to reproductive, and these lower cells in turn produce an increased amount of auxin and cause the rest of the apex to become transformed.

For a complete review of auxin and anti-auxin effects upon floral initiation see the review of Bonner and Liverman (10).

Curios:

Wareing reports (68) that he has found a new photoperiodic phenomenon in the SDP, soybean and Perilla. He states that there is a relationship between the light and dark periods such that when the dark period is abnormally long the length of the associated light period must be shortened if flowering is to occur. Extension of the dark period to 39 hours reduces the critical light period to 10 hours above which no flowering occurs. Further lengthening the dark period does not cause any further reduction in the critical light period. If one examines Wareing's data it is found that he has repeated some experiments performed by Allard and Garner (2) with essentially the same results.

Rather than interpreting this as a new photoperiodic phenomenon, as did Wareing, the author prefers to explain the results as indicating that any extension of the natural photocycle is deleterious to flowering; if the cycle is extended then an extension with dark is less harmful than an extension with light, as discussed previously. This explanation of Wareing's results applies only to SDP for it is well known that many LDP respond best in continuous light, which may certainly be considered an extension of the natural photocycle.

Schwabe (64) has shown that the response of one variety of Chrysanthemum to short-day induction is greatly enhanced by pre-treatment with low temperatures; in one of his experiments there is a qualitative requirement for the cold treatment.

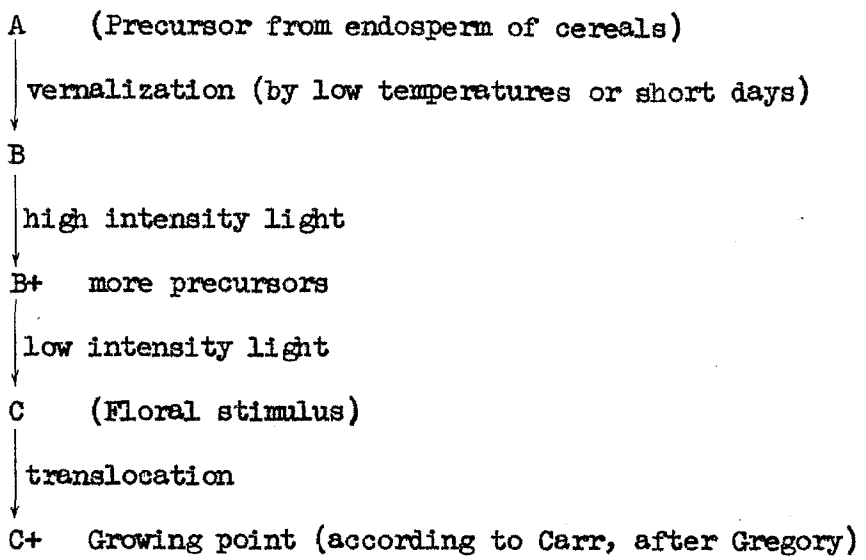
Lang (41) has quoted some reports which indicate that the vernalization requirement in some winter cereals may be replaced by short-days, this is termed short-day vernalization. Another curious report relating short-days to vernalization is that by Wellensiek for Campanula Medium (70, 21a). Short-day treatment (3 months) reduces the time required for the plant to attain the ripeness-to-flower age, at which time it becomes sensitive to long-day induction. The short-day treatment can be replaced by low temperatures of the same duration. Whether or not these are typical photoperiodic phenomena is not known.

5. Theories of Floral Initiation (resulting from vernalization and photoperiodic induction).

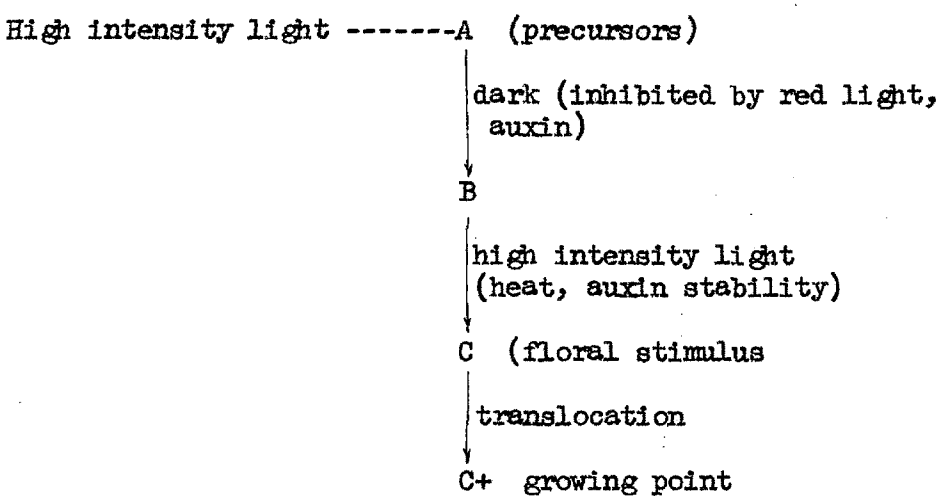
a) Partial Processes

This is nothing more than a description of experimental facts in which symbols, representing unknown chemical substances, are inserted.

1. Long-Day Plants



2. Short-Day Plants



Auxin inhibition occurs mainly during the dark period in SDP although inhibition occurs prior to and immediately after the dark.

Adding the "growing point" to the scheme is to accommodate the arguments of Carr in favor of the final synthesis of the floral stimulus occurring at the bud.

b) Borthwick, Hendricks, and Parker (43) -

The floral stimulus is produced in both light and dark but considerably faster in the dark. SDP have a relatively low rate of production and require much dark; LDP, on the other hand, are equipped for a high rate of production and require no dark period. The stimulus may be inhibitory at high concentrations and LDP require long days to prevent the accumulation of inhibitory concentrations. As Leopold has indicated (43) this theory does not explain why it is possible to induce long-day plants with short days interspersed between the long days. One would expect under such conditions that the stimulus would have regained its inhibitory level during the intervening SD's.

c) Bakhuyzen (5, 6). This theory combines some parts of the "partial process" and "Borthwick" theories. It assumes that there are two substances produced in the leaves of plants which combine stoichiometrically to produce the flower-forming stimulus (FFS)

$$A+2B \text{ -----} BAB \text{ (FFS)}$$
$$\downarrow$$
$$LFS$$

A is produced only during the light phase and is stable to light; B is produced in the light and dark phases but is unstable to light. If there is an excess of B then this will serve as a precursor for the formation of the leaf forming stimulus, also produced in the leaves and competing with the FFS for morphogenetic control of the apical and axillary meristems.

At the ripeness-to-flower stage the ratio of rates of production in the light of B/A in SDP is less than 2 and LDP equal to 2. It is also

important to assume that the complex BA is light labile, only BAB is stable, and that the back reaction for the formation of BAB from BA and B is negligible.

In continuous light long-day plants accumulate the FFS faster than is possible in any other light regime; as the length of the dark period increases, the supply of A becomes limiting and B accumulates, leading to the formation of the LFS. The LD requirement of different species is determined by the number of dark hours that pass by before the formation of LFS. The reason why light applications during a long night stimulate flowering in LDP is that the concentration of B is kept below that necessary for production of LFS.

If the ratio of B/A is less than 2, as it is in SDP, then no BAB can accumulate in continuous light. Only with some amount of dark is it possible to gather enough B to combine with A to form the light stable BAB. The length of the required dark period is determined by the ratio of rates of production of B/A. One would predict an optimal night length equal to that time required to reach a concentration of B twice that of A; any dark period beyond this time permits an accumulation of B to serve in the production of LFS, and, therefore, to compete with FFS and reduce the strength of induction. Optimal night lengths are well known for all SDP, and this theory explains this phenomenon with ease.

Perhaps the best indication of the ingeniousness of this theory is that Bakhuyzen was able to calculate a ratio of B/A for both Xanthium and Kalanchoë on the basis of their critical night lengths, and then use these ratios to predict the shape of the long night light-interruption curves.

Though the author has serious objections to Bakhuyzen's theory on the basis of his observations with Cestrum, he believes that no other theory has been proposed which can cope with as many of the facts of photoperiodism.

d) Bünning (16) - There exists, in some plants, an endogenous daily rhythm of light and dark favored reactions. The synchronization of this rhythm is determined in nature by the daily alternation of day and night in a 24-hour cycle, and the two phases are of about 12 hours duration.

The light favored reactions occur in the "photophile" phase, and the dark reactions in the "scotophile" phase. In long-day plants the photophile phase begins approximately 12 hours after the beginning of the light period, and light falling in the scotophile phase is without effect in promoting floral initiation. In SDP the photophile phase begins immediately after the start of the photoperiod, and light falling within the scotophile phase inhibits flowering.

This theory stands or falls on whether or not "endogenous rhythm" exists in plants, particularly with respect to the perception of light. There have been a number of experiments performed since the time of Garner and Allard's first paper which seem to indicate that the time of application of light is as important as the duration; the light interruption experiments with short and long-day plants are perfect examples of investigations concerned with the time of application of light.

Lang (41) and Melchers (46) have reviewed light interruption experiments with 48-hour cycles and they concluded that a 72-hour cycle

would be necessary to determine whether or not a rhythm of light and dark-favored phases existed. The results of the 48-hour cycles with both long and short-day plants indicated that there were two periods during a long night when low-intensity light irradiations were maximally effective in stimulating or inhibiting flowering (41, 34). The objection to the 48-hour cycle was that the two maxima occurred within 5-10 hours of the high-intensity light periods and it was possible to interpret the light interruption effectiveness as a continuation of the main light periods. If a rhythmic process existed in plants then a 72-hour cycle would be expected to show three maxima during the long night; if not, then one would expect only two maxima, near the beginning and end of the long dark period.

Recently there have been two papers investigating the effects of light interruptions during the long night of a 72-hour cycle. Carr (17) working with Kalanchoë, a SDP, has shown that there are, indeed, three maxima occurring during a 60-hour dark period, at which times light applications stimulate or inhibit stem elongation. Under the conditions of this experiment the control plants, 60-hour dark period, did not flower, and consequently, as far as flower formation is concerned, there are only two peaks. The occurrence of the peaks for flower formation, far removed from the high intensity light periods, indicates that there really is an optimal time for light applications having no relation to duration.

Hussey (34) working with Anagallis arvensis, a qualitative LDP, and Arabidopsis thaliana, a quantitative LDP, also investigated the effects of low-intensity light interruptions during the long night of a 72-hour

cycle. With Anagallis he found that low intensity light was effective only when given as a continuation or prelude to the high intensity light period, as suggested by Claes and Lang. Low intensity light applied at any other time during the long night was entirely ineffective, even when coinciding with Bünning's postulated photophile phase. In the case of Arabidopsis there was some slight stimulatory effect of shifting a high intensity light period during a long night so that it would coincide with a photophile phase. However, the effectiveness of this break was greatly increased (ca. 300 per cent in number of days to budding) by a supplementary 4-hour low intensity light period preceding or following the high intensity light. The additional low intensity light was given during postulated scotophile phases when light is either ineffective or inhibitory according to Bünning's theory.

Evidently there is no way as yet to decide which plants will or will not show an endogenous rhythm. The best test so far devised to determine alternating light-dark phases is the 72-hour cycle. However, there are many plants which will not survive this treatment, and it is possible that it will be some time before enough material has been investigated to permit a generalized statement. Bünning (16) has reported that it is possible to correlate the sleep movements of the leaves of some plants with their photoperiodic requirements for floral initiation. His investigations have shown that five soybean varieties with rhythmic leaf movements fitting the scotophile-photophile pattern of SDP were indeed SDP. Five other varieties without rhythmic leaf movements were day-neutral plants.

Galston (24, 21c) believes that any theory explaining all the facts of photoperiodism and floral initiation will include some scheme accounting

for endogenous rhythms. He has proposed the existence of a constantly oscillating system of IAA synthesis and destruction. Since both of these processes are markedly affected by light, and, since auxin has a great effect upon the flowering response of plants, Galston suggests that this mechanism can control flowering and explain endogenous rhythms.

Summary:

It is plain that there is very little agreement on the mechanisms responsible for photoperiodic control of floral initiation. There is agreement on the existence of a specific flower-initiating substance, although this point in particular will be criticized as a result of some experiments with Cestrum. The reasons for disagreement in some cases are probably due to differences in experimental technique and/or material. The chemical factors responsible for floral initiation are completely unknown, and there must necessarily be great variations in speculations pertaining to the mechanism of synthesis of unknown factors. The "partial processes" serve to divide the inductive period into a number of separate processes; whether or not single limiting reactions are involved at each phase is not known.

6. The Nature of Induction

The nature of induction has been discussed in detail by Salisbury (62); this discussion follows many of the suggestions contained in his thesis in the section on the "induced state".

The initiation of flowers by photoperiodic treatment is characterized by the fact that the conditions necessary for initiation need not be continued up to the time of initiation. For example, a single long night will cause Xanthium to change from the vegetative to the reproductive state

even though the succeeding conditions may be completely dark-free.

A Xanthium plant is obviously in a different condition after a long dark treatment, and it is said to be in the "induced state". The long night represents the inductive treatment. In general, any treatment resulting in a permanent change in the condition of an organism (and not required continuously), is called an inductive treatment.

This definition does not account for degrees of induction. One is led to believe that once a plant is induced to flower nothing can be done to modify this condition, but it is known that the rate of growth and the structure of flowers of some plants can be influenced by the strength of the inductive treatment. A flower is a flower no matter what its growth rate, yet it is true that the same treatment which determines whether or not flowers will be formed also determines the growth rate, and sometimes the form, of the flower.

Induction is the act, "induced" is the state. Owing to the fact that there are many different strengths of induction there are many different "induced" states.

Salisbury found that there was a direct relationship between the length of an inductive long night (or the number of inductive cycles) and the rate of growth of the terminal male flowers of Xanthium; prior to this Naylor (53) had reported that the rate of growth of both the male and female inflorescences of Xanthium was increased by increasing the number of inductive cycles. In addition, any treatments which interfered with induction were effective in reducing the growth rate of the flowers (62). For example, it is possible to completely prevent induction by applying light in the middle of a long night; if either the intensity of the light is reduced or the time of application shifted, some plants will

flower, but these flowers will grow at a much lower rate than those of the controls. The rate of growth of inflorescences is determined by defoliation at various times after induction. The earlier the defoliation the slower the growth, the later the more rapid. If defoliation occurs too soon after induction, then the plants remain completely vegetative.

Salisbury's experiments, summarized above, suggest that the same substance that causes floral initiation determines the growth rate of the flowers; the more initiating stimulus translocated to the bud the faster the bud will grow. Other experiments, with varying numbers of buds per plant, led Salisbury to conclude that it is the concentration of stimulus and not the amount that determines the growth rate of the flower. It is important to emphasize that the same substance which causes a qualitative change apparently causes quantitative changes as well.

At present there is no model to explain how such a system would work for the initiation and development of flowers. Quantitative induction, as expressed by rate of flower development, is an established fact in Xanthium, but what other cases are reported in the literature? Are there any measures of quantitative induction other than rate of flower development? What effects do post-inductive treatments have upon flower initiation and development? The following discussion is a literature review on these questions.

Rudbeckia (48), dill (52), spinach (72), aster (72), barley (11), etc. are all LDP, and in every case it has been found that continuation of LD past the minimum for induction causes a more rapid development of flowers.

The author has found the same to be true for the opium poppy and Nicotiana grandiflora. In all of these cases there is probably a saturation effect, i.e., beyond a certain number of LD's there is no great increase in development rate, however, there is no photoperiodic treatment which will cause a higher rate of development than continuous LD. This is definitely the case for the opium poppy and N. grandiflora; between 11 and 21 LD's N. grandiflora responds quantitatively in the rate of growth of the flowers, whereas, there is no difference in time of appearance of flowers between plants receiving 21 or continuous LD's. In the case of the opium poppy, Papaver somniferum var. polycephalum, there is a three-day difference in time of appearance of first flower between the 20 and 14 LD groups, whereas there is a 22-day difference between the 20 and 10 LD groups. Four additional LD's cause a 19-day stimulation up to 14 LD's; after 14 LD's 6 additional LD's cause a 3-day stimulation. The greater the number of LD the faster the growth of the flowers. The effectiveness of LD decreases as the number of treatments is increased.

Among the SDP, Xanthium (49, 53, 62), Cosmos (30), Chrysanthemum (64, 65), and soybean (57) show a higher rate of flower development with continuous SD's. The effectiveness of SD treatments decreases as the number increases showing that there is a saturation similar to that for LDP.

Generally hyperbolic saturation curves have been interpreted as evidence for the formation of a complex between two substances. The tail of the curve reflects the fact that the limiting concentration of one of the reactants has been reached. In enzyme kinetic studies the hyperbolic curve indicates a union of an enzyme with its substrate and the asymptotic leg of the curve at high substrate concentrations represents a saturation of the enzyme by its substrate. It is possible that saturation curves for

photoperiodically sensitive plants may be analyzed as hyperbolas and be interpreted as an indication of the union of the flower initiating stimulus with a receptor at the apical and axillary meristems. One expects that the flower initiating substance combines with some other substances within cells in order to exert its influence. But how may a substance effect a qualitative change (initiation) in a quantitative manner (rate of development). This is analogous with money activating bricklayers to construct a wall, twice the amount of money, twice the number of bricklayers, and presumably double the rate of construction. In this case money by action on a single bricklayer starts a qualitative change which may become quantitative by an increase in the amount of money. Such an analogy requires that there be a number of identical flower building sites that may be activated by the flower initiating substance.

Hartmann (32) has shown that strawberry flowers will develop under conditions not favorable for initiation; however, he does not report on the relative rates of flower development between the favorable and unfavorable conditions. Sironval (41, 21d) has extracted a substance from the leaves of photoinduced strawberry plants that will promote the development of initiated flower buds. The substance(s) remains in the unsaponifiable fraction of leaves. Apparently it is active in promoting development of those flowers which would ordinarily remain dwarfed or abort, and a necessary condition for its activity is that the treated plants be subjected to photoperiodic treatments favorable for initiation and development. From Sironval's most recent reports one would conclude that this unsaponifiable fraction contains substances active in floral initiation, but his previous papers indicate that the fraction

contains only accessory factors necessary for the development of the flower.

The Camellia definitely requires different conditions for floral development than for initiation. Bonner (8) found that Camellia plants require relatively high temperatures for floral initiation and low temperatures for development. It is the only case known to the author that shows a clear separation of initiation and development.

The effects of post-inductive treatments upon floral morphology have been reviewed by Naylor (52). Bryophyllum, given periods of low intensity light after induction, will develop small flowers lacking stamens; if these conditions for development are continued the plants will regress to the vegetative state. Salisbury reports similar low-intensity light effects upon the development of the Xanthium flower. Ambrosia develops a greater number of female flowers if the number of inductive cycles is decreased, whereas Xanthium shows an increased ratio of female to male flowers as the number of cycles is increased (53). Hyoscyamus will form small defective flower buds if given suboptimal vernalization or photoperiodic induction (41). Rudbeckia and Cosmos (47) will form "vegetative flowers" with suboptimal photoperiodic induction. Schwabe (65) reports that Chrysanthemum, a SDP, flowers will fail to develop normally in LD. By transferring plants to LD after floral initiation has occurred it was possible to suppress development or cause the formation of floral abnormalities (to the extent that the distinguishing features of the Chrysanthemum flower are lost and the flower no longer resembles others belonging to the same Substribe). Galinat and Naylor (23) report that the normal development of stamens in one strain of maize requires the proper photoperiodic conditions.

The purpose of this rather detailed review of the effects of inductive treatments upon the flowering behavior of plants was to emphasize that the problem of induction is concerned with the problems of initiation and development of flowers, and not one or the other alone. It is possible that there is no single specific substance controlling floral initiation (or development) and that a large class of compounds exist which are all involved in the processes of differentiation leading to the production of flowers. Since all measurements of floral initiation are dependent upon the development of the flower, it is not possible to separate these two phenomena (except in the case of Camellia). In many cases the development of flowers is not all or nothing, i.e., either a normal flower or no flower at all. The light and temperature conditions following the treatments necessary to produce the minimum morphologic transition of the growing points, have a great effect upon the size, structure, and/or rate of growth of the flowers. It is impossible to say from the experiments the author has reviewed that the substances necessary for floral development are not produced under the conditions necessary for accumulation of the floral stimulus. Until one can clearly show that the floral stimulus and the developmental stimuli can operate separately and be produced by different mechanisms, there does not appear to be sufficient evidence to speak of the initiation and development processes as separate phenomena. This problem will be presented again in the Discussion (Chapter X).

7. The Nature of the Stimulus

Deduction of the nature of the substances causing floral initiation and development from a study of the induced state has probably been as fruitful as attempts at the direct approach.

To begin with it has not been possible to isolate by extraction or diffusion any substances which can cause floral initiation outright. It has been possible, by growing plants slightly below the critical photoperiod to cause floral initiation in Hyoscyamus plants by auxin applications (21e). However, it is not generally believed that auxin exerts a direct effect upon the growing points, rather it is involved in the synthesis or destruction of the floral stimulus.

One of the most remarkable facts of photoperiodic induction in Xanthium is the persistence of the inductive stimulus. Leopold reports that Xanthium plants were kept flowering for 18 months after the inductive treatments were given, long after the "induced" leaves had fallen off. The quality of persistence has led to the belief that the stimulus may be a substance capable of perpetuating itself, such as a nucleic acid.

A theory has been developed by Salisbury and Bonner to explain how Xanthium plants are maintained at different levels of induction incorporating the idea that the stimulus is self-perpetuating. In addition, this theory includes the idea that the original concentration of stimulus, resulting from the inductive treatment, determines the level of stimulus that is maintained as long as no further inductive treatments are given.

This idea is similar to the facts which have recently been discovered explaining the development of crown-gall tumors. These tumors are formed on plants as a result of infection with certain strains of the bacterium, Agrobacterium tumefaciens. It is known that the bacteria are necessary for tumor formation for the first 30 hours after inoculation (15). Beyond this 30-hour period the size and rate of growth of the tumor formed is

unaffected by high temperature treatments; however within the 30-hour period, heat treatments can affect both the size and rate of growth of the tumor. Sufficiently long treatments at elevated temperatures completely prevent tumor formation. By varying the length of the heat application it is possible to obtain a graded series of tumors with varying growth rates.

The fact that the bacteria are necessary for a limited time only has led to the designation of tumor formation as an inductive process (36, 15), i.e., the conditions necessary for tumor initiation are not required for development. The strength of induction, as modified by heat treatments, determines the rate of growth of the resultant tumor - making the analogy between crown gall initiation and floral initiation in Xanthium complete.

Since the initiation of tumors is believed to be caused by desoxy-nucleic acids(36, 37), it is tempting to speculate that a similar substance is involved in floral initiation.

The tumor-inducing principle is heat labile, as shown by Braun (15), and any experiments showing that the floral stimulus is heat labile would be further evidence that the stimulus for initiation is high molecular weight. Lockhart and Hamner (44) have shown that at some stage in the synthesis of the floral stimulus heat labile compounds are involved, and experiments to be reported in this thesis support the contention that the floral stimulus is heat labile.

Prior to Klein's recent experiments showing that the tumor-inducing principle could be collected in plant wound juice media in which crown gall bacteria have been grown, it was impossible to extract the substance

from the bacteria. To date it has not been possible to extract the active substances in floral initiation from leaves or from phloem tissue through which the stimulus is transported. This has been used as further evidence of the high molecular weight nature of the floral stimulus, for it is argued that extraction and reapplication under conditions not causing denaturation is virtually impossible.

It is believed that the only means by which tumor cells can be forced to increase their growth is to expose them to products of the combined action of virulent bacteria and wound juice, i.e., increased induction is required.

Explants of tumors of various growth rates preserve the growth rate of the parent tumor showing that the growth rate is some function of the original inductive treatment (Klein - discussion at the California Institute of Technology).

Here again the analogy between crown gall and floral initiation is quite clear. Whether it is justified to speculate that the substances involved in the two processes are similar is a matter of taste. A number of experiments have been suggested to the author by the crown gall investigations, and this may be sufficient justification for making the analogy.

Braun (14) has attempted to explain the Kalanchoë teratomas produced as a result of infection of young tissues with crown gall bacteria on the basis of incomplete induction, i.e., young tissues possess a high concentration of morphogenetic factors which compete with the tumor-inducing factors for control of the fate of the cell, as a result of this competition it is possible to get intermediate normal-tumor tissues forming teratomas. Similarly one may explain "vegetative flowers" as a

competition between leaf forming and flower part-forming factors with the resultant production of intermediate stages when induction is incomplete.

8. Terminal and Axillary Meristems and Floral Initiation

In a recent review (28), Gifford summarizes two current theories explaining the transition of growing points from the vegetative to reproductive phases of development. One view holds that there is a gradual change from the pattern characteristic of the vegetative shoot apex to that of the reproductive pattern. This explanation comes from the school of morphologists that hold that the growth of a shoot can best be explained by the two-layered, or tunica-corpus, concept of apex structure. During the onset of reproductive activity in Bellis and Succisa a gradual transition takes place: "the peripheral zone of the corpus extends down the flanks of the enlarging apex and the central zone of the corpus is replaced by the peripheral meristem which becomes continuous over the apex. The tunica and the extended peripheral zone of the corpus become a 'meristematic mantle'".

Buvat recognizes a central region which includes the upper median portion of the tunica and the upper portion of the corpus; these two areas together constitute the "meristeme d'attente" which is considered to be inactive during vegetative growth. The lower or peripheral portion of the two-layered tunica constitutes the "anneau initial", the main organo-genetic region during vegetative growth. Subjacent to the lower layer of the "meristeme d'attente" is a zone in which the cells undergo active transverse divisions characteristic of the rib meristem, accounting for most of the longitudinal extension of the central axis during vegetative growth. Only at the onset of flowering does the meristeme d'attente

return to activity: the upper layer gives rise to the petals, carpels, and stamens. The sepals owe their origin to the last vegetative activity of the "anneau initial". The lower layer of the "meristeme d'attente" gives rise to the axis of the flower or inflorescence, and Buvat concludes that there is no ontogenetic continuity between the vegetative stem and the peduncle of the flower (this is interesting since the flower is commonly regarded as a modified stem structure).

Bakhuyzen (5) has proposed that the leaves produce a leaf-forming as well as a flower-forming stimulus and that these two substances compete with each other for control of the destiny of growing points. At the onset of flowering the concentration of the leaf-forming stimulus is reduced and the first signs of flowering in many plants reflect this reduced concentration, i.e., the floral bracts have narrow bases and as a result of the reduced leaf area there is a lowered production of auxin, the lowered auxin level in the bracts reduces the inhibition of the terminal and axillary buds permitting them to grow, the enlarged growing points then come under control of the flower-forming stimulus. It is true that many buds show the same first stages of flowering, enlargement of the terminal and axillary buds (46, 62, Cestrum).

Struckmeyer (74) reported that floral initiation in the soybean is accompanied by reduced meristematic activity. She has shown this by direct observation and by an ingenious experiment using Boron deficiency symptoms. Soybeans were grown in long days with a boron-deficient nutrient supply and these plants developed severe deficiency signs such as abnormal cambial activity and apical necrosis. When soybeans were grown on short days and induced to flower there were few or no deficiency

signs. It is known that boron deficiency is particularly harmful in actively growing plants at regions of active cell division. Therefore, Struckmeyer interpreted these results as indicating a reduced meristematic activity and, therefore, a reduced boron requirement.

Perhaps the occurrence of vegetative or otherwise abnormal flowers is an indication that the transition of the growing points from vegetative to reproductive is not an all or none process and that a continuous supply of transforming agents either from the leaves or from the floral initials is necessary to complete the transition. Whatever the explanation it is clear that the present state of our knowledge regarding the structural changes occurring during floral initiation and development is not sufficient to permit any general statements. It would be interesting to apply some of the surgical techniques to developing flowers that animal embryologists have applied to salamander embryos and determine whether or not the development of a flower is epigenetic in character, i.e., one organ dependent upon the previous formation of another.

III. Description of Cestrum Nocturnum

A. Taxonomic relationships

Cestrum nocturnum, the Night Blooming Jessamine (pronounced jasmine) is a member of the Solanaceae. This family is best known for its herbaceous members among which are the tobacco (Nicotiana tabacum), the tomato (Lycopersicum esculentum), the potato (Solanum tuberosum), the red pepper (Capsicum), and the eggplant (Solanum Melongena). All of these are valuable commercial plants.

There are about 70 genera and 1600 species in the Solanaceae distributed mainly in the tropical and warm temperate regions, the greatest number being in central and South America. Cestrum species number about 150 and are indigenous to tropical and subtropical America. The horticulturally important species are divided into three main groups on the basis of flower color.

Cestrum nocturnum, a woody perennial, is reported by Schulz (63) to be indigenous to the West Indies. He reports that it is found growing in wooded mountainous areas of Cuba and Martinique. The one case of recorded altitude shows that the plant occurs at 500 m. It is widely grown in Southern California for ornamental purposes

B. Morphology

1. Vegetative

Henceforward all references to Cestrum nocturnum will be made by the generic name of Cestrum.

Cestrum plants during the course of their growth present several different leaf sizes and shapes, more or less in a continuous series, progressing from an elliptic leaf of less than 35 mm. in length to an

ovate or lanceolate leaf up to 200 mm. long on the mature plant. The cotyledons are nearly circular and are opposite, never greater than 15 mm. in diameter. The mature leaves are alternate and are supported by petioles approximately one-tenth the length of the blade.

Schulz describes the branches as slender, flexuose, and brownish in color. The stems of laboratory grown plants are never brown; during the first year they are greenish, and in the period following, grayish (this color owing mainly to the increased crenulation and scarring of the stem). The girth of the stem is quite variable, including slender as well as thick branches. In all cases, if the branches become long enough, they are flexuose.

Though Cestrum occurs in nature as a shrub about 3 m. tall, it has been cultivated mainly as a single-shooted plant for the purpose of this work. During the first three to five months of growth of a seedling there is a strong tendency for the main shoot to dominate. After this time the axillary shoots grow as rapidly as the main shoot and the many branched habit of a shrub becomes apparent. Single-shooted plants are raised by constant pruning of all axillary buds.

The axillary buds are usually flanked by one or two smaller buds (called sub-axillary buds) which have bract-like leaves at their base (Fig. 1). When the sub-axillary buds are not at the base of the axillary shoot they may be seen as the first buds a short distance up from the base on the axillary shoot.

Among the anomalies of vegetative growth seen were:

1) Stem fasciation - the broadening of the stem and disturbed phyllotaxy, characteristic of fasciations was often observed. In all

cases in which special attention was paid to the phenomenon it was seen that the fasciated shoot was an outgrowth from a cutback stem and root stock. One of the means of inducing fasciations in beans is to decapitate a seedling, forcing the cotyledonary buds to begin rapid growth and become fasciated. The appearances of the anomaly in Cestrum are probably cases of induced fasciations similar to the case in beans.

2) In three plants a curious reduction in size of axillary buds was observed. The reduction in bud size was accompanied by reduced leaf size and stem girth and finally culminated in cessation of growth from the terminal apex. In the span of 4-5 nodes the axillary buds decreased from a visible to invisible stage of development. A cursory inspection of transverse sections in the axillary region failed to show any submerged buds. Apparently this infrequent anomaly is not correlated with any particular environmental treatment.

3) White leaves - on one plant, and on a short stem segment of this plant, completely white leaves appeared. By forcing the outgrowth of one of the axillary buds of an "afflicted" leaf it was possible to continue the production of white leaves. This phenomenon is characteristic of somatic mutations.

2. Floral morphology

The flowers of Cestrum are white to greenish-yellow in color, about 15-20 mm. long from the base of the ovary to the tip of the pistil. The corolla is tubular, usually having 5 ovate lobes about 3-4 mm. long (the number of corolla lobes, stamens and $calyx$ lobes varies from 4-7). When the flowers are fully open the corolla lobes span 8-10 mm. The anthers are brown-black and are divided into two pollen sacs; the filaments become distinctly separate from the corolla tube about 5 mm. up

from the base and in the mature flower support the anthers just below the plane of the open corolla lobes. The gynoecium is bicarpellate although the pistil does not appear bifurcated anywhere along its length. The stigmatic surface extends about 1-2 mm. above the plane of the corolla lobes. The ovary is superior with two chambers bearing up to 5 ovules. The sepals are the least conspicuous organs of the mature flower forming a small, 5-lobed calyx tube around the base of the ovary. This ring remains green throughout the life of the flower and is present around the base of the mature fruit. (See Fig. 1)

The fruits are at first green, then during the ripening period they become white, and finally are brown. They attain a maximum size of about 8-10 mm. and generally contain 2-5 seeds which are black and crescent-shaped at maturity.

Each flower has at least one bract at its base varying in size from 2-5 mm. long. The flowers occur in clusters of 3-5 with as many as 20 clusters on one axillary branch.

During the development of the flower different structures assume importance. The sepals, anthers and stigmatic surface, though inconspicuous in the mature flower, are the main distinguishing features in the early stages of development. These are described more fully in Fig. 7.

IV. Propagation of Plants

A. Cuttings

The first Cestrum plants grown in the Earhart Laboratory were derived from a one-year-old nursery plant. Cuttings were chosen about 5-10 mm. in diameter, about 150 mm. long, including at least two mature

leaves and their axillary buds. They were dipped in a dry powder of talc and auxin ("Rootone" - a commercial product containing synthetic auxin) and planted to a depth of 60-80 mm. in a mixture of Vermiculite and gravel.

The flats containing the cuttings were placed in the shade in the orange greenhouse. Within two weeks the majority of slips had developed root systems sufficient to support growth of the axillary buds.

Terminal cuttings were found to be much more uniform in their growth than were the subterminal. Possibly this fact may be correlated with the more uniform diameter of the terminal apex of these cuttings as compared with those of the axillary buds of the subterminal cuttings.

After three to four weeks the cuttings were transplanted to plastic pots (97 x 97 x 147 mm.) or to plastic cups (75 mm. diameter, 90 mm. high) and were ready for use in experiments.

B. Seedlings

Owing to the great variability in the growth rate of cuttings and to the time required to raise large numbers necessary for quantitative experiments, it was decided to sacrifice the genetic uniformity of cuttings for the greater vegetative uniformity of the seedling. Seeds were collected from a number of plants in the Pasadena vicinity.

It is interesting that attempts to raise seed in the greenhouses were generally unsuccessful, at least from the standpoint of seed quantity. The difference in seed bearing between plants grown either in the greenhouse or in nature can probably be attributed to two facts. Plants grown outdoors receive generally lower night temperatures than do Cestrum plants grown in the Laboratory for experimental purposes and lower night

temperatures are definitely correlated with increased fruit set, probably by retarding abscission. It was found that there was absolutely no fruit set at a night temperature of 20°C. and essentially complete set at 10-14°C. Secondly, Earhart Laboratory is insect free, and since it is well known that insects aid in the pollination of many plants it is quite possible that many flowers go unpollinated. There are two pieces of evidence which suggest this to be the correct interpretation. Even when laboratory plants have a high fruit set it is generally found that the fruits are without seeds and/or are very small. If plants are grown and induced to flower in the laboratory and then placed outside, it is found that the number of fruit is normal and the seed content and fruit development are also normal. The flowers of this plant are very fragrant at night and the corolla lobes are fully open during most of the nocturnal hours. If one were to give an explanation for the selection of such traits in a plant, he would probably suggest that the fragrance is useful for attracting insects to the flower to insure pollination (and the fact that the flower is presented in such an inviting position is a further aid to the insect to insure pollination). Some evenings while walking past these Cestrum plants in nature it was possible to see insects darting in and out among the branches. These facts suggest that insect pollination is one of the chief reasons for the greater seed bearing capacity of plants grown in nature. Hand-pollinating Cestrum flowers never produced as many seeds as the simple procedure of placing the plants outdoors.

Seeds were soaked 24 hours in water and then germinated in Vermiculite at 26°C. Within three days it was possible to detect the emergence of the radicle, and after 19 days the cotyledons were fully grown and the

first node was apparent. These figures are samples of some of the seeds and are not averages. There was a wide range in growth rate of seedlings, particularly during the first six weeks of growth, but this was not as serious a factor as it was in the case of the cuttings, owing to the fact that there was no difficulty in selecting suitable numbers of uniform seedlings from the large number sown.

Transplanting usually took place three weeks after sowing and at that time the seedlings were placed in Vermiculite in plastic cups and grown at 26°C. day, 20°C. night, 8-hour photoperiod until ready for experimentation. All seedlings judged to be healthy were employed and there seemed to be a very high percentage of the seeds initially sown that survived the selection of fast germination and normal growth, perhaps as high as 70-80 per cent.

C. Cut-Back Plants

By following the horticultural practice of cutting shrubs back at the end of a growing season and permitting new shoots to grow from the root crown, it was possible to follow individual plants through a series of experiments and check for constant flowering or vegetative characteristics which might reflect a genetic rather than an environmental effect. Very few plants were discarded during the course of this work and consequently some of the plants used were $3\frac{1}{2}$ years old and appeared in as many as five experiments.

On the average, three months elapsed between the cutting back and re-use of the plants. No carry-over effects of the previous treatments on the new shoots were ever noted; however there may be some normal aging effect related to the ripeness-to-flower that will be discussed in the

section on the selection of plants.

By this technique of raising plants it is possible to determine how much of the variability of an experiment is a result of the treatment or of the selection of plants of different physiological state, and how much is due to genetic variability.

However, the number of repeat experiments with each plant was insufficient to determine whether differences in response were due to genetic or physiological factors. From the experience gained in this investigation the author believes that at least 18 months would be required to raise a stock of plants of known genetic response. About the same amount of time would be required to raise a clone of cuttings from a single plant. These estimates are for growth in a greenhouse averaging 22°C. the year round.

V. Optimum Growing Conditions

The determination of the correct temperature and light requirements necessary to support maximum vegetative growth of Cestrum was the first and least difficult problem approached.

A. Temperature

It was known that Cestrum was indigenous to the American tropics and grew most rapidly in Pasadena from late Spring to early Fall. Quite naturally the warmest temperatures available in the laboratory were tried first.

It turned out that the best, or at least one of the better, temperature combinations was a 26°C. day and a 20°C. night. There is probably a fairly broad range of temperatures that will support growth rates

equal or nearly equal to that of the above. Night temperatures should not exceed 23°C. nor be lower than 17°C. and day temperatures should not exceed 26°C. nor be lower than 17°C. for optimum growth. Stem growth rates and leaf sizes are determined by temperature, and in the case noted below, particularly the night temperature.

Twenty cuttings were measured for height and placed in the temperature conditions indicated in Table I. A 16-hour photoperiod (pp.) was used in all four treatments. After 106 days stem heights and leaf sizes were recorded and the results appear in Table I.

TABLE I

Temperature vs. Vegetative Growth

Temp. Cond.	Stem Elong. (cm.)	Leaf Size (mm.) (length x width)	# of Plants
26D, 20N	84	158 x 50	4
26D, 7N	32	134 x 32	8
20D, 20N	64	158 x 50	4
20D, 7N	27	134 x 32	4

In addition to effects upon rate of stem elongation and leaf size, temperature has been shown to affect the rate of unfolding of leaves from the terminal apex. Since this parameter is somewhat easier to measure than stem height or leaf size, leaf counts were used to keep check on vegetative growth. The number of visible leaves on Cestrum plants differs from the actual number by 6-8 leaves, meaning that there is some variability among plants as to their arrangement and number of terminal leaves. It was not desirable to dissect the plants and, therefore, the number of visible leaves was a convenient count both before and

after the experimental treatments. If, during the course of the experiment, the pattern of leaf arrangement in the terminal apex should have changed, in some regular fashion, then the count of visible leaves may have varied by 2-3 from the true number of leaves unfolded. For this reason differences of greater than two were necessary for significance.

The following experiments, summarized in Table II, show the effects of temperature on the rate of leaf unfolding from the terminal apex. In one case, Experiment 56, three and one-half-month-old seedlings were used and in the other two cases, Experiments 66 and 72, cut back seedlings and cuttings with new shoots were used. For Experiments 66 and 72 the plants were selected for a uniform number of leaves and in Experiment 56 the size of the largest leaf was the basis of selection. In all three experiments the plants received 16 hours of artificial light and 8 hours of complete darkness for a period of 18 days, the night temperature was the same for all the plants (20°C.), and the day temperatures were varied as shown in Table II. The light intensity in Experiment 56 was 500-700 ft. candles and for Experiments 66 and 72 it was 1000-1200 ft. candles at plant height.

TABLE II

Phototemp. vs. Vegetative Growth (#lvs. unfolded)

Day Temp.	No. of Leaves Unfolded (No. of plants)			
	Exp. 56	Exp. 66	Exp. 72	Exp. 81
30		7.8(5)		
26	8.6(7)			
23		9 (6)		8 (8)
20	11.3(7)	10 (6)	12.8(8)	
17	9.7(7)			11.4(7)
14			8.8(8)	
10	5.1(7)	4.5(6)	5.8(8)	
7	3.9(7)	3.7(7)		

Experimental conditions: 18 days (16 hr. pp; X°C. 16 hrs.; 20°C. 8 hrs.)

In Experiment 66 leaf lengths were recorded, and the data show that the amount of growth was greatest at 20°C. and lowest at the 10 and 14°C. treatments, showing that there is a similar effect of temperature upon leaf growth and rate of leaf unfolding. The data for growth of the #7 leaf (counting from base to apex) are given in Table III.

TABLE III
(Data from Exp. 66)

Phototemperature vs. Vegetative Growth (Growth of Leaf)

Day Temp.	Average Growth of #7 Leaf (Length in mm.)
30	57.5
20	75.3
10	39.2
7	44

Experimental conditions: 18 days (16 hr. pp.; X°C. 16 hrs.; 20°C. 8 hrs.)

B. Photoperiod (PP)

Though this thesis is mainly concerned with the photoperiodic control of floral initiation, there is a pronounced effect of light period on vegetative growth.

It was recognized within the first year of experimentation that the only plants which flowered were those that received either the natural daylengths of Spring and Summer or 16 hours of light, 8 hours artificial added to 8 hours natural. Plants receiving no more than 8 hours of light per day remain vegetative so that for experimental purposes all plants were raised in 8-hour pp. with no thought given to what would be the optimum photoperiodic conditions for vegetative growth.

There is an increased growth rate with increased pp. from 8 through 24 hours on a 24-hour cycle (Table IV). This increased growth rate is reflected only in the stem height and not in the rate of leaf unfolding. In a 24-hour pp. Cestrum plants show a behavior quite different from that of plants grown in other pp. That is, the axillary buds show an enormous elongation, as if the terminal bud had ceased to exert any inhibitory effect upon them. The increased stem growth with the same number of leaves means that the internode length of plants grown in a 24-hour pp. is greater than that of plants grown in a 16-hour pp. Long internodes are characteristic of etiolated plants, whereas axillary bud development is not. The auxin explanations of these two phenomena would be as follows: long internodes mean high auxin concentrations; increased axillary bud development means reduced auxin from the terminal bud. Perhaps there is an intermediate auxin level in plants which could produce both of the observed 24-hour pp. effects.

TABLE IV

A. Vegetative Growth of Plants in Different PP's
Temperature: 23°C. 8 hrs. nat. light; 17°C. X hrs. art. light

PP	Growth in stem length (cm.)	# of plants
8	17.3	5
16	25.6	5
24	37.2	4

B. Vegetative Growth of Plants in 16 hr. and 24 hr. PP's
Temperature: 26°C. 8 hrs. nat. light; 20°C. X hrs. art. light

PP	Increase in stem length (cm.)	# of lvs. unfolded	# of plants
24	21.6	12.6	8
16	16.4	12	8

VI. Measurement of Induction

A. Review of Methods

Many of the problems concerning floral initiation involve comparison of two or more treatments and are necessarily concerned with problems of measurement.

Frequently the effectiveness of inductive treatments is represented as the percentage of plants flowering, and all plants with flowers are considered equal in their response, regardless of other variations. "Per cent flowering" is most suitable for judging differences among treatments bracketing the critical requirements for floral initiation and development in which qualitative differences in response may occur. If one must compare the effectiveness of treatments, all of which are inductive, it is necessary to employ some other method of measurement. There is another difficulty with the "per cent flowering" method which becomes particularly apparent when investigating the control of floral initiation of genetically homogeneous material selected for uniform vegetative characteristics. Material such as this should elicit a uniform response, and theoretically should flower completely or not at all as a result of inductive treatments. Salisbury (62) has pointed out that the "per cent flowering" criterion is only valuable for quantitative measurements when the material investigated is variable.

There are other criteria for judging strength of induction which permit differentiation among plants, all of which have initiated flowers.

Borthwick and Parker (12), working with soybean, have shown that the number of floral primordia initiated reflects the strength of the inductive treatment. It is assumed that the number of floral primordia initiated is

a function of the amount (or concentration) of the floral stimulus and that increased induction results in an increased amount of stimulus. It is known that inductive treatments may have a pronounced effect upon the rate of development, form, and final size of flowers. By counting all flower buds as equal, the above facts are overlooked, and in some cases these effects of induction are as important as the number of flowers initiated.

The effect of induction upon the rate of flower development has been well studied in the case of Xanthium by Naylor (47), Mann (45), Salisbury (62), and has been reviewed on pages 35 to 38. The stage of development at any time after induction is a reflection of the preceding rate. Determination of the stage represents the rate of development (in stages per day). For this system to be effective one must be able to identify a series of stages in the development of the flower and must be certain that a constant rate of development has prevailed prior to dissection. Salisbury has shown that one must be able to control all factors affecting the response of the plant to induction and all factors affecting the rate of bud development.

The fact that the rate of development of a flower can be determined by the strength of induction may mean that floral differentiation is a quantitative process. This fact was not realized before Braun suggested the possibility in crown gall initiation (15) and it apparently is true for the initiation of flowers in Xanthium. The study of the quantitative nature of differentiation is necessary if one wishes to devise experiments in which the mechanism of floral initiation is concerned, and, therefore, with every plant investigated some attempt should be made to determine the relationship between the rate of development and induction. The effect of

induction upon rate of development of flowers of Cestrum is summarized in Chapter VII, Sec. F.

Measurement of the number of days to flower from the beginning of induction has been used quite frequently to determine the effectiveness of inductive treatments. This criterion would be most useful if one were certain that the time to floral initiation and the time to appearance of the flower were independent. Since the rate of flower development may be affected by the same conditions affecting floral initiation, the "number of days to flower" is a measure of the time required for the accumulation of a concentration of stimulus sufficient to cause floral initiation and of the ensuing rate of development of the flower. It would seem better then to employ some criterion which permits separation of these two factors.

Another method used to determine the effectiveness of inductive treatments is the "earliness-to-flower" as measured by the first node to develop flowers. This method is particularly valuable if one is dealing with plant material possessing uniform ripeness-to-flower characteristics, as is the case with many cereal varieties and with some other food crops in which the earliness to flower is an important genetic marker. In many instances the factors affecting ripeness-to-flower are not known, or, if they are, there are difficulties in obtaining seed with uniform growth characteristics and the attainment of ripeness-to-flower is spread over a long period of time.

B. Methods Used

For many of the problems investigated with Cestrum it was important to have some criteria for determining the relative effectiveness

of inductive treatments. Almost all of the experiments were performed with seedlings of unknown genetic composition and with variable growth rates. This eliminated from consideration the "earliness to flower" method, but made "per cent flowering" determinations valuable.

There were a number of experiments performed in which all of the treatments resulted in 100 per cent flowering, however they could be resolved on the basis of the number of nodes bearing flowers. (The total number of nodes flowering divided by the number of plants in a group was used to represent the number of nodes flowering.) It is assumed that increased induction produces increased amounts of stimulus causing a greater number of nodes to bear flowers. The difficulties presented by the node counting system are discussed below.

Cestrum requires the presence of a large number of leaves for optimal response to inductive treatments. For this reason each plant is a population of leaves of different physiological age. Since the number of nodes flowering is dependent upon the response of the leaves to induction, it must be recognized that different leaves will have a different response to the same treatment, and the resultant node count will reflect the combined effect of leaf age and inductive treatment. An attempt was made to select plants of similar physiological age and minimize the effects of this factor upon increasing the variability of the response to induction. The problem of selection of plants will be discussed in a separate section, however it is unusual to obtain a uniform response among identical inductive treatments. Some of the reasons believed responsible for the variability within treatments are presented below.

Since the plants used are derived from seeds of unknown origin, it can be assumed that some of the variability is due to genetic inhomogeneity, not only in response to photoperiodic induction but in growth rate and ripeness-to-flower.

Another reason for variability in the flowering response, based on the number of nodes flowering, is the terminal inflorescence. In the case of Cestrum the terminal inflorescence includes many nodes but few flowers. A comparison of the number of flowers on a single axillary shoot further down the main axis with the number of flowers at the terminal ten nodes, shows that there are almost 10 times as many flowers per node on the lower axillary shoots. This comparison shows that a disproportionate weight is given to the terminal node if each is counted as equal to each basal node. Since the terminal 7 nodes always flower as a cluster, the decision was made to count them as a single node. Whether or not this is a sufficient correction factor is not known; however, it is a conservative estimate in the right direction.

No consistent attempt was made to count the number of flowers per plant, however the results of a few experiments in which the number of flowers and number of nodes flowering appear in Figure 2.

It is apparent that there is a fairly good correlation between number of nodes flowering and number of flowers; however, in the case of terminal flowers there is often a large deviation from a straight line relationship. In general, plants with terminal flowers have fewer flowers in proportion to the number of nodes flowering indicating that there is some justification for counting the terminal seven as one node. Figure 2. also shows that there is a close similarity between systems counting the

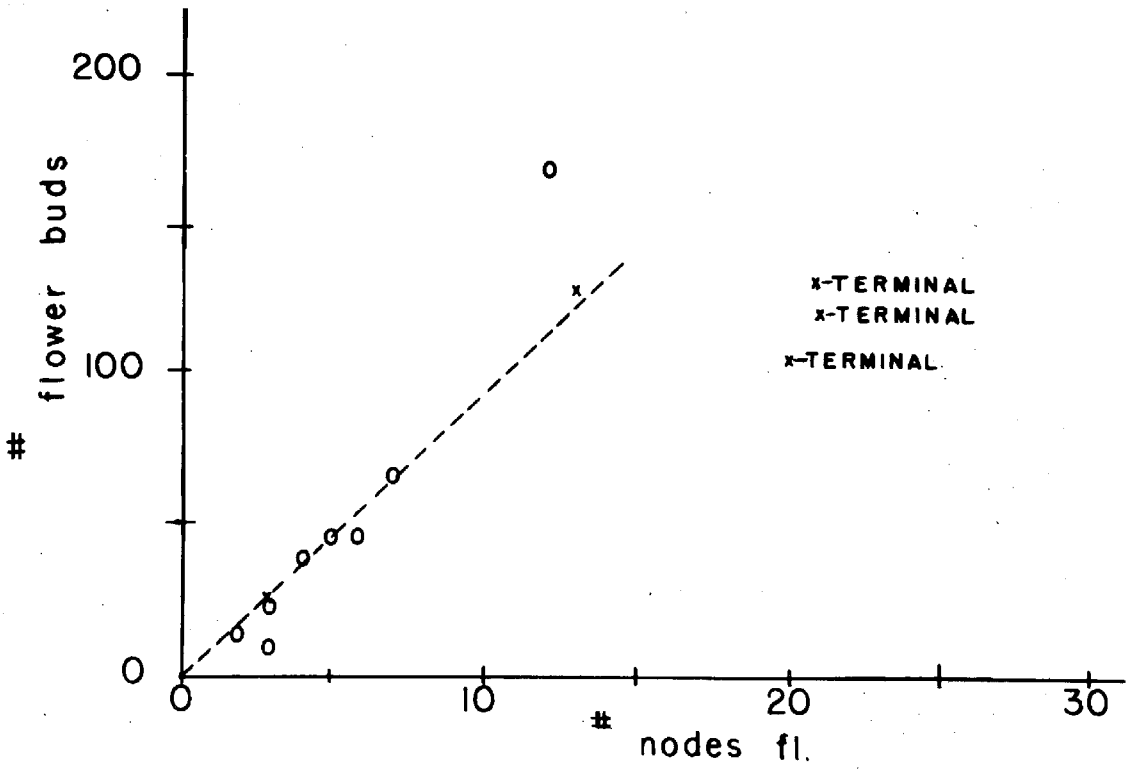


Fig. 2 Number of flower buds vs. number of nodes flowering.

Showing the proportionality between the number of flower primordia and the number of nodes bearing flowers within the range of 0-15 nodes bearing flowers. Terminal flowering deviations (marked "terminal") result from an overweighting of the terminal 7-10 nodes. All future measurements of "number of nodes flowering" count the terminal 7 nodes as one.

number of floral primordia and those counting the number of floral nodes.

To test the significance of differences among treatments on the basis of the number of nodes flowering, the data were analyzed by a statistical method suitable for small samples. The "t" test as described by Goulden (73) was employed. This method enabled one to judge the probability of chance occurrence of differences taking into consideration the variability of the data. Fundamental to the "t" test is the assumption that the means of a number of identical experiments are from a normal distribution. It has not been shown that this criterion is met owing to the relatively few repeat experiments. For this reason some doubts are held as to the propriety of using the "t" test.

In all experiments the 5 per cent level of probability was taken as significant and the probabilities corresponding to the various values of "t" are quoted in all cases along with the standard deviation, the 5 per cent level means that, in a homogeneous population, in 5 out of every 100 experiments this result would be expected on the basis of chance alone.

Since terminal flowering reflects strength of induction (see Chapter VII, Sec. B,2), the per cent of plants with terminal flowers was used as a standard for comparison among different groups.

Summary

Methods used for judging strength of induction:

1. per cent of plants with flowers (per cent fl.)
2. per cent of plants with terminal flowers (per cent term. fl.)
3. number of nodes flowering per plant (# nodes fl./pl.)

There is a separate discussion of the effects of induction upon rate of development in Chapter VII, Sec. F.

VII. The Selection of Plants

A. Ripeness-to-Flower

It is a well known fact that plants must attain a minimum amount of vegetative growth before they are capable of initiating flowers. The "ripeness-to-flower" stage is completely unpredictable among plants, yet is an important factor in determining the response of plants to photoperiodic induction. One should know when the leaves of a plant in question become sensitive to induction and/or when the apices can initiate flowers.

No absolute minimum age, or minimum number of nodes, for response to induction was determined for Cestrum. A rough estimate was made of the age at which plants attained maximum sensitivity to photoperiodic treatments.

Tables V and VI record the results of a series of experiments with seedlings of different ages given varying degrees of LD induction. The seedlings were raised in short days (8-hour photoperiod, 26°C. natural light 8 hours, 20°C., dark 16 hours). It can be seen that sometime between the second and third month after sowing, Cestrum plants attain maximum sensitivity to long day induction.

Three-month-old seedlings, grown under the conditions specified above, have at least three leaves with a maximum length of 140 to 200 mm. The number of nodes varies from 30 to 50 depending upon the rate of growth of the seedling.

Table VII summarizes the data comparing the response to induction with the number of nodes. The node count starts with the cotyledons and ends with the smallest visible leaf. Experiments 48 and 49 were performed with three-month-old seedlings. There is no obvious correlation between

TABLE V

An Estimate of the "Ripeness to Flower" Age:

1) Seedlings from seed lot sown 7/2/53

2) LD treatment - X days 24 hr. pp. (26°C., 8 hrs. nat. light; 20°C., 16 hrs. art. light)

3) SD Treatment - 7 days

4) Results:

Date of Entry and Exit for LD Treatment	% flowering Number of long days	
	10	15
8/25 - 9/4/53	0	
10/1 - 10/11/53	67	
9/6 - 9/21/54		100
9/25 - 10/10/54		80

TABLE VI

An Estimate of the Effects of Age Upon Response to LD Treatment

1) Seedlings from seed lot sown 7/7/54

2) LD Treatment - X days, 16 hr. pp. (26°C., 8 hrs. dark)

3) SD Treatment - 7 days, 8 hr. pp. (26°C., 8 hrs. natural light; 20°C., 16 hrs. dark)

4) Results:

DATE OF ENTRY AND EXIT FOR LD TREATMENT	# NODES FL/PL NUMBER OF LONG DAYS		
	12	15	18
10/6 - 10/18/54	12.1 ± 5		
11/2 - 11/14/54	10 ± 7		
12/13 - 12/25/54	1.4 ± 2.6		
10/13 - 10/28/54		21.9±6.8	
11/5 - 11/20/54		13 ± 11.9	
11/10 - 11/25/54		14.4±8.9	
10/6 - 10/24/54			20.4±3
10/27 - 11/14/54			21±8
12/22 - 1/9/54			22±3

TABLE VII

Number of Nodes Vs. Response to LD Treatment

1) Seedlings from seed lot sown 7/7/54; experiment began 10/12/54.

2) LD treatment - 15 days, 24 hr. pp. (26°C., 8 hrs. natural light; 20°C., 16 hrs. artificial light).

3) SD treatment - 2,3,4, or 5 days; 8 hr. pp. (26°C., 8 hrs. natural light; 20°C., 16 hrs. dark).

4) Results:

Number of Nodes Visible at Start of LD Treatment	Number of Nodes Flowering			
	Number of Short Days			
	2	3	4	5
18				18
20	0	0, 23		
21	0		23	1
22	0		16	16
23		1	0, 24	
24	10	3		
25	0, 2	7		0
26			8	8, 16
27	5	6, 10	12	12
28		1		
29			9	9
30			9	
33				1
34	8		1	
35			24	

number of nodes and response to induction in plants of this age. Since the many experiments were performed with secondary growths from the crown of seedlings, some criterion, other than chronological age, was required to judge ripeness-to-flower. Again, no minimum requirements were determined. An arbitrary decision was made to select "cutback" plants which resembled seedlings having maximum response.

B. Number of Leaves Required

Since the response of plants to photoperiodic induction is determined by the response of its leaves, a number of experiments were performed to determine the influence of leaf number and size upon induction.

For convenience in handling, and for prevention of undue amounts of shading, it is necessary to reduce the number of leaves to a minimum.

The results of two experiments, recorded in Table VIII, show the basis for selecting plants according to number of leaves visible. The leaves are removed from the base of the plant and the number of leaves visible is obtained by counting acropetally to the smallest visible leaf. Experiments 50 and 98 show definitely that there is a relationship between number of leaves and response to photoperiodic induction. The reasons why the older leaves are required is investigated in other experiments to be reported in another section.

The optimal response to long day induction is obtained with 13 to 16 leaves.

All experiments, unless otherwise noted, utilized plants with 14-16 visible leaves and at least three months old. In the case of "cutback" plants the size of the largest leaves exceeds 140 mm. prior to the beginning

TABLE VIII

Number of Leaves Visible vs. Response to LD

A. Experiment 98

1) Defoliation performed acropetally so that oldest leaves are removed. # lvs. vis. refers to number at start of LD treatment.

2) LD treatment - 15 days; 16 hr. pp. (26°C., 8 hr. nat. light; 20°C., 8 hrs. art. light; 20°C., 8 hrs. dark)

3) SD treatment - 7 days; 8 hr. pp. (26°C., 20°C., 16 hrs. nat. light)

4) Results:

# lvs. visible	Per cent fl.	Per cent term. fl.	# nodes fl/pl	# pls.
10	67	33	8.7	6
15	100	42	19.2	12

B. Experiment 50

1) Defoliation same as in Experiment 98 (above)

2) LD treatment - 15 days, 24 hr. pp. (26°C., 8 hrs. nat. light; 20°C., 16 hrs. art. light)

3) SD treatment - same as in Experiment 98 (above)

4) Results:

# lvs. visible	Per cent fl.	Per cent term.fl.	# nodes fl/pl	# pls.
10	0	0	0	10
16	70	0	4.8 ± 3.7	10

Errors quoted are standard deviations of the mean.

of inductive treatments.

The plants ranged in height from 45 to 100 cm., and to prevent excessive shading all plants in the same experiment were of similar height.

All attempts with Cestrum to correlate leaf size with response to induction have failed. The leaf size factor has been used with success for Xanthium (62) and soybean (12) in selecting plants with approximately the same physiological response to induction.

There is no simple estimate of the physiological condition of Cestrum plants based upon leaf size since the number of leaves required is too large. In a few experiments an attempt was made to correlate the size of the largest leaf with the response to induction. However, in every case there was a greater correlation with the number of leaves than with the size of the largest leaf.

Summary

Plants are selected on the basis of age (three leaves greater than 140 mm. long), number of leaves visible (14-16 including 3-4 leaves greater than 100 mm. long), and for equal height.

Although no relationship has been found between leaf size and photoperiodic response plants were distributed in the following experiments equally according to the size of the largest leaf.

VIII. Experimental

A. Preliminary

In the vicinity of Pasadena, Cestrum plants flower from late spring to fall. It is possible that low winter temperatures may affect the production of flowers in the spring by fulfilling vernalization requirements; however, cold treatment of plants did not have any stimulatory effect upon flowering.

Since Cestrum has few leaves remaining throughout the winter, it must be the new spring and summer vegetation which responds to the environment and causes the production of flowers.

In the greenhouses, regardless of temperature, flowering occurred from the end of May to the middle of September. Since all plants had sufficient vegetative growth to bear flowers in the greenhouse during the winter months, it was clear that Cestrum flowered in response to the long photoperiods of the summer months. Cestrum plants did not flower if they were kept on an 8-hour photoperiod. In this manner plants were kept vegetative for 18 months and reached a height of nine feet.

If plants were given continuous 16-hour daylengths, a few flowers developed after one to two months. However, the number of flowers produced was very much below that of plants grown in nature or in the greenhouse. Flowering occurred only on the lower branches of plants kept in artificial long days, whereas in natural daylengths, flowering was general, occurring at the terminal as well as basal portions.

For the time being these facts were disregarded and an attempt was made to discover approximately how many long days were required to get a minimum number of flowers. Four groups of plants were given 10, 20, 30,

and 40 long days respectively. All groups entered the long day conditions on the same date, were removed at 10-day intervals and placed in short day conditions. Observations on the appearance of flowers were made daily and recorded in Table IX.

Surprisingly, the order of appearance of flowers began with the 10 and ended with the 40-day group. Flowers did not appear until after the plants were removed to short days. Shortly thereafter, in other experiments, it was discovered that no morphological change was apparent on plants remaining in long days. Flower buds begin their development three to four days after removal to short day conditions and not before. Floral initiation in Cestrum requires long days followed by short days. In one experiment plants remained completely vegetative for 68 long days (24 hr. pp. continuous art. light). The long day treatment must be discontinued for floral initiation to occur, and is by definition an inductive phenomenon. In an attempt to determine whether or not the short day requirement is also an example of induction, i.e., are short days required continuously for the initiation of flowers, it was found that only two short days are required for floral initiation. Plants were given 20 long days, placed in short days for a designated time, and then moved back to long days. Observations on the appearance of flowers were made 12 days after the beginning of the short day treatment. The results are recorded in Table X.

Evidently both the short and long-day requirements fit definitions of inductive phenomena. Most of the following experiments are concerned with describing the two processes.

The sequence of long-short day requirements explains the flowering

TABLE IX

The Number of Long Days Required and the Short Day Requirement for Floral Initiation

1) LD treatment - X days; 24 hr. pp. (26°C., 8 hrs. natural light; 20°C., 16 hrs. artificial light).

2) Transferred to SD - after LD treatment - 8 hr. pp. (26°C., 8 hrs. natural light; 20°C., 16 hrs. dark)

3) Results:

# long days	# days to appearance of fl. from beginning of LD
10	18
20	27
30	37
40	> 45

TABLE X

Number of Short Days Required for Floral Initiation

1) LD treatment - 20 days; 24 hr. pp. (23°C., 8 hrs. natural light; 17°C., 16 hrs. art. light).

2) SD treatment - X days; 8 hr. pp. (26°C., 8 hrs. natural light; 20°C., 16 hrs. dark).

3) After X short days plants transferred back to X long days (conditions same as above).

4) Dissection of all plants 7 days after beginning of SD induction.

# short days	Per cent fl.	# pls.
0	0	5
1	0	5
2	100	13
3	100	12
6	89	19

in autumn, however it remains to explain why flowers develop in May and June, when the daylengths are continuously increasing. The final section of the thesis considers this problem.

Two other facts may be added now:

1) Complete defoliation of plants before long-day induction prevents flowering.

2) Complete defoliation of a plant after long and before short-day induction prevents flowering.

As is the case with most other photoperiodically sensitive plants it is the leaves of Cestrum that respond to both the long and short-day treatments.

B. Long-Day Induction

The most important questions to be answered were:

1) Is this long-day requirement similar to other long-day phenomena, i.e., will low intensity light satisfy part of the light requirement, and will a short light interruption of long night satisfy the requirements for a long day?

2) Is there a critical daylength?

3) What is the minimum number of long days required?

These questions are answered in order.

1. a. Long-day induction by supplementary low intensity light.

A single experiment was performed to show that the effect of supplementary light in a 24-hour photoperiod could be satisfied by 16 hours of low intensity light added to 8 hours of high intensity light.

Ten plants were given 8 hours of natural light (26°C.) and 16 hours of artificial light (20°C., less than 10 foot candles by Norwood light meter). The length of the long day treatment was 12 days.

In another experiment, performed at a different time, 8 plants were given 12 days of 24-hour pp. (8 hours natural light 26°C., 16 hours artificial light, 20°C., 700-1000 foot candles.)

Both groups received seven short days following the long-day treatment, and were dissected after this period.

Results

Supplementary Light Treatment	Percent fl.	Percent term. fl.	# nodes fl/pl
1. No light	0	0	0
2. Low intensity	40	0	1 ± 1.6
3. High intensity	100	0	5.7 ± 3.2

Though supplementary low intensity is very much less effective than high intensity light, for some of the plants it was sufficient to satisfy the long-day requirement. The light intensity measurements were made at the tops of the plants. In the low intensity group, the light intensity at the basal leaf was too low to be recorded by the light meter. The 10 ft. candle measurement applies only to the top three or four leaves on each plant and is very much lower at the remainder of the leaf surfaces. It is not surprising then that the response to "low intensity" light is very weak.

b. Long-Day Induction by Interruption of a Long Night

Ten plants were given eight hours of natural light at 26°C. and 16 hours darkness at 20°C. The 16-hour dark period was interrupted with one hour of light (700 ft. candles) $7\frac{1}{2}$ hours after the beginning. The experimental period was 18 days followed by seven short days (8 hr. pp.). This treatment is compared with plants receiving 18 long days (24 hr. pp.) 8 hours natural, 16 hours artificial (700 ft. candles) followed by seven short days (8 hour pp.)

Results

Light Treatment	Percent fl.	Percent term. fl.	# nodes fl/pl.
1. No light	0	0	0
2. One hour interruption	60	0	2.9 ± 3.8
3. Continuous	100	75	17 ± 6.6

Errors quoted are standard deviations of the mean.

Although the effectiveness of light interruption is very much less than continuous supplementary light, there is no doubt that this treatment is sufficient to satisfy the long-day requirement.

2. Critical Photoperiod for Long-Day Induction

The results quoted in Table XIA and Figure 3 show an increase in flowering response with increasing photoperiod between 11.5 and 16 hours of light per day. There is a photoperiod below which none of the plants will flower (11.5 hrs.) and a photoperiod (12.5 hours) above which maximum response is recorded not only for "per cent flowering" and "number of nodes flowering". However, increasing the photoperiod beyond 12.5 hours does result in an increased response in the parameter "per cent terminal flowering". The increase in terminal flowering is a reflection of an increase in the amount of stimulus available for translocation to the upper parts (see experiments on translocation, Section C this Chapter).

One of the most remarkable effects of increasing photoperiod upon long-day induction was the reduced effectiveness of a 24-hour as compared with a 16-hour photoperiod. The results of two experiments in which a comparison was made between 24-hour and 16-hour photoperiods are quoted in Table XIB. Two facts are apparent. Sixteen hours is greatly superior to continuous light, and, secondly, the effectiveness of 24-hour induction approaches that of 16 hours as the number of cycles is increased.

Since there is no inhibitory effect of continuous light upon vegetative growth, this is a specific inhibition of floral initiation. The importance of the 8-hour dark period has not been investigated. It will be shown in other experiments that varying the temperature during this period has very little effect upon induction.

3. Number of Long Days

All of the data reported in Table XII is a summary of experiments in which the long-day treatment consisted of a 16-hour photoperiod

TABLE XI-A

Photoperiod vs. LD Induction

A. 1) LD induction at a) 26°C., 8 hrs. nat. light; 20°C., X hrs. art. light, 16-X hrs. dark.

or

b) 20°C., X hrs. art. light; 24 - X hrs. dark.

2) SD induction - 7 days, 8 hr. pp. (26°C., 8 hrs. nat. light; 20°C., 16 hrs. dark).

3) Results:

Photoperiod	% fl.	% term. fl.	#nodes fl/pl	# pls.	Exp.#	Conditions of LD Induction
11	0	0	0	6	39	a
11.5	17	0	0.5 ± 1.2	6	82	a
12	17	17	2.7 ± 6.9	6	84	b
12.5	100	33	14	9	102	b
12.5						a
13	94	31	13.6 ± 7.1	15	61	a
14	100	54	16 ± 9.7	13	53	a
16	100	100	20.4 ± 3	8	47	a
16	100	67	18 ± 7	8	72	b
24	100	75	17 ± 6.6	8	47	a

Errors quoted are standard deviations of the mean.

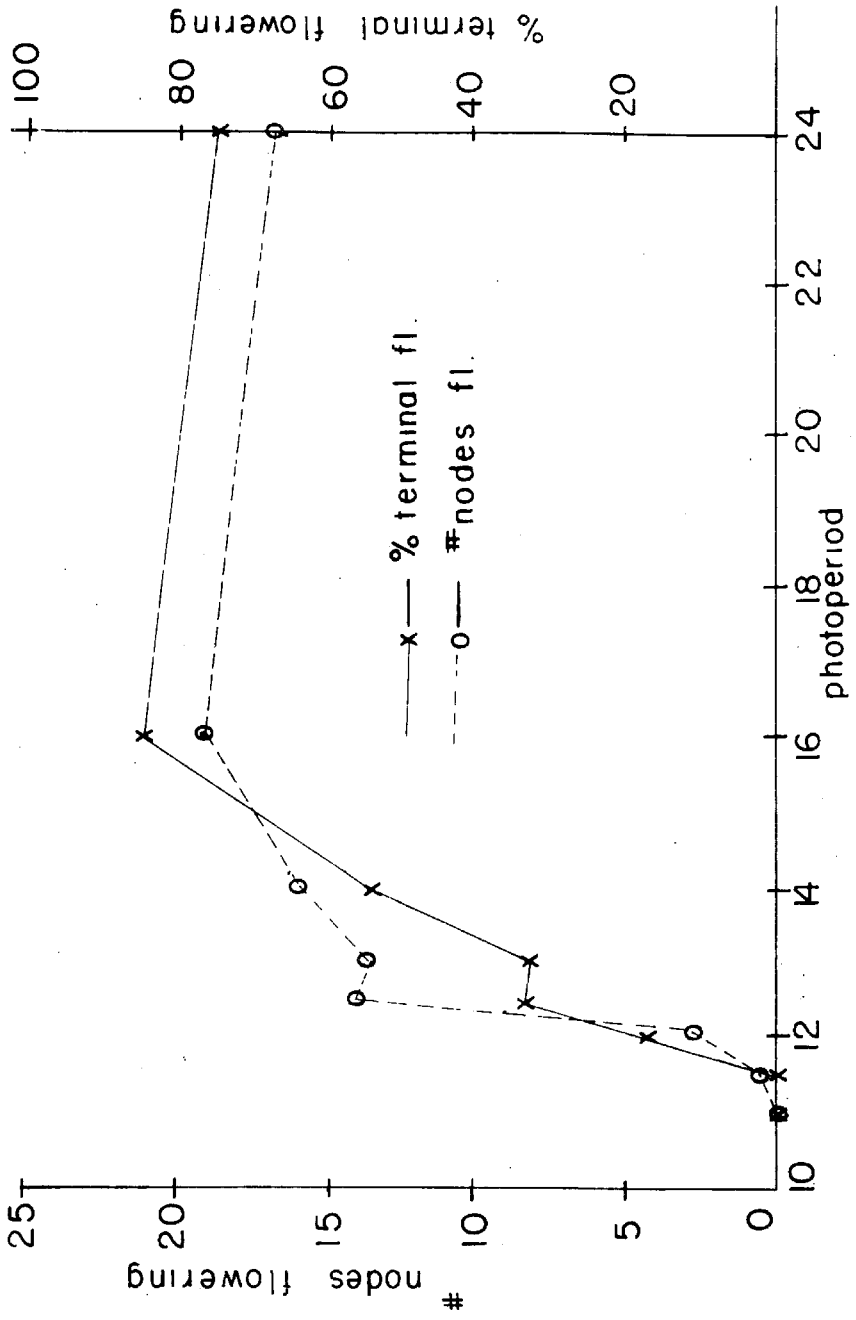


Fig.3. Photoperiod(pp) vs. LD induction. The sharp rise in the "number of nodes fl." curve between 11.5 and 12.5 hrs. represents the critical daylength.Data taken from Table XI.

TABLE XI-B

24-hr. vs. 16-hr. pp. for LD Induction

B.

1) LD conditions
   a) 24 hr. pp., X days (26°C., 8 hrs. nat. light; 20°C., 16 hrs. art. light)
   b) 16-hr. pp., X days (26°C., 8 hrs. nat. light; 20°C., 8 hrs. art. light; 20°C. 8 hrs. dark)

2) SD conditions
   8-hr. pp., 7 days (26°C., 8 hrs. nat. light 20°C., 16 hrs. dark)

3) Results:

pp.	# LD's	% fl.	% term. fl.	# nodes fl/pl.	# pls.	Expt.
24	12	100	0	* 5.7± 3.2	8	47
16	12	100	57	*12.1± 5	7	47
24	15	88	0	t 5.5± 3.9	7	58
16	15	75	43	t 13± 11.9	7	58
24	18	100	75	t. 17± 6.6	8	47
16	18	100	100	t. 20.4± 3	8	47

* Significant at 5% level
t. Not significant at 5% level

Errors quoted are standard deviations of the mean.
* t = 2.64,13 DF ,P.= 0.02.

(8 hours nat. light, 26°C.; 8 hours artificial light, 20°C.; 8 hours dark, 20°C.) for the designated number of days. After long-day induction the plants were shifted to short-day conditions (8 hours pp.; 8 hours natural light 26°C.; 16 hours dark, 20°C.).

The minimum number of cycles required is somewhere between 5 and 9; the enormous increase in response that occurs between 7 and 9 cycles indicates that the "critical" number is in this range. In this case the increase in response can be measured by three parameters: per cent flowering, per cent terminal flowering, number of nodes fl./pl.

4. The Effect of Temperature Upon Long-Day Induction

The conditions available in the Earhart Laboratory permitted separate temperature treatments during the photo and nycto-periods. The following experiments made use of these facilities.

a. Phototemperature

All plants received 18 long days (16 hour photoperiod, phototemperature varying from 7-30°C.; nycto-temperature, 20°C.). The photoperiod was supplied by artificial light of 700-1000 ft. candles intensity.

The results of a number of experiments are summarized in Table XIII. Two conclusions can be drawn from these experiments.

Temperatures below 17°C. definitely are inhibitory to long-day induction, and, secondly, there is a rather broad optimum temperature which extends beyond the upper range for optimal vegetative growth (column listing number of leaves unfolded).

TABLE XII

Number of LDs vs. LD Induction

1) LD induction - X days, 16 hr. pp. (26°C., 8 hrs. nat. light; 20°C., 8 hrs. art. light; 20°C., 8 hrs. dark)

2) SD induction - 7 days, 8 hr. pp. (26°C., 8 hrs. nat. light; 20°C., 16 hrs. dark)

3) Results:

# LD's	% fl.	% term. fl.	# nodes fl/pl.	# pls.	# Expt.
3	0	0	0	7	62
4	0	0	0	7	62
5	14	0	0.3	7	62
7	29	0	0.4	7	62
9	93	17	7.6	14	55,62
10	100	29	12.5	6	55
12	86	29	10	7	55
14	86	57	11.4	7	55
18	100	86	18	7	55
5	67	0	1.3	6	104
7	100	17	6.7	6	104
8	100	0	9	6	104
9	100	50	17.3	6	101
10	100	80	20	6	104
12	100	50	19.2	6	104
14	100	83	25.8	6	104
18	100	100	31.8	6	104

There are three criticisms of these experiments:

1) The temperature control in the artificial light rooms is dependent upon efficient air circulation. Since Cestrum plants are quite large and leafy, there is no doubt that they greatly impede circulation and the leaf temperatures may exceed the recorded air temperature. No leaf temperatures have been recorded to permit correction.

2) The length of the long-day treatment was 18 days, and it was shown previously that this amount of induction is sufficient to saturate the requirement even under sub-optimal conditions (24 hr. versus 16 hr. photoperiods). If these experiments were to be repeated, nine to twelve cycles would be used, insuring that the long-day requirement would be limiting.

3) The light intensity in the artificial light rooms is not sufficient to prevent partial etiolation (elongated internodes, thin stems), and it is not advisable to have both light intensity and temperature be limiting factors in a single experiment.

b. Nycto-temperature

A single experiment was performed in which plants received 18 long days (16 hour photoperiod, 26°C. artificial light, 700-1000 ft. candles) and the nycto-temperatures ranged from 4 to 26°C. Table XIV summarizes the results.

The temperature during the 8-hour dark period has very little effect upon the response of the plant to long-day induction. This is so even when vegetative growth is severely inhibited, as in the 4°C. treatment in which the number of leaves unfolded is approximately one-third that at other temperatures.

TABLE XIII

Phototemp. vs. LD Induction

1) LD Induction - 16 hr. pp. at indicated phototemp.; nyctotemp. 20°C.

2) SD Induction - 8 hr. pp. 7 days

Phototemp.	% fl.	% term. fl.	# nodes fl/pl	# pls.	Expt. #	# leaves unfolded
30	100	100	9.6± 1.5	5	66	7.4
26	100	50	13.6± 8	16	71,90	9.4
23	100	78	17.5± 6	13	66,81	8.8
20	100	57	14.5± 9	14	66,72	11.4
17	100	29	15 ± 8	14	81,96	11.4
14	75	0	2.8± 2.6	8	72	8.8
10	34	0	2.0± 3.1	14	66,72	4.2
7	0	0	0	13	56,66	3.8

See text for further description

TABLE XIV

Nyctotemp. vs. LD Induction

1) LD Induction - 18 days, 16 hr. pp. (phototemp. 26°C., nyctotemp. as indicated)

2) SD Induction - 7 days

Nyctotemp.	% fl.	% term. fl.	# nodes fl/pl	# pls.	# lvs. unfolded
26	83	67	10.7± 9.6	6	11.5
23	100	67	14 ± 8.5	6	10.2
20	100	67	14.2± 3.3	6	9.4
17	100	50	13.2± 6.8	6	9.8
14	100	67	15.8± 7.3	6	9.5
10	100	67	14.5± 9.6	6	10.3
4	100	50	13.5± 6.4	6	3.7

See text for further description

Errors quoted are standard deviations of the mean.

The one criticism of this experiment is that the length of the induction period was such as to obscure small effects of nycto-temperature. It would have been better to limit the induction period to fewer cycles.

5. Heat Stability of Long-Day Induction

The nature of the product of long-day induction is unknown; it has been suggested that the floral stimulus in both long and short-day plants is a high molecular weight substance. Substances of this character generally have very low heat stability.

Although it is known that the product of long-day induction, in Cestrum, is not by itself the floral stimulus (short days must follow before initiation occurs), the nature of the product is certainly of interest in that it probably represents an immediate precursor or catalyst in the synthesis of the stimulus.

Kunkel (39) has shown that peach trees may be freed of a number of virus infections, by short exposure to temperatures ranging from 38 to 52°C. Braun (14) demonstrated that the crown gall inducing principle was destroyed by temperatures in excess of 32°C. In these examples, the substances inactivated are thought to be nucleic acids or nucleo-proteins, and it can be seen that there is a wide range of destructive temperatures.

There is no one temperature that can be chosen, a priori, upon which the determination of the nature of the substance to be destroyed, or to be found resistant, is based. Kunkel has shown that peach mosaic virus (40) is resistant to heat treatments as high as 50°C.

To test the effect of elevated temperatures on the product of long-

day induction plants were given 15 days with a 16-hour photoperiod and then transferred to 42-45°C. ovens (with light, 700 ft. candles) for different periods of time. After the high temperature treatment plants were subjected to seven short days and then dissected. The results of two experiments are summarized in Table XV. The "control" groups represent plants treated in the same manner as the high temperature groups, with exception of the period in the ovens.

The product of long-day induction is heat labile; the longer the exposure to high temperatures the greater the inhibition of flowering with no apparent effect upon vegetative development.

From the data showing an increase in response with an increase in number of long days one would conclude that something is accumulated in a plant given long-day induction. The heat destruction evidence is further proof that a substance is accumulated, a substance which probably has a high molecular weight. Whatever the substance may be it does not affect the development of a plant unless it is further modified or added to by short-day induction.

No experiments were performed to test whether or not the effectiveness of a heat treatment decreased as the number of long-day cycles increased; such an experiment should indicate the extent, or rate, of accumulation of the long day substance during inductive treatments.

6. Translocation of the Long-Day Product

If a substance is accumulated as a result of long-day induction, one wishes to know where the accumulation occurs and whether or not the substance can be translocated throughout the plant.

TABLE XV

Heat Stability of the Long Day Stimulus

A. Long-Day Induction - 15 days, 16. hr. pp.
Heat treatment - 120 min.; 42-45°C.
Short-Day Induction - 7 days, 8 hr. pp.

Treatment	% fl.	% term. fl.	# nodes fl/pl.	# pls.
Heat	30	0	1.8 ± 3.6	10
Control	78	11	11 ± 7.6	9

Difference between Heat and Control groups significant at 5% by "t" test. t = 3.35 ,17 DF ,P. =0.01

$$\text{Inhibition} = 100 - \frac{\text{Heat (\# nodes fl/pl)}}{\text{Control (\# nodes fl/pl)}} \times 100 = 84\%$$

Errors quoted are standard deviations of the mean.

B. Long-Day Induction - 15 days; 16 hr. pp.
Heat treatment - 1) 30 min.; 42-45°C.
2) 70 min.; 42-45°C.
Short-Day Induction - 7 days; 8 hr. pp.

Treatment	% fl.	% term. fl.	# nodes fl/pl.	# pls.
Heat, 30 min.	67	50	10 ± 9	6
Heat, 70 min.	83	33	6.2 ± 3.8	6
Control	86	43	14.4 ± 8.9	7

Difference between "Control" and "Heat, 70 min." treatments significant at 5% level by "t" test. t = 2.21 ,11 DF ,P. = 0.05

$$\text{Inhibition} = 100 - \frac{\text{Heat, 70 min.}}{\text{Control}} \times 100 = 57\%$$

Errors quoted are standard deviations of the mean.

It is known that the production of the substance takes place in the leaves since defoliated plants do not respond to long-day induction and partial defoliation reduces the response. The question of transport of the substance is difficult to answer, since the effect of long-day induction can be noticed only after the short-day requirement is fulfilled.

However, there is some evidence attesting to the fact that the "long day" substance remains in the induced leaf.

a) If plants are completely defoliated after long-day induction then no flowers are ever produced on these plants even though they develop other leaves and these leaves are given short-day induction. The axillary buds at the defoliated node produce normal vegetative structures. This experiment suggests that the long day substance is not translocated to other portions of the plant and is only effective for short-day induction in the leaf in which it was produced.

b) Unless a leaf previously receives long-day induction it will not respond to short-day treatment. Or, stated differently, it is not possible to cause floral initiation by giving plants long and short-day induction simultaneously. An experiment was performed in which one group of plants was treated so that three leaves received short days at the same time the remainder of the plant received long days. This was accomplished by covering the "short day" leaves with aluminum foil for 16 hours daily. The treatment was continued for 25 days.

Another group of plants was treated so that the whole plant received long-day induction for 18 days. After this treatment three leaves, corresponding to those covered on the first group, were covered with

aluminum foil so that they received an 8-hour photoperiod while the rest of the plant was kept on long days.

In this fashion the two groups of plants were given the following treatments:

1) simultaneous long and short-day induction; short-day induction of leaves not previously receiving long-day induction

2) successive long then short-day induction; short-day induction of leaves previously receiving long-day induction

The results of this experiment appear in Table XVI.

Two facts are brought out by this experiment:

1) A leaf must receive long-day induction before it will respond to short-day induction. It is not possible to give a single plant both treatments simultaneously, possible meaning that the product of long-day treatment does not move to short day leaves.

2) After short-day induction something moves from the treated leaves which can cause flowering at nodes not receiving both long and short-day induction. That is, the final product of short-day induction is translocatable (as shown in column headed by "# nodes fl. above covered leaves").

There is one major criticism of this experiment. By covering the leaves with aluminum foil there is some mechanical injury and the possibility of heat injury. No measurements were recorded of the temperature increase of the air under the aluminum foil; however, as long as the temperature remains below 30°C. this should not inhibit any accumulation of the long day product (as shown by experiment in which the

TABLE XVI

LD and SD Induction Given to Different Leaves

Long-Day Induction - 16 hr. photoperiod, 8 hrs. nat. light, 8 hrs. artificial light, 8 hrs. dark 23°C.

Short-Day Induction - leaves covered with aluminum foil before entry into artificial light room and after end of 8 hr. dark period - 16 hrs. dark.

Treatment	% fl.	# nodes fl/pl	# nodes fl. above covered leaves	# pls.
Long and Short-Day induction simultaneously	0	0	0	7
Long then Short-day induction	86	3.4	8	7

phototemperature was raised to 30°C.). The control group receiving short days after long-day induction shows that accumulation of the floral stimulus is possible in leaves covered with aluminum foil.

7. Which Leaves Respond to Long-Day Induction

Experiments pertaining to the number of leaves required for long-day induction were discussed in the chapter "Selection of Plants". It was found that at least 14-16 leaves were required for maximum response, but in these experiments leaves were removed from the base to the tip, never from tip to base. Therefore, in every case there were young, developing leaves as well as older, more mature leaves remaining on the plant.

The following experiments were designed to find out which leaves were required for long-day induction. Leaves were arbitrarily placed in three categories:

a) Older (mature) - greater than 110 mm. long.

b) Middle-aged - between 60 and 110 mm. long

c) Young - less than 60 mm. long.

a) Requirement for mature leaves:

In most cases the lowest 4-10 nodes of Cestrum plants do not flower even though the leaves adjacent are healthy throughout the experimental period. Yet removing these older leaves severely inhibits flowering at the upper nodes. These two facts suggest that the lower leaves do not ordinarily respond to long-day induction, but nevertheless produce substances required for the response of the younger leaves. It is possible that the older leaves supply the younger with photosynthates during the period when they are incapable of supplying themselves. The photo-

synthates are probably necessary for synthesis of materials engaged in the photoperiodic response.

However, it is possible that the older leaves do respond to long-day induction, but the nodes in their axils are in a relatively non-receptive state to the floral stimulus and do not develop flowers. It is known that buds must be actively growing before they will initiate flowers (62, 75); if the older buds of Cestrum are in a relatively non-growing condition, perhaps they are not receptive to the floral stimulus produced by adjacent leaves.

To decide between these two possibilities the following experiments were performed (the assumption is made that any leaf greater than 110 mm. is mature.)

1). Three groups of plants were treated as described below. (At the start of long-day induction all plants have 14-16 visible leaves, five of which are greater than 100 mm.).

a) Remove the five lowest leaves; largest remaining leaf averages 100 mm. in length. In this manner the plant was cut off from photosynthate supply from older leaves.

b) Cover the five lowest leaves with aluminum foil so that they receive only 8 hours of natural light per day. (Covered only during long-day induction). The plants of this group still received a considerable amount of photosynthate but no floral stimulus from the older leaves.

c) Control plants intact.

The three groups received nine long days (16 hr. pp.) 26°C., 8 hrs. natural light; 20°C., 8 hrs. art. light; 20°C., 8 hrs. dark). After this

they were transferred to short days (8 hr. pp.; 26°C., 8 hrs. nat. light; 20°C., 16 hrs. dark) for seven days and then dissected. The results are recorded in Table XVIIA.

2) Two groups of plants were treated as follows after receiving 14 long days (16 hr. pp.; 26°C., 8 hrs. natural light; 17°C., 8 hrs. artificial light; 17°C., 8 hrs. dark). At the start of long-day induction all plants have 14-16 leaves visible.

a) Remove lowest five leaves

b) Control plants intact

The results of this experiment are summarized in Table XVIIB.

It may be that the lower leaves serve two functions in the response to long-day induction:

1) Synthesizing and supplying the floral stimulus for the upper regions of the plant

2) Synthesizing food supplies for the younger leaves and thus enabling them to synthesize the floral stimulus.

If this were the case and the lower leaves served a dual function, then one would expect to obtain the results recorded in Table XVIIA in which the "covered" group fell midway between the "leaves removed" and "control" groups.

In the first experiment the lowest five leaves were not permitted to receive long-day induction and could not form the floral stimulus (see previous experiments showing that long days required for short day response). However, there are at least two criticisms of this experiment:

1) Covering the lower leaves may cause some mechanical or heat injury reducing their photosynthetic efficiency.

2) The control plants receive 8 hours high intensity light over and above the 8 hours that the "covered" plants receive. This extra amount of light may stimulate an extra supply of photosynthates in the "control" and account for the difference between the two groups.

The second experiment was designed to avoid these errors by permitting the lower leaves to receive the same long-day treatment as the control but removing them before short-day induction begins. Since there is fairly good evidence that the long day product is not translocated, this procedure does not involve the risk of providing the younger leaves with anything but photosynthates from the older leaves. Experiment "2" Part B, Table XVII, is still open to criticism on the basis that removing the lower leaves prior to short-day induction decreases the food supply for short day processes; however, at the time the lowest five leaves are removed, the plants have more than 16 leaves remaining which should be a sufficient source of food (see chapter on "Selection of Plants" in which it was shown that optimum response to long-day induction required no more than 14-16 leaves).

The results, recorded in Table XVIIB show that there is relatively much less inhibition resulting if the lowest five leaves are removed after rather than before LD induction.

	Removed before (per cent inhibition)	Removed after (per cent inhibition)
(# nodes fl/pl)	73	20
(per cent fl)	17	0
(per cent term.fl)	33	0

It is concluded, therefore, that the main function of the "older" leaves in long-day induction is that of supplying the "younger" with food supplies, and, secondarily, synthesizing additional LD product (converted to floral stimulus in SD).

These experiments do not permit an unequivocal interpretation of the function of the older leaves in long-day induction. However, it is indicated that part of the requirement for mature leaves is to feed the younger leaves with nutrients enabling them to respond to long photoperiods.

b. Requirement for middle-aged leaves

To test the requirement for middle-aged leaves the following experiment was performed. In one group of plants all leaves between 60 and 110 mm. were removed; there were 4-5 leaves greater than 110 mm. and 5-7 leaves less than 60 mm. remaining. In the second group all plants were left intact and selected for approximately the same distribution of leaf sizes; there were 15-16 leaves visible at the beginning of long-day induction. Both groups received 12 long days (11 hr. pp.) and were then transferred to short days.

The results are listed below:

Treatment	Per cent fl.	Per cent term. fl.	# nodes fl/pl	# pls.
Middle-aged leaves removed	50	0	2.6 ± 5	10
Intact	43	0	1.4 ± 3.8	7

Errors quoted are standard deviations of the mean.

Although the response was low, this experiment indicates that the middle-aged leaves are not required for the response of a plant to long-day induction.

TABLE XVII

A. Which Leaves Respond to LD Induction?

Group: 1) Five lowest leaves removed; 16 hr. pp.

2) Five lowest leaves covered so as to receive 8 hr. pp., whereas rest of plant receives 16 hr. pp.

3) Control, intact; 16 hr. pp.

Group	% fl.	% term. fl.	# nodes fl/pl	# pls.
1	83	17	* 6.5± 8.3	6
2	100	0	11 ± 4.7	6
3	100	50	*17.3± 8.6	6

* Difference significant at 5% level

t = 2.21 , 10 DF ,P.= 0.05

B. Experiment 105

Group: 1) Remove 5 lowest leaves after long-day induction.

2) Control, intact.

Group	% fl.	% term. fl.	# nodes fl/pl	# pls.
1	100	56	*17.7	9
2	100	56	*22	9

* Difference not significant at 5% level

Errors quoted are standard deviations of the mean.

c. Requirement for young leaves

The following experiment was performed in an attempt to show whether or not leaves less than 60 mm. were required for long-day induction. One group of plants was treated as described below:

All leaves less than 60 mm. long were removed prior to the start of the long-day treatment and new leaves unfolding were removed throughout the course of the inductive period. There were approximately 11 leaves remaining.

Another group was left intact with 15-16 leaves visible and served as the control.

Both groups were given 18 long days (16 hr. pp.; 23°C., 8 hrs. natural light; 17°C. 8 hrs. artificial light; 17°C. 8 hours dark) followed by seven short days (8 hr. pp.; 26°C. 8 hrs. natural light; 20°C. 16 hrs. dark).

The results are summarized below.

Treatment	Per cent fl.	Per cent term. fl.	#nodes fl/pl	# pls.
Young leaves removed	31	0	1.9± 3.3	13
Intact, control	100	71	18.4± 4.5	7

Errors quoted are standard deviations of the mean.

It is mainly the nodes with leaves that have flowers in the "young leaves removed" group.

There is no doubt that young leaves are required for optimal response to induction although it is not clear that the response of the upper nodes is dependent upon the presence of these leaves during long-day induction or afterwards for short-day treatments. The experiments concerned with translocation of the short-day stimulus indicate that removing the young leaves after long-day induction is also inhibitory to flowering at the

upper nodes. In this case the reason may lie in reduced translocation to the apex or decreased auxin supply, both of which are increased by young leaves.

C. Short-Day Induction

Most of the experiments describing the long-day requirement were duplicated for short-day induction.

1. Is the short-day requirement typical of short-day phenomena in other photoperiodically-sensitive plants?

a) Inhibition of SD induction by interruption of a long night.

Plants were exposed to 15 long days (16 hr. photoperiod; 26°C., 8 hrs. natural light; 20°C. 8 hrs. artificial light; 20°C. 8 hrs. dark) and divided into two groups:

1) Received two uninterrupted 16-hour nights (8 hr. pp; 26°C. 8 hrs. natural light; 20°C. 16 hrs. dark)

2) Received two 16-hour nights, interrupted in the middle by 10 minutes of light (900 ft. candles).

Results:

Treatment	Per cent fl.	Per cent term. fl.	# nodes fl./pl.	# pls.
Interrupted	80	30	*10.1± 9.6	10
Uninterrupted	100	60	*21.9± 6.8	10

Errors quoted are standard deviations of the mean.

* Difference significant at 5 per cent level.

$t = 2.9$, 18 DF , $P = 0.02$.

In another experiment the length of the light interruption was increased to 60 minutes.

Results:

Treatment	Per cent fl.	Per cent term. fl.	# nodes fl./pl.	# pls.
Interrupted	0	0	0	8
Uninterrupted	100	100	22 ± 3	8

Errors quoted are standard deviations of the mean.

The conclusion drawn from these experiments is that light interruption inhibits the effectiveness of a long night. In other experiments (see "Critical night length") it was demonstrated that a 15-hour night was as effective as a 16-hour night for induction, and, therefore, the inhibitory effect of the light interruption was not due to a reduction in the total length of dark.

b. Inhibition of SD induction by auxin application.

Many short-day plants are known to be inhibited by auxin applications (62) and an experiment was performed to determine the effect of auxin upon Cestrum.

Salisbury (62) has shown that auxin inhibits floral initiation in Xanthium if applied to the leaves just before or during a long night and a similar treatment was employed with Cestrum.

Cestrum plants were given 18 long days (16 hr. pp.; 17°C. 16 hrs. artificial light, 20°C. 8 hrs. dark) followed by two short days (8 hr. pp.; 26°C. 8 hrs. natural light; 20°C. 16 hrs. dark). Prior to entry into the dark period of the short-day treatment one group of plants was sprayed with a solution of naphthalene acetic acid until the leaves were wet. Twenty minutes after auxin treatment, both the "auxin-sprayed" and "control"

plants were dipped in tap water and then given their long night.

This procedure was repeated before each long night. The plants were then moved back to long-day conditions and dissected five days later.

Results:

Treatment	Per cent fl.	Per cent term. fl.	# nodes fl./pl.	# pls.
Auxin	57	0	* 0.7± 0.8	7
Control	100	14	*10.1± 9.8	7

Errors quoted are standard deviations of the mean.

* Difference significant at 5 per cent level.

t = 2.9 ,11 DF , P.=0.02

There was no indication that the auxin sprays had any lasting effect upon the vegetative development of the treated plants. It was concluded that short-day induction is inhibited by auxin.

Note: Naphthalene acetic acid solution - 5×10^{-4} M. (adjusted to pH 7, containing some detergent).

2. Critical Night Length

The range to be investigated lay between 8 and 16 hours of darkness per long night. Though it is recognized that a determination of the critical night length should refer to continuous cycles, three cycles were chosen for convenience. Three cycles are used as the basis for comparison of inductive night lengths.

In the case of the two experiments recorded in Table XVIII (and illustrated in Fig. 5) plants were exposed to the following treatments:

Experiment 70 - 18 long days (16 hr. pp.; 20°C., 16 hrs. artificial light; 20°C., 8 hrs. dark) followed by the indicated night lengths (26°C., 8 hrs. natural light; 20°C., "X" hours art. light; 20°C., "X" hours dark).

TABLE XVIII

Nyctoperiod vs. SD Induction

# SD's	nyctoperiod	% fl.	% term. fl.	# nodes fl/pl	# pls.	Expt. #
3	10	0	0	0	6	70
3	11	0	0	0	6	70
3	11.5	33	0	2.5± 4	6	86
3	12	67	0	2.7± 4.1	6	86
3	12.5	67	50	12.8± 13.7	6	86
3	13	67	33	10.5± 11.4	6	70
3	15	83	17	11.5± 11.7	6	86
3	16.0	100	33	14 ± 3.8	6	70

(See text for description of Table)

Errors quoted are standard deviations of the mean.

Experiment 86 - 18 long days (16 hr. pp.; 26°C., 8 hrs. natural light; 17°C., 8 hrs. artificial light; 17°C., 8 hrs. dark) followed by the indicated night lengths (temperature and light conditions same as for Experiment 70).

The minimum night length required for short-day induction is 11.5 hours. There is a considerable increase in response between 12 and 12.5 hours, and perhaps the critical night length, for the majority of plants, should be placed in this range. Since these experiments contain very few plants, it is impossible to define the exact shape of the response vs. night length curve. For the purposes of discussion it is assumed that the critical night length lies between 11 and 13 hours (between 13 and 11 hour photoperiods, respectively).

3. The Number of Short Days

The minimum number of short days required is two. (See Preliminary Experiments, Chapter VIII-A and Table X). Four experiments were performed in which the effect of increasing the number of short days was investigated.

A description of the experiments follows and the results are recorded in Table XIX.

Experiment 48 - Long-Day Induction - 15 days, 24 hr. pp. (26°C., 8 hrs. natural light; 20°C., 16 hours artificial light.

Short-Day Induction - X days 8 hr. pp. (26°C., 8 hrs. natural light; 20°C., 16 hrs. dark).

Plants dissected 7 days after beginning of SD induction.

Description of Experiments in Table XIX.

Experiment 48 — Long-Day induction - 15 days, 24 hr. pp. (26°C., 8 hrs. natural light; 20°C., 16 hrs. art. light.

Short-Day induction- X days 8 hr. pp. (26°C., 8 hrs. nat. light; 20°C., 16 hrs. dark.)

Plants dissected 7 days after beginning of SD induction

Experiment 58 — Long-Day induction

a. 15 days, 16 hr. pp. (26°C., 8 hrs. nat. light; 20°C., 8 hrs. art. light; 20°C., 18 hrs. dark)

b. 15 days, 24 hr. pp. (26°C., 8 hrs. natural light; 20°C., 16 hrs. art. light)

Short-Day induction-

a.)

1. 2 days, 8 hr. pp. (26°C., 8 hrs. nat. light; 20°C., 16 hrs. dark)

2. 7 days, 8 hr. pp. (26°C., 8 hrs. natural light; 20°C., 16 hrs. dark)

b.)

1. 2 days, 8 hr. pp. (26°C., 8 hrs. natural light; 20°C., 16 hrs. dark)

2. 7 days, 8 hr. pp. (26°C., 8 hrs. nat. light; 20°C., 16 hrs. dark)

Plants dissected 7 days after beginning of SD induction.

Experiment 75 — Long-Day induction - 18 days, 16 hr. pp. (26°C., 8 hrs nat. light; 17°C., 8 hrs. art. light; 17°C., 8 hrs. dark)

Short-Day induction -X days, 8 hr. pp. (26°C., 8 hrs. nat. light; 20°C., 16 hrs. dark)

Plants dissected 7 days after beginning of SD treatment

Experiment 76 — Long-Day induction - 21 days, 16 hr. pp. (26°C., 8 hrs. natural light; 20°C., 8 hrs. art. light; 20°C., 8 hrs. dark)

Short-Day induction- X days, 8 hr. pp. (26°C., 8 hrs. nat. light; 20°C., 16 hrs. art. light)

Individually, these experiments are not clear-cut in showing the effect of increasing short-day induction. However, together there is no doubt that increasing the number of short days increases the response up to about four days.

There is another interesting fact illustrated in these experiments. As the strength of long-day induction increases, the required strength of short-day induction decreases. For example, in Experiment 58, compare the effect of increasing the number of short days upon plants receiving 16 hr. pp. long-day induction with that of plants receiving 24 hr. pp. induction. For 24 hr. pp. long days there is a three-fold increase in the number of plants responding to seven SD (see per cent fl. column) over the number responding to two SD. There is relatively little difference in response for plants induced with 16 hr. pp. long days.

TABLE XIX

Number of Short Days vs. SD Induction

Expt. 48 -15 long days, 24 hr. pp.	# SD's	% fl.	% term fl	# nodes fl/pl	# pls	MAS
	2	50	0	* 3.1± 4	8	
	3	88	0	7.2± 7.5	9	
	4	90	20	*13.2± 9	10	
	5	88	25	10.4± 8	8	

* Difference significant at 5% level

t = 3.0, 16 DF, P.= 0.01

Expt. 58 -

a) 16 hr. pp. LD induction;						
b) 24 hr. pp. LD induction	2a)	75	25	10.4± 8	8	
	7a)	75	38	13 ± 11.9	8	
	2b)	25	0	2.1± 4.9	8	
	7b)	88	0	5.5± 3.9	8	
Expt. 75 - 15 long days, 16 hr. pp.						
	2	100	29	15 ± 10.8	6	
	3	100	43	18 ± 11.7	6	
	4	100	58	22 ± 9.5	6	
	7	100	72	23 ± 6.1	6	
Expt. 96 - 21 long days, 16 hr. pp.						
	2	100	43	19.4± 12.3	7	
	3	100	50	19.5± 13.7	6	
	4	100	86	28.3± 7.6	7	
	7	100	71	28.6± 13.1	7	

Errors quoted are standard deviations of the mean.

A comparison between Experiment 48 and Experiment 75 shows the same relationship between long and short-day induction.

Single long dark periods ranging from 48 to 168 hours duration are completely ineffective for SD induction. Since Cestrum can survive such treatment, it may be an ideal plant for studying endogenous rhythms. By applying light interruptions at various times during an extended dark period it will be possible to test for "scotophile" and "photophile" phases.

4. The Effect of Temperature Upon Short-Day Induction

a. Nyctotemperature

Parker and Borthwick (56, 58) have shown that the temperature of the long night has a great effect upon the response of soybean to short-day induction. The effects of night temperatures were confined mainly to the leaf blade. Treating the entire plant produced very nearly the same results as treatment of single leaves.

In view of these facts an experiment was performed with Cestrum in which the nyctotemperatures during short-day induction ranged from 7 to 26°C. Plants exposed to 18 long days (23°C., 8 hrs. natural light; 17°C., 8 hrs. artificial light; 17°C., 8 hrs. dark) and then given two short days 8 hr. pp. (26°C., 8 hrs. natural light; X°C., 16 hrs. dark). After short-day induction all plants were moved back to long-day conditions and dissected 5 days later. The results are summarized in Table XX and illustrated in Fig. 4.

It is concluded that the temperature of the dark period is important in determining the effectiveness of SD induction. The optimum is probably between 20 and 23°C.; the minimum is between 10 and 14°C.

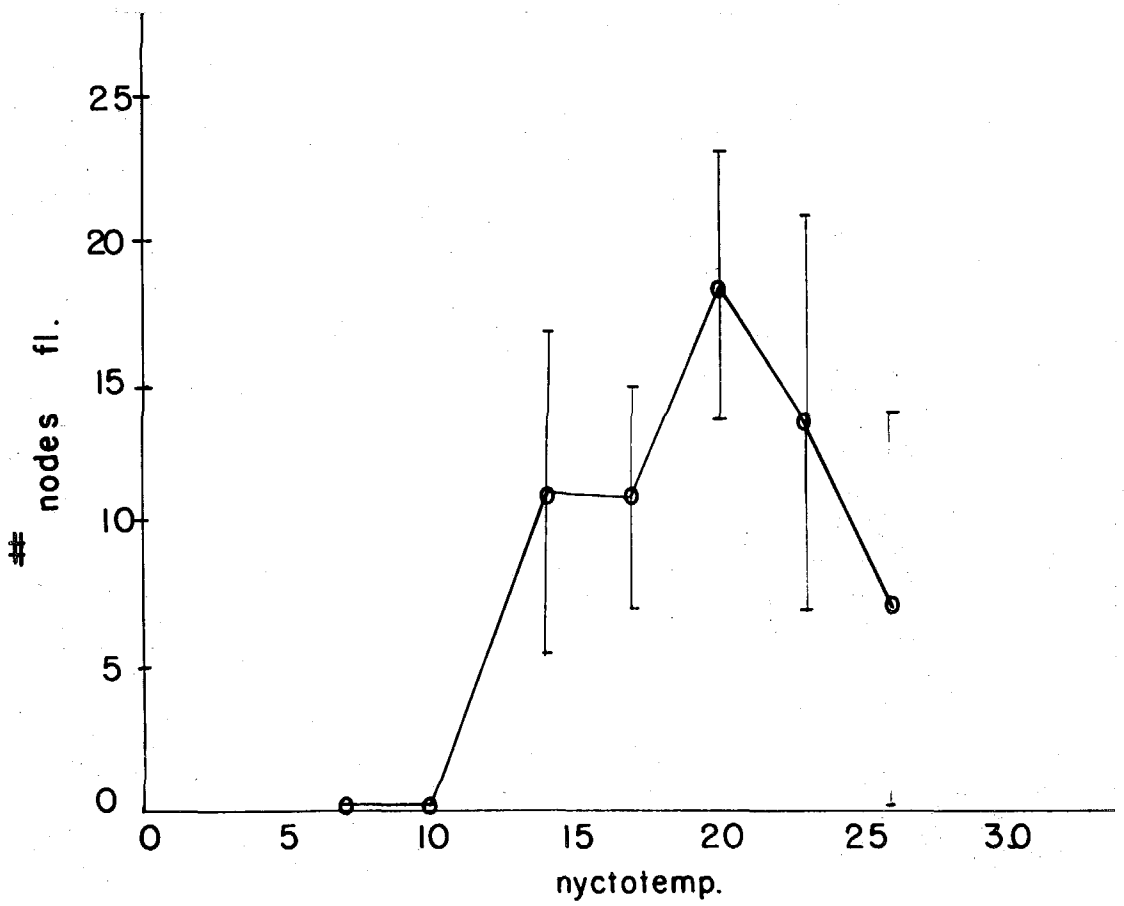


Fig.4. Nyctotemperature vs. SD induction.

Nyctotemperature in ^{o}C.Data taken from Table XX.

b. Phototemperature

It has been demonstrated for soybean that there is very little influence of the phototemperature upon short-day induction (56).

An experiment testing the effect of phototemperature upon SD induction was performed with Cestrum. Plants were exposed to 20 long days (16 hr. pp. 26°C., 8 hrs. natural light; 20°C., 8 hrs. artificial light; 20°C., 8 hrs. dark) and then transferred to short-day conditions (8 hr. pp; X°C., 8 hrs. artificial light; 20°C., 16 hrs. dark) for three days. All groups were moved back to long day conditions and dissected four days later. The results are summarized in Table XXI.

There is very little relationship between the phototemperature and response. Possibly 7°C. inhibits although the variability of the data precludes any definite conclusions.

There are two criticisms of this experiment:

1) The strength of long-day induction is such that short-day induction is very efficient (see experiment relating response to # SD's.) It is preferable to give minimal LD induction so that the effect of varying SD conditions is emphasized.

2) It is better to give two short days instead of three, to insure that the short day requirement is limiting and the effect of variations in short-day induction be more pronounced.

5. Translocation of the Short-Day Stimulus

The fact that flowering occurs at a distance from the point of perception of induction suggests that a stimulus is translocated. It has been shown for Xanthium (62) that plants may be defoliated 20 hours after the end of a single long night with no inhibitory effect upon floral initiation at the terminal apex. If the leaves are removed prior to this

TABLE XX

Nyctotemp. vs. SD Induction

Nyctotemp(°C.)	% fl.	% term. fl.	# nodes fl/pl	# pls.
26	71	14	* 7.1± 7	7
23	86	43	13.7± 7.4	7
20	100	58	*18.4± 4.5	7
17	100	29	*10.9± 4.2	7
14	100	29	*11.1± 5.7	7
10	0	0	0	7
7	0	0	0	7

* The difference between the 20° and 26, 17, and 14° groups is significant at 5% level. 20 - 26, t=3.52, 12 DF, P.=0.01 20-17, t=3.23, 12 DF, P.=0.01 .20-14, t=2.66, 12 DF, P.=0.02.

Phototemp. 26°C., 8 hrs. natural light.

TABLE XXI

Phototemp. vs. SD Induction

Phototemp(°C.)	% fl.	% term. fl.	# nodes fl/pl	# pls.
26	100	60	16± 7.9	5
23	100	100	20.2± 13.3	5
20	100	60	13.8± 8.7	5
17	100	80	18.4± 3.2	5
14	100	100	17.6± 2.6	5
10	100	50	16.4± 10.2	5
7	67	50	14.2± 9.2	6

Errors quoted are standard deviations of the mean.

time then inhibition is observed.

Salisbury (62) concluded that the floral stimulus is translocated from Xanthium leaves during the 20-hour period following induction.

Experiments analogous to those described above have been performed with Cestrum. Instead of removing single leaves, as in the case of Xanthium, 20-30 leaves are removed.

Experiment 99 - Plants were exposed to 18 long days (16 hr. pp., 20°C., 16 hrs. artificial light; 20°C., 8 hrs. dark) followed by two short days (26°C., 8 hrs. natural light; 20°C. 16 hrs. dark). They were completely defoliated at the times indicated in Table XXII.

Experiment 74 - Plants were exposed to 18 long days (16 hr. pp., 26°C., 8 hrs. natural light; 20°C., 8 hrs. artificial light; 20°C., 8 hrs. dark) followed by three short days (8 hr. pp.; 26°C., 8 hrs. natural light; 20°C., 16 hours dark). They were then transferred back to long-day conditions and dissected four days later. Results are recorded in Table XXII.

Experiment 60 - Plants received 18 long days (16 hr. pp.; 26°C., 8 hrs. natural light; 20°C., 8 hrs. artificial light; 20°C., 8 hrs. dark) followed by seven short days (8 hr. pp.; 26°C., 8 hrs. natural light; 20°C., 16 hrs. dark). All plants were dissected after the seventh day. Results are recorded in Table XXII.

Experiment 99 is perhaps the most unequivocal of the three in that all plants received the same number of short days. The data show that the leaves contain some of the stimulus(i) up to 48 hours after short-day induction and all of the stimulus immediately after the second long night.

TABLE XXII

Translocation of the SD Stimulus

A. Expt. 99 2 SD - Hour of defoliation is recorded as hour after end of second long night

Hour of defoliation	% fl.	% term. fl.	# nodes fl/pl	# pls.
0	0	0	0	7
8	29	0	0.6± 1	7
25	29	0	0.3± 0.5	7
48	71	0	10.7± 8.9	7

B. Expt. 74 3 SD - Hour of defoliation is recorded as hour after end of second long night.

Hour of defoliation	% fl.	% term. fl.	# nodes fl/pl	# pls.
8	100	0	4.3± 5	6
22	83	33	14.5± 11.3	6
31	100	17	13.2± 5.6	6
46	100	67	22.3± 3.9	6
54	100	17	17.8± 7.8	6
70	100	100	28.5± 1.9	6
128 (Control)	100	67	25.7± 7	6

C. Expt. 60 7 SD - Hour of defoliation recorded as hour after end of second long night. Negative value is for defoliation time preceding end of second long night.

Hour of defoliation	% fl.	% term. fl.	# nodes fl/pl	# pls.
-21	0	0	0	7
3	0	0	0	7
27	14.3	0	1.1± 3	7
51	100	43	11.1± 6.3	7
123	100	71	15.4± 8.2	7

Errors quoted are standard deviations of the mean.

This statement is extreme in that it is probable that some of the stimulus is translocated from the leaves prior to the second long night, but the amount translocated is in sub-minimal concentrations required for initiation. Within eight hours after the end of induction some stimulus has been translocated from the leaves and there is continuous translocation up to 48 hours after the second night (as seen by increase in response).

Experiment 74 and Experiment 60 are variations of Experiment 99 in which the number of short days is increased. They show essentially the same results as Experiment 60: prior to 8 hours after the end of the second night no stimulus has left the leaves. In addition Experiment 74 suggests that all of the stimulus has left the leaves by 70 hours after the end of the second night.

6. The Number of Leaves Required for Short-Day Induction

For Xanthium and soybean it is possible to cause floral initiation by exposure of a single leaf to short-day induction (this is true even if every other leaf is removed).

An experiment was performed with Cestrum in which two groups of plants were selected for 14 visible leaves and given 15 long days (16 hr. pp., 26°C., 8 hours natural light; 20°C., 8 hrs. artificial light; 20°C., 8 hrs. dark). At the end of long-day induction one group was defoliated to a single leaf, #15; the other group remained intact and served as a control. The size of the #15 leaf was recorded for both the defoliated and control plants.

Both groups were placed in an 8 hr. pp. for seven days and then dissected.

Results:

Treatment	Per cent fl. at 15th node	#15 leaf length(mm)	# pls.
#15 leaf	0	103.5	6
All leaves	83	102.2	6

Buds at the 15th node can flower, but they, or the #15 leaf, require something from the other leaves remaining on the plant.

However, it is possible to discover which leaves are supplying most of the floral stimulus or accessory factors.

To discover which leaves are required for short-day induction a series of experiments was performed in which a number of leaves from different portions of plants was removed after long-day and before short-day induction.

a. Leaf removal from tip to base

Plants were selected for 16 visible leaves and exposed 21 long days (16 hr. pp. 20°C., 16 hrs. artificial light; 8 hrs. dark). After the long day treatment the plants were defoliated as described in Table XXIII-A, and moved to short days (8 hr. pp.; 26°C., 8 hrs. natural light; 20°C., 16 hrs. dark). After seven short days they were dissected.

Defoliation began with the first visible leaf (10 mm. long or less) and proceeded basipetally. The number of leaves removed and the results of the experiment are indicated in Table XXIII-A.

There is no inhibitory effect of removing the terminal five leaves prior to SD induction. This was shown in an experiment (Exp. 105) in

which plants were exposed to 12 LD (16 hr. pp.; 26°C. 8 hrs. natural light; 17°C. 8 hrs. art. light; 8 hrs. dark) followed by seven SD's (26°C., 8 hrs. natural light; 20°C., 16 hrs. dark). The five terminal leaves (5-25 mm. long) were removed immediately after LD induction. The results of the experiment are recorded in Table XXIII-C; compare treatments #1 and #3.

The results show that removal of more than five terminal leaves prior to SD induction severely inhibits flowering in the terminal regions but has little effect upon flowering at the lower nodes. The lowest nine to nineteen leaves respond to short-day induction regardless of the response of the more apical or basal leaves.

6. Leaf removal from base to tip

In another experiment, in which leaves were removed acropetally, it was shown that some of the "lowest 10" leaves are required for optimal response.

Plants were selected for 14 visible leaves, exposed to 15 long days (16 hr. pp.; 26°C., 8 hrs. natural light; 20°C., 8 hrs. artificial light; 20°C., 8 hrs. dark), defoliated as described in Table XXIII-B, and then transferred to short days (16 hr. pp.; 26°C., 8 hrs. natural light; 20°C., 16 hrs. dark). After seven short days the plants were dissected. Results are recorded in Table XXIII-B. There is a marked reduction in response if 10 leaves are removed and a complete absence of initiation if 15 leaves are removed. These experiments are not sufficient to determine the function of the lower leaves. Perhaps the leaves serve a dual function: supplying food to younger leaves permitting them to respond to short-day induction, and translocating excess floral stimulus to upper nodes and

TABLE XXIII

Which leaves respond to short-day induction?

A. Expt. 78 Defoliation from tip to base

Treatment	% fl.	% term. fl.	# nodes fl/pl	# nodes fl. by transloc.	# lvs. remaining after defol	#pls.
Control	100	71	22.6± 13.4		29	7
Top 10 lvs. removed	71	14	11.4± 11.8	5.9	19	7
Top 15 lvs. removed	71	0	8.9± 9.2	3.4	14	7
Top 20 lvs. removed	29	0	0.4± 0.8	0	9	7

B. Expt. 85 Defoliation from base to tip.
Note: # nodes fl/pl not counting lowest 18 nodes.

Treatment	% fl.	% term. fl.	# nodes fl/pl	# lvs. rem. after defol.	length of lgst. remaining lf.	# pls.
Control	100	83	8.0	25	165 mm.	6
Lowest 10 lvs. rem.	50	0	1.5	15	164 mm.	6
Lowest 15 lvs. rem.	0	0	0	10	84	6
Lowest 18 lvs. rem.	0	0	0	7	42	6

C. Expt. 105 Defoliation acro- and basipetally

Treatment	% fl.	% term. fl.	# nodes fl/pl	# pls.
1) Control	100	56	22	9
2) Lowest 5 removed	90	50	17.7	9
3) Top 5 removed	100	63	22.3	8

Errors quoted are standard deviations of the mean.

causing initiation. By subjecting the lower leaves to SD induction while the upper receive long days, and vice versa, it should be possible to determine whether the upper leaves are capable of producing specific short-day substances causing floral initiation.

The results of Exp. 105 (Table XXIII-C) show that removal of the lowest five leaves has little inhibitory effect upon floral initiation (compare treatments #1 and #2).

The drastic reduction in terminal flowering by removal of 10 leaves even though there are 15 leaves remaining, three of which are greater than 110 mm. suggests that the lower leaves supply most of the floral stimulus. Since the leaves which respond to short-day induction must have received preceding long-day induction, this experiment indicates that it is the lowest 10-15 leaves which respond to long-day induction. Removal of the lowest five leaves had very little inhibitory effect so that it may be possible to localize the response to LD induction in the 5th through 15th leaves.

7. The Effect of High Temperatures After Short-Day Induction

a) Cestrum

The previous experiments show that there is a stimulus(i), translocated from the leaves to the growing points responsible for floral initiation (and possible floral development).

The heat lability of the stimulus is tested in the following experiment. For a suitable introduction see discussion on heat lability (Chapter VIII, B, 5). In the case of a labile stimulus, heat treatments may destroy sufficient amounts to inhibit floral initiation.

A single experiment was performed testing for heat lability of the short day stimulus. The high temperature was applied five hours after the end of the second long night. This time was chosen to permit the second high light intensity process (44) to come to completion, and precede the time of translocation to the growing point.

Plants were exposed to 15 long days (16 hr. pp; 23°C., 8 hrs. natural light; 8 hrs. artificial light; 8 hrs. dark) followed by two short days (8 hr. pp.; 8 hrs. natural light; 20°C., 16 hrs. dark). Five hours after the end of the second dark period one group of plants was placed in a darkened 50°C. oven for 10 minutes and the other group was placed in a 23°C. darkroom for the same period of time. Both groups were then transferred back to long day conditions and dissected 10 days later.

Results:

Treatment	Per cent fl.	Per cent term. fl.	# nodes fl/pl	# pls.
Control	100	43	*18.7± 8.2	7
10 min., 50°C.	86	14	* 8± 9.5	7

Errors quoted are standard deviations of the mean.

* Difference significant at 5 per cent level.

t=2.23 ,12 DF ,P.= 0.05 .

Although there was no wilting or damage to the leaves or growing points, the phloem tissue in a 6 cm. band around the crown of the heat-treated plants was killed.

The short heat treatment is inhibitory and one may conclude that the process of floral initiation is heat labile 5 hours after short-

day induction. The experiment is suggestive of the fact that the stimulus(i) involved in floral initiation is destroyed, and may be of high molecular weight.

b) Xanthium

A similar experiment was performed with Xanthium plants.

Experiment 106 - Plants were selected for approximately equal #3 leaves (all other leaves were removed) between 55 and 95 mm. long. They were exposed to a single long night (15 hrs. 23°C.) and then transferred to high intensity light for 6 hours (23°C., greater than 2000 ft. candles). One group was placed in a 50°C. oven for 10 minutes and the control plants were placed in a 23°C. dark room for 10 minutes.

Both groups were then moved back to high intensity light (23°C., 8 hrs. natural light; 17°C., 16 hrs. artificial and natural light) and dissected 7 days later.

Results:

Treatment	Per cent fl.	Per cent inhibition	Stage	# pls.
Control	100	--	6.3 ± 0.5	10
10 mm. 50°C.	100	*25	4.7 ± 1.7	10

Errors quoted are standard deviations of the mean.

* Per cent inhibition = 100 - Heat/Control x 100 = 25

The 25 per cent inhibition is significant at the 5 per cent level.

t=6.1 ,18 DF ,P.= 0.000X

The inhibition is less than that observed with a similar post-inductive heat treatment of Cestrum. Since there was no sign of any damage to vegetative structures, it is concluded that the floral stimulus is heat labile in Xanthium. Further experiments, comparing Xanthium

and Cestrum with increased exposure times may give evidence as to the relative heat lability of the stimulus in the two plants. Heat lability, or heat inactivation, studies have been employed to determine the molecular weight of enzymes and nucleic acids. Perhaps similar experiments pertaining to the stimulus for floral initiation will aid in determining the relative molecular weights of the stimulus in different plants. If the differences are great, it would suggest that a number of different molecules may be involved as stimuli for floral initiation.

D. Intermediate Daylengths and the Relationship Between Long and Short Day-Induction in Cestrum

Allard (3) found a few plants which produce flowers only when the photoperiod was kept within narrow limits. For example, sugar cane flowers when the day length is 13 hours; no flowers are produced if the photoperiod is greater or less than 13 hours.

If "intermediate daylength" plants had two overlapping photo-periodic requirements for floral initiation (as does Cestrum), they would be expected to produce flowers only in photoperiods within the range of overlap.

Conversely, since the daylengths for the long and short day requirements overlap between 11 and 13 hours, it is possible that Cestrum would produce flowers if subjected to continuous photoperiodic induction in this range.

Figure 5 illustrates the response of Cestrum to photoperiod for both long and short-day induction. The data are derived from Tables XI and XVIII.

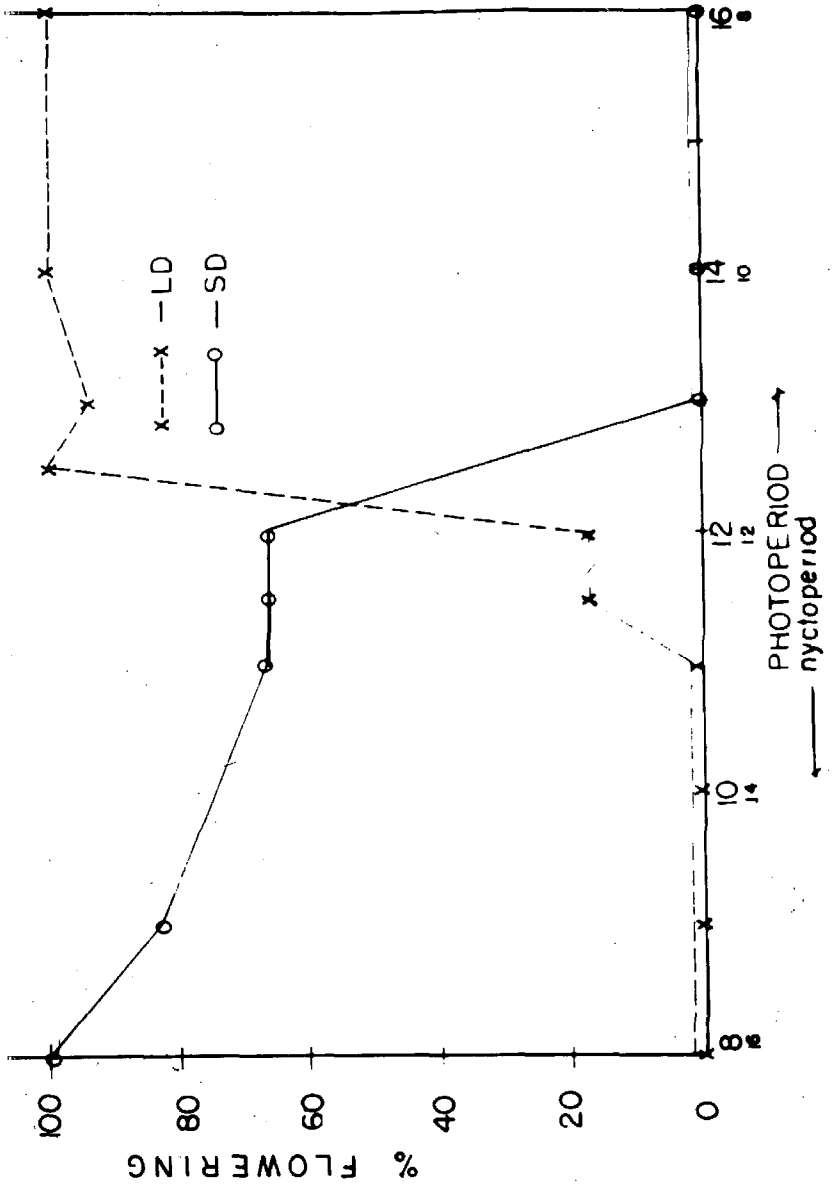


Fig.5. Critical photo- and nyctoperiod for LD and SD induction, respectively.

Showing overlapping region between 11.5 and 12.5 hr. photoperiod.
Data taken from Tables XI and XVIII.

Three daylengths were applied continuously:

1) 11.5 hours continued for 42 days

2) 12 hours continued for 40 days

3) 12.5 hours continued for 30 days

No flowers were produced on any plants remaining in these conditions. However, if, at any time after 18 days, they were removed to eight-hour daylength conditions then flowers developed within seven days. Evidently plants receiving these daylengths have been long-day induced.

It is known that 11.5 - 12.5-hour night lengths are sufficient for minimal short-day induction; apparently night lengths in this range are only inductive when plants have been long-day induced in more favorable photoperiods. What is the explanation for this anomaly?

One would expect that the effectiveness of minimal long-day induction would increase as the number of long days increases, and, if the number of long days were extended sufficiently, there would be no difference between the minimal and optimal inductive treatments.

This is not the case as illustrated by the data in Table XXIV, comparing the increase in terminal flowering by increasing the number of long days of 11.5, 12, and 16-hour photoperiod. Terminal flowering is a reflection of an accumulation of stimulus, and evidently there is little chance for accumulation in 11.5 and 12-hour photoperiods. In this case the limiting substance determining terminal flowering must be the long-day product. An 8-hour photoperiod is employed after all three treatments, and the difference in response must be due to differences in long-day induction.

TABLE XXIV

Photoperiod of LD Induction vs. Terminal Flowering

Photoperiod (Hours)	# LD's	% term. fl.	# pls.
11.5	18	0	6
	28	0	6
	40	0	6
12	18	17	6
	28	0	6
	42	17	6
16	7	0	7
	14	70	13
	18	83	21

Why is a 12.5 or 12-hour night inductive following long-day induction by 16 hr. pp. and not inductive following an 11.5 or 12-hour photoperiod? Less long-day product is synthesized in 11.5 and 12 than 16-hour photoperiods, regardless of the number of long days.

Possible there is a quantitative relationship between long and short-day induction such that the product of the long day processes determines the efficiency of the short-day requirement or the amount of short-day product. In short, there may be a mass action relationship between the product of long-day induction (L) and the product of short-day induction (S).

1). $L + S \rightarrow LS$ (floral stimulus)

2). $S \xrightarrow{L} C$ (floral stimulus)

3). $L \xrightarrow{S} C$ (floral stimulus)

It is possible that Cestrum flowers in the intermediate daylengths if the treatments and prolonged past the 40-day limit. Even if this were the case, an explanation is required for the difference between 16 and 12 or 11.5-hour photoperiods.

Flowering in intermediate daylengths, in plants having two overlapping photoperiodic requirements, may depend upon the efficiency of the long day reactions in the range of overlap. In the case of sugar cane, sufficient long day product is formed in 13-hour photoperiods to permit short-day induction by 11-hour night lengths. A hypothetical relationship between the critical photo- and nycto-periods in sugar cane on the basis of the proposed scheme is represented in Fig. 8 (Discussion).

E. The Flowering Behavior of Cestrum in Natural Daylengths

In the Pasadena area, Cestrum plants produce flowers from May to October. Since floral initiation is dependent upon long followed by short days, autumn flowering is understandable. However, it is not easy to explain why flowering occurs in May and June when the daylengths are constantly increasing and short-day induction is impossible. Plants in nature have already received their long-day induction by this time (See Table XXV) but do not receive day lengths below 13 hours until the end of August (See Fig. 6, recording sunrise-sunset interval). Excluding the possibility of "intermediate daylengths" through March and early April causing floral initiation (see section D of this Chapter), what factors may contribute to plants receiving the proper photoperiodic sequence in the long days of May and June?

We generally refer to natural daylengths as the sunrise-sunset interval without regard to light intensity. In fact the natural daylength is extended by the twilight hours at dawn and dusk, and is reduced by cloudy days and shading by buildings and trees. The light intensity required to stimulate long day and inhibit short-day reactions is of a very low order, in some cases, as low as 1 ft. candle applied continuously (72). However, the reduction in light intensity in a dense forest or within the branches of a shrub can be quite significant. For example, when the light intensity at the top of a Cestrum plant is 10 ft. candles the intensity at the lowest leaf is less than 1 ft. candle. This measurement is for plants with a main axis and no branches; in nature, Cestrum plants grow as densely foliated shrubs, and the light intensity reduction is at least as severe.

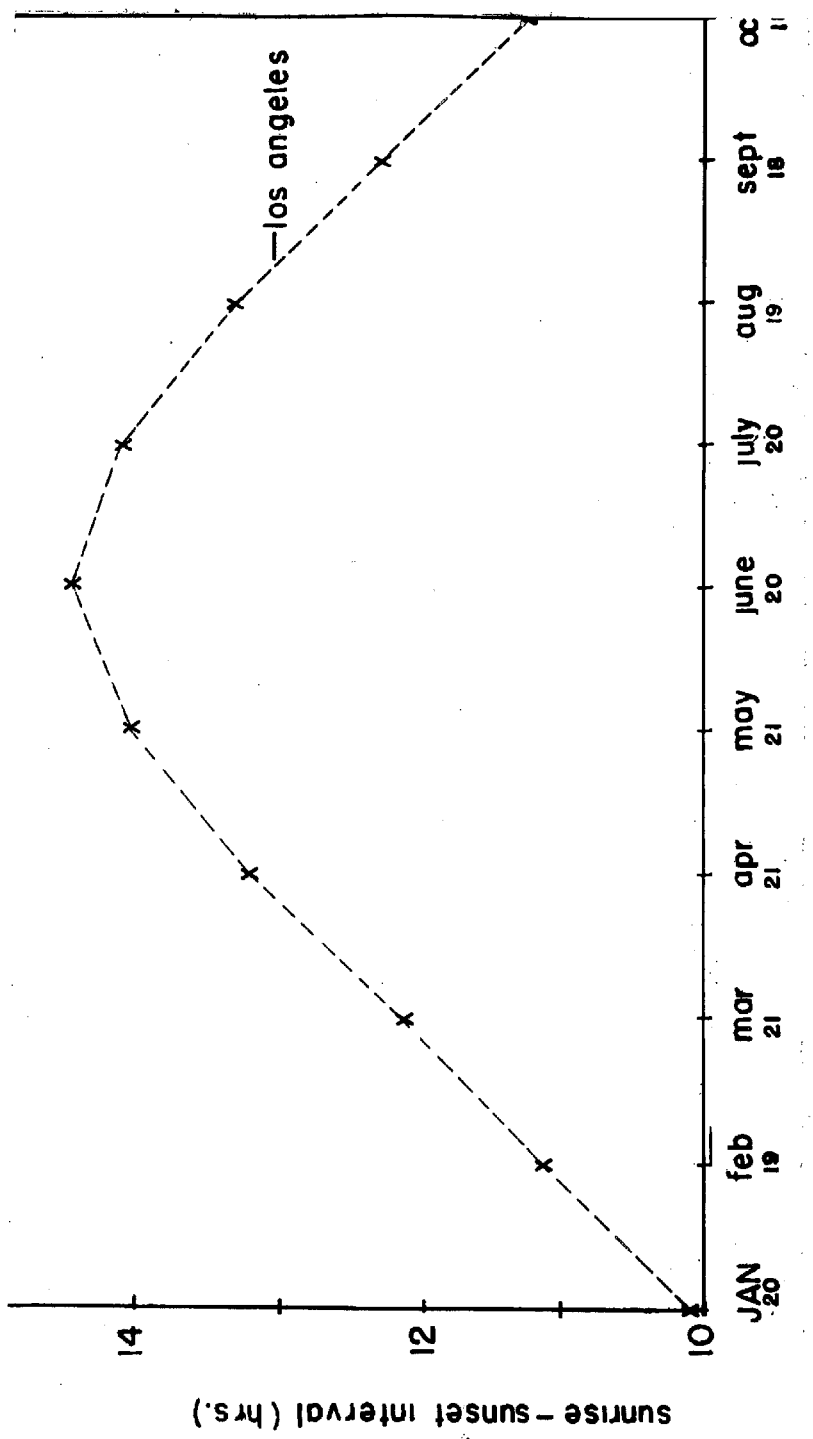


Fig.6 Natural daylength.Data recorded by U.S. Weather Bureau for Los Angeles,1948.

TABLE XXV

Long-day induction by natural daylengths

Plants placed in greenhouse (26°C. 8 hrs, 20°C., 16 hrs.) for number of days indicated and then transferred to short days (8 hr. pp. 26°C., 8 hrs. natural light; 20°C., 16 hrs. dark). Seven days later plants were dissected.

# Days in Greenhouse	Dates of Entry and Exit	% fl.	% term. fl.	# nodes fl/pl.	# pls	sunrise-sunset-int. hrs.
22	3/6 - 3/28	33	0	2	12	11.6-12.4
18	3/12 - 3/30	17	0	0.3	6	11.8-12.5
18	3/30 - 4/17	100	17	16	6	12.5-13.1
18	4/5 - 4/23	83	50	19.5	6	12.7-13.3
18	4/11- 4/29	100	67	27.7	6	12.9-13.5
18	4/17 - 5/5	100	100	29	6	13.1-13.7

If plants are grown in the greenhouses throughout the year and care is taken to prevent shading, flowering does not occur until August and September. If shading is permitted then flowering during May and June is confined to the lower branches at the regions of greatest shading.

In the latitude of Pasadena it is probable that Spring and Summer flowering is a result of a reduction in light intensity below the limit of perception by Cestrum, thereby shortening natural daylength and permitting short-day induction.

Cestrum is indigenous to the islands of Cuba and Martinique. At the latitudes of these islands the natural daylength ranges from 13 hours in June to 11 hours in December (4). It is interesting to note that Schulz (63) recorded flowering on Cuba and Martinique in January and February, coinciding with the periods following short-day induction (11 hours in December). If there were an intermediate daylength satisfying the requirements for floral initiation, flowering would be expected throughout the year in the tropics. A large part of the year is spent with daylengths between 11 and 13 hours in the range of overlap of the critical day and night length.

F. The Rate of Development of Cestrum Flowers

1. A stage system

The rate of development of the terminal male inforescence of Xanthium is constant for a limited time during the early stages following floral initiation (62). That is, it is possible to describe a series of morphological changes in the inflorescence which follow one another at constant time intervals. The rate is determined by the strength of induction and reflects the concentration of stimulus causing floral initiation.

Since the rate after initiation is constant, for a certain period of time one may dissect plants after induction, determine the stage of development, the rate in stages per day, and estimate the concentration of floral stimulus produced by an inductive treatment. It is not the absolute concentration, rather the relative concentration produced by different inductive treatments that is determined by this method.

For Cestrum the problem of measuring the rate of development of flowers is complicated by the fact that there are a number of flowers initiated on a large number of axillary shoots. (Only the terminal male inflorescence of Xanthium was considered).

Not all of these flowers have the same growth rate, and as yet no completely satisfactory system has been divised by which it would be possible to select one flower on one node of a plant for measurement.

For the sake of simplicity, the stage of development of the most advanced flower represents the floral stage of the entire plant, regardless of the development of flowers at other nodes. Criticisms of the method will be made at the end of this chapter.

By dissecting plants on successive days after the beginning of short-day induction, and recording the morphological changes occurring at the apical and axillary meristems, a series of stages were selected. The stages follow one another at constant intervals, and therefore, reflect a constant rate of development of the flower. The dissections were made daily four to eight days after the beginning of short-day induction.

The morphological changes occurring in the transition from vegetative to floral during the eight-day period are described in Fig. 7.

Fig. 8 is a graph plotting the stage of development against the number of days after short-day induction begins.

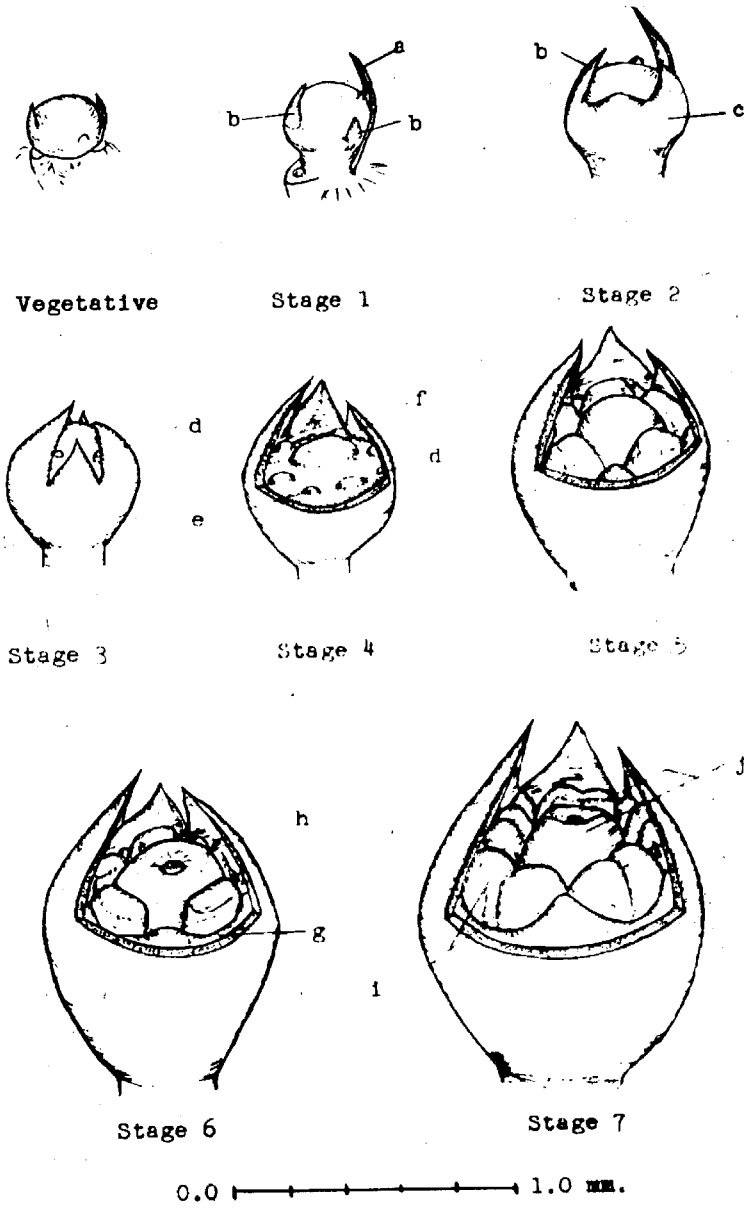


Fig.7 Cestrum floral ontogeny.Stage system described in text.Letter references described on following page.

Description of Stages and Letter References Appearing in Fig. 7

Stage 0 — vegetative bud; apex flattened; youngest leaf primordia are stubs on either side of apex; axillary buds are undeveloped.

Stage 1. — floral; apex expanded and raised; youngest leaf primordium elongated and arched around bud (bract) (a); calyx lobe primordia apparent (b); axillary buds slightly swollen.

Stage 2. — floral; apex enlarged; calyx lobe primordia forms ring around bud (c); 5 lobes evident (b).

Stage 3. — floral; calyx lobes almost enclose central mass; stamen primordia (d) appear on central mass;

Stage 4. — floral; calyx lobes enclose central mass (two are removed); stamen primordia enlarged and corolla lobe primordia (e) appear; central mass defined as hemisphere (f).

Stage 5. — floral (two calyx lobes removed); stamen and corolla lobe primordia cover entire space between hemisphere and calyx; corolla lobes enlarged.

Stage 6. — floral (two calyx lobes removed); corolla lobes linked to form ring (g) around stamens; central hemisphere invaginates forming ovary (h).

Stage 7. — floral (two calyx lobes removed); corolla lobes enlarged; stamens bifurcating to form pollen sacs (i); carpels (j) become evident enclosing ovary.

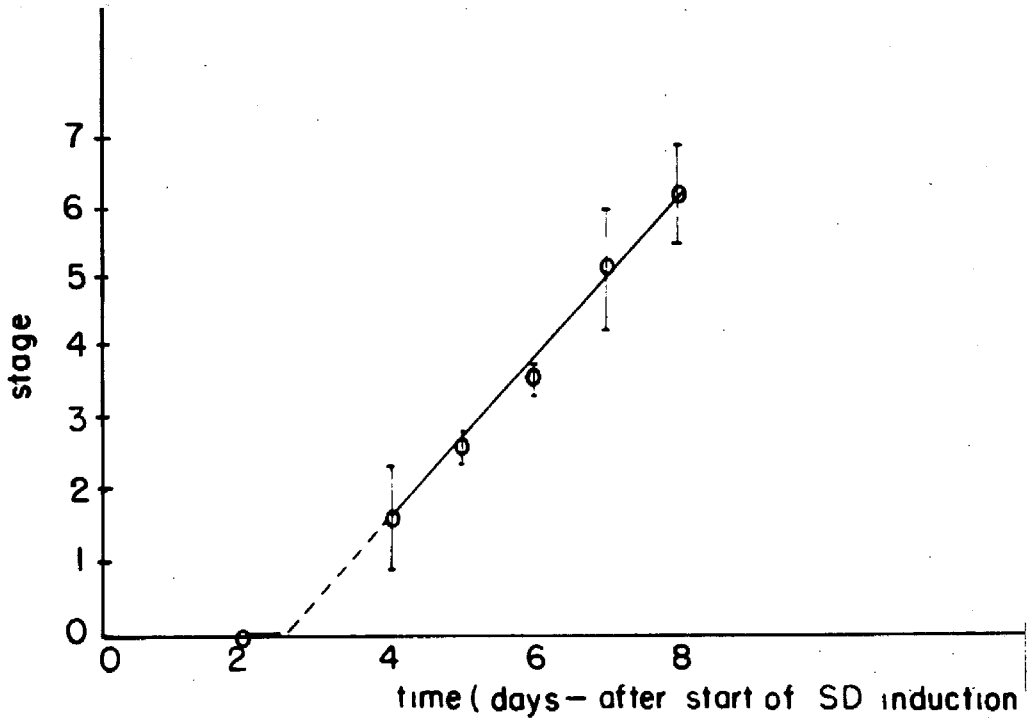


Fig. 8

Stage vs. Time. The rate of development (stage/day) is equal to the slope of the straight line. The stages correspond to those pictured in Fig. 7. (Development in 8 hr. pp.; (26°C. 8 hrs. natural light; 20°C., 16 hrs. dark)

If the rate of development of a flower is dependent upon the initial concentration of floral stimulus, the stimulus must be limiting. There is probably a concentration of stimulus which, if exceeded, has no effect upon the flower. The stage system for Cestrum is based upon the rate of development of the most advanced flowers, and if these flowers are initiated by the optimum amount of stimulus, the maximum rate is determined. If it is assumed that some leaves produce an excess and others limiting concentrations of the floral stimulus, a determination of the most advanced flower reflects this fact alone, and does not provide any information regarding the strength of induction.

For the Cestrum stage system to be useful it must be concerned with treatments in which the concentration of stimulus is limiting the rate of development of the flower, as in the case of Xanthium. If it is possible to induce one leaf and consider one node, the present stage system will be valid for determining the effect of induction on the rate of development.

2. The Effect of Inductive and Post-Inductive Treatments on the "Most Advanced Flower".

a. Inductive

Acknowledging the fact that the Cestrum stage system is not comparable to that for Xanthium, a survey was made of the effects of various treatments on the rate of development of the "most advanced flower" (MAF).

Varying the strength of induction had little effect upon the stage of development of the MAF. Compared with the effect upon the number of nodes flowering or per cent flowering, there was little evidence that the MAF reflected the strength of induction.

Table XXVI summarizes results of three experiments showing the effect of induction upon the rate of development of flowers, number of nodes flowering, and per cent flowering.

Note: Abbreviations for Table XXVI

nodes fl/pl. fl. equals the total number of flowering nodes divided by the number of plants with flowers.

MAF/pl. fl. equals the sum of the stage of the "most advanced flower" divided by the number of plants flowering.

It was concluded on the basis of experiments such as the above that the MAF is not a good criterion for comparing the effectiveness of inductive treatments.

b. Post-Inductive

That the rate of development of Cestrum flowers is not determined solely on the basis of inductive conditions, is shown by the effect of increasing the length of day following short-day induction.

To test the effect of increasing the number of short days upon floral initiation, it is necessary to transfer plants back to long days after receiving the desired number of short days (described in Section C of this chapter). The comparative effect of continuous short days or short days followed by long days upon the rate of flower development is summarized in Table XXVII (described in Section C, 3).

The effect of the long days following short-day induction is to increase the rate of development of the "most advanced flower", even though terminal flowering indicates an excess of floral stimulus. Whether this effect of long day is a specific long day phenomenon or due to increased temperature and/or photosynthates arising from the additional 8 hours high intensity light is not known. An experiment in which the

TABLE XXVI

Inductive Treatments vs. Rate of Development of Flowers

	# nodes fl/pl. fl.	MAF/pl.fl.	% fl.
1) Heat treatment after LD induction			
Control	14 ± 5	3	78
Heat	6± 4	2.5	30
2) Number of Long Days			
7	1.5± 0.5	6	29
9	7.5± 5	6.5	100
3) Defoliation after SD induction			
Hr. of Defoliation after end of second long night	# nodes fl/pl.fl.	MAF/pl.fl.	% fl.
27	8	5.5	14
123	15.4	4.8	100

Errors quoted are standard deviations of the mean.

TABLE XXVII - Post-inductive Treatments vs. Rate of Development of Flowers

Comparative Effect of Long and Short Days Following SD Induction

Legend # LD's - number of long days following SD induction

Expt. 58

# LD's	# nodes fl/pl.fl.	% term. fl.	MAF/pl.fl
5(a)	16	25	6.8
0(a)	21	38	5.0
Expt. 75			
5	15 ± 10.8	29	4.9
4	18 ± 11.7	43	5
3	22 ± 9.5	58	4.6
0	23 ± 6.1	72	3.9
Expt. 96			
5	19.4 ± 12.3	43	5.8
4	19.5 ± 13.7	50	5.8
3	28.3 ± 7.6	86	6.1
0	28.6 ± 13.1	71	5.1

Errors quoted are standard deviations of the mean.

supplementary light of the long day was of low intensity would permit separation of the two possibilities.

IX. Discussion

A. Summary

The results of experiments with Cestrum listed below are pertinent for the discussion which follows:

1. Cestrum requires LD induction followed by SD induction for floral initiation.

2. Interruption of a long night with high intensity light or extension of a short day with low intensity light satisfies the long day requirement. The same is true for Hyoscyamus, spinach, and aster, all LDP not requiring SD after LD induction.

3. Interruption of a long night with high intensity light or auxin application prior to a long night inhibit SD induction. The same is true for Xanthium and soybean, both SDP not requiring LD before SD induction.

4. Short days are completely ineffective in causing floral initiation prior to LD induction.

5. Continuous LD induction following extended periods in short days does not cause floral initiation.

6. There is a relationship between LD and SD induction such that if one is strengthened the other may be weakened. E. G. A 12-hour nyctoperiod is sufficient for SD induction after LD induction in 16 but not 12-hour photoperiods.

7. Floral initiation may be inhibited by high temperatures after LD and SD induction.

B. Relationship of Cestrum to Long and Short Day Plants

It is customary to create schemes accounting for the flowering behavior of plants; the discussion that follows attempts to explain floral initiation in Cestrum with the assumption made that specific chemical substances are the causal agents.

For Cestrum the product of long-day induction serves as a precursor in the synthesis of, or a co-factor with, the product of short-day induction. The end product of long and short-day induction is translocated from the leaf and causes floral initiation. Both the long and short-day requirements possess characteristics typical of other photoperiodic phenomena.

If one were to generalize on the basis of these results for Cestrum, at least two schemes could be suggested to account for floral initiation:

1) $\xrightarrow{LD}$ L $\xrightarrow{SD}$ S (floral stimulus)

In this case "L" is a precursor in the synthesis of "S", and "S" may be identical with the floral stimulus. Both "L" and "S" are heat labile. The reason why SD before LD induction is ineffective is simply because there is no precursor available for SD reactions.

"L" is produced independently of daylength in SDP and by LD in other LDP. The conversion of "L" to "S" is daylength independent in LDP and requires SD in other SDP.

2) L + S ---> LS

L (long-day product) - the production of "L" is stimulated by long days in Cestrum and other LDP, but is independent of daylength in SDP. It is produced and accumulated in leaves and is not identical with photosynthates.

S (short-day product) - the production of "S" is stimulated by short days in Cestrum and other SDP, but is independent of daylength in LDP. It is probably a labile product which cannot be accumulated except in combination with "L".

LS (floral stimulus) - the production of "LS" is determined by the relative concentrations of "L" and "S" and is translocated from leaves soon after synthesis. It has to be assumed that "S" is labile for it is known that "short" preceding long days will not cause floral initiation.

These schemes may be made more complex by the addition of the partial processes discovered for other long and short day plants and by the results of low temperature and short-day vernalization discovered for some biennials and winter annuals.

Is it possible to test the validity of either scheme? Floral initiation is inhibited by exposure of plants to high temperatures following long-day induction, suggesting that the long-day product, "L", is heat labile. If other short day plants have a requirement for "L", high temperature treatment prior to short-day induction may inhibit floral initiation. This is providing, of course, that "L" exists in limiting concentrations or the mechanism controlling its synthesis is limiting.

If Cestrum can be grafted to other short or long day plants, it will

be possible to test the identity of the floral stimulus (LS) in Cestrum with other response types. Since "L" (and possibly "S") is not translocatable, grafting data will not provide evidence pertaining to this part of the scheme.

There is no experimental evidence known to the author that precludes the possibility that in other photoperiodically-sensitive plants a sequence of long and short day reactions is essential as suggested for Cestrum.

C. Relationship of Cestrum to Intermediate Day Plants

The response of intermediate day plants (3) may be explained on the basis of two overlapping photoperiodic requirements (Scheme 2, above). If the production of "L" and "S" can occur simultaneously, it will be in a range in which the critical day and night lengths overlap. If there is a high efficiency of production in this daylength range, sufficient floral stimulus, "LS" will be formed to cause floral initiation. In the case of Cestrum, the rates of production of "L" and "S" are too low in the 11.5 - 12.5-hour photoperiod to attain the required concentration of LS. The overlap of photo- and nycto-periods required to explain the sharp 13-hour daylength requirement for sugar cane (Allard) is represented graphically in Fig. 9 (the shaded area represents the "intermediate daylength").

For sugar cane the hypothetical critical photoperiod for LD induction is between 12 and 13 hours and the critical nyctoperiod between 10 and 11 hours. The "intermediate daylength" region can be expanded, contracted, or re-shaped by shifting the curves for the critical photoperiods along the abscissa or by changing the slope of the curves.

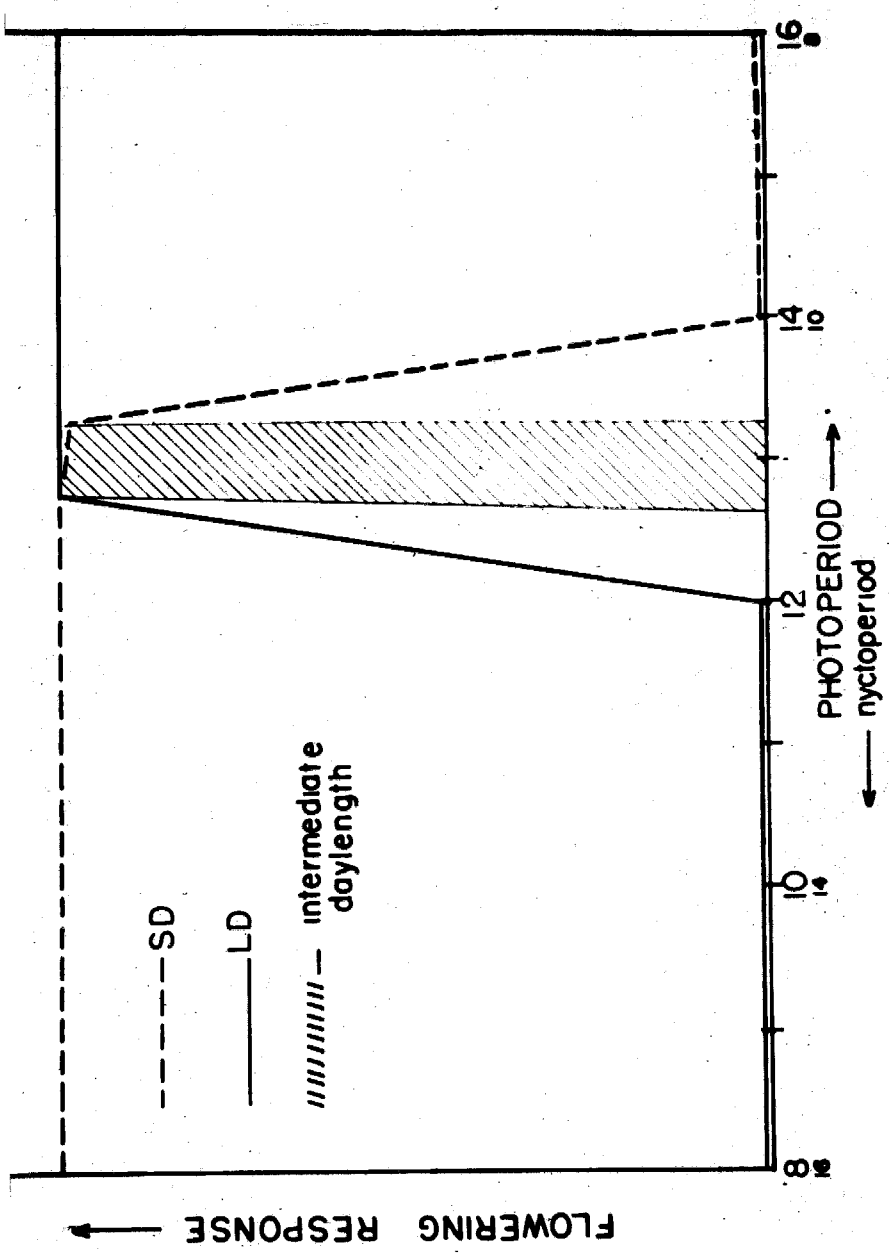


Fig. 9 Hypothetical "Intermediate Daylength Plant". Critical photo- and nycto-periods overlap between 12 and 14-hour photoperiod. Region of maximum efficiency for simultaneous LD and SD induction centered around 13-hour photoperiod.

Allard's experiments were with extended inductive treatments in a constant daylength, i.e., plants were placed under certain photoperiodic conditions and remained there until flowers were produced. No attempt was made to determine the effect of long days followed by short days. If the scheme proposed for Cestrum has general validity, it should be possible to induce Allard's intermediate day plants with two photoperiodic treatments, applied in sequence.

D. The Nature of the Floral Stimulus

An extensive discussion of this topic was included in the literature survey, and only the new data with Cestrum and Xanthium will be reviewed here.

Heat treatments of Cestrum and Xanthium plants after short-day induction inhibit floral initiation (see Section C, 8, Chapter VIII). The time of exposure to high temperature is sufficiently beyond the end of the dark period to remove the possibility that some heat-labile precursor or synthetic mechanism is destroyed (Lockhart and Hamner).

It is assumed that the inhibition results from the heat-inactivation of the floral stimulus(i). Heat lability is characteristic of high molecular weight substances and these experiments suggest that the floral stimulus is a large molecule. However, it is possible that there are a number of high molecular weight substances involved in floral initiation.

These studies were stimulated by previous discussions suggesting that floral initiation is controlled by proteins or nucleic acids. In Xanthium the persistence of induction may indicate that the stimulus or mechanism controlling its production is self-reproducing (properties of nucleic acids and/or nucleo-proteins). It has been impossible to extract

the stimulus from plants, which may indicate that extraction and re-application causes denaturation and inactivation (properties of proteins). The parallel between the nature of induction of crown gall tumors (probably due to a nucleic acid) and floral initiation in Xanthium is remarkable (see Chapter II, page 37). Braun, as a result of his heat-inactivation studies provided the first clue as to the nature of the tumor-inducing principle, and the present "elevated-temperature" experiments were in search of the nature of the floral stimulus.

Floral initiation is an example of tissue differentiation, a general phenomenon in biology, and perhaps it is not too far afield to examine results pertaining to differentiation in amphibian embryos. The theory of epigenesis (71) developed by animal embryologists suggests that differentiation is a gradual process. The differentiation of one tissue is dependent upon the previous development of another underlying or adjacent tissue. How this operates is not known, but it has been suggested that chemical inductors produced by one cell type diffuse to nearby tissues and cause them to differentiate. Recently Niu and Twitty (54) have shown that the differentiation of cells from gastrula ectoderm of salamander embryos, can be accomplished by culturing them in media previously supporting the growth of axial mesoderm. Presumably these substances diffused from the axial mesoderm and are the effective agents in differentiation of the ectodermal tissue.

Is it reasonable to expect the floral stimulus to be the same in all plants? The little grafting evidence available suggests that this is true.

"Floral stimulus", is always used in reference to the substance causing initiation, the onset of flowering. No mention has been made of

the possibility that other factors are involved in the development of flowers. The morphological changes occurring after the first sign of initiation are quite complex. In some cases the flower does not develop beyond the first stages (62, 65), and the control of further development is dependent upon increased induction. This implies that something other than the stimulus for initiation is synthesized in the inductive conditions or that the initiating stimulus is functional in the later development of the flower. If the development of a flower is automatic, and the initiating stimulus is analogous with sperm-initiating development of an egg, the stimulating influence of continuous induction beyond that required for initiation is not easily understood. On the other hand, floral development may depend upon the continuous supply of specific factors produced in the leaves; in some plants the formation of these factors may require the same conditions required for the synthesis of the initiating substance. Murneek (47) suggested that the occurrence of "vegetative flowers" was strong support in favor of specific developmental substances.

With the techniques at hand, a certain amount of morphological change is required before floral initiation is recognized, i.e., some amount of development must occur after initiation, otherwise the effects of induction go unnoticed. If there are developmental stimuli, it is possible that some plants are not limited by the amount of initiating substance.

Induction, in such cases, supplies developmental, not initiating stimuli; as induction is continued more developmental stimuli are provided until, finally, the flower reaches maturity. This is exactly what Schwabe (65) found to be true for Chrysanthemum.

There is no estimate of the number of daylength (or low intensity light) controlled reactions functioning in plants. Since photoperiodic treatments affect pigment formation (52, 47) the pH of cell sap, tuberization, dormancy, and stem elongation (52), it must be assumed that many reactions are affected by low intensity light, and floral initiation may be the end result of a general change in the metabolism of the plant.

In searching for a floral initiating substance we may overlook the effects of photoperiodic induction upon floral development. In so doing we ignore the possibility of a multiplicity of substances controlling "initiation" by virtue of their effect upon development.

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