# AN ATTEMPT TO EXTRACT A FLOWER-INDUCING STIMULUS FROM CERTAIN PHOTOPERIODICALLY SENSITIVE PLANTS

Thesis for the Degree of M. S.
MICHIGAN STATE COLLEGE
James Arthur Lockhart
1951

This is to certify that the

thesis entitled

[illegible]

presented by

[illegible]

has been accepted towards fulfillment
of the requirements for

[illegible] degree in [illegible]

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Date [illegible]

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# AN ATTEMPT TO EXTRACT A FLOWER-INDUCING STIMULUS FROM CERTAIN PHOTOPERIODICALLY SENSITIVE PLANTS

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A THESIS

Submitted to the School of Graduate Studies of Michigan
State College of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Horticulture

1951

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# ACKNOWLEDGEMENT

The author wishes to express his sincere appreciation to Dr. Charles L. Hamner under whose direction this study was made. His suggestions and criticisms have been of great value and the writer is sincerely grateful for his guidance throughout the course of this work.

# I INTRODUCTION

Until about the beginning of the present century it was generally believed that; since the genetic constitution of the plant determines not only the type of reproduction, but, also, to a considerable extent, the time of its onset, sexual reproduction in its entirety was under the control of the so-called "germ-plasm".

The earlier hypothesis of Sachs (109), that hormones control organ formation lacked experimental evidence; and it was Klebs who took the early lead in the investigation of the physiological causes of flowering. Klebs showed that the environment, especially light and temperature, could greatly influence the transformation from vegetative to reproductive growth (53, 55). He came to the conclusion that a balance between carbohydrates and minerals, especially nitrogen, is the primary internal cause of flowering (54).

Soon Kraus and Kraybill (59) reemphasized the problem of the relationship between carbohydrates and nitrogen, and their work stimulated research which has continued to the present day. In the subsequent studies, it has sometimes been forgotten that the results of Kraus and Kraybill's experiments with the tomato were based on fruit-set and not flowering. Although a great deal of work has been done on the relationships between the internal nutrients and metabolites of the plant and the subsequent growth responses, the definitive work on this phase of the physiology of flowering remains to be done, in the opinion of this author.

Shortly after the work of Kraus and Kraybill came the discovery of an environmental factor with which the initiation of flowering in some plants could be qualitatively controlled. This was the discovery by Garner and Allard of the phenomena of photoperiodism. This presented for the first time a technique with which to study the physiological factors and the biochemical processes which are the direct cause of the flowering response.

Rather slow progress has been made in the elucidation of the mechanisms involved in flowering however; partly because of the complexity of the problem and partly because the problem was not adequately defined until the work of Hamner and co-workers in the late thirties. They introduced into this field, for the first time, a point of view which produced immediate advances and it appears that their approach will be the one which will ultimately resolve the problem.

## II REVIEW OF THE LITERATURE

### EARLY HISTORY

Nearly all of the early work relating to the influence on plants of the daily duration of light was directed toward determining the extent to which both the growth and the development of the plant may be stimulated by lengthening the daily light period. Considerable prominence was given the question of whether plants would thrive under continuous light or if they need a daily rest period.

According to Smith (112), apparently the first reference in the literature to the influence of length of day on plants is found in Carl von Linne's "Ron omvaxters plantering grundat, pa naturen" (64), published in 1739. However, Linne ascribes the rapid growth and early maturity attained by plants in polar regions to additional heat supplied by the continuous sunlight, rather than the additional light as such. Schubeler (110) in 1879, advanced the idea that the cereals and other species of plants, when gradually transferred from lower to higher latitudes, undergo definite changes in growth characteristics, and ascribed the observed effects to direct or indirect action of additional sunlight. Siemens (111) in 1881, reported results of growing plants under electric-arc lamp to replace or supplement sunlight and he reached the conclusions that, under suitable conditions it can replace sunlight, and the plants apparently do not require a daily rest period.

Kjellman (52) during an expedition to the north coast of Siberia in 1878 and 1879, conducted experiments with the arctic species Catabrosa algida and Cochlearia feulestrata which were exposed to continuous northern light or a twelve hour day. As opposed to the shortened photoperiod the continuous light resulted in a more rapid growth and earlier and more profuse flowering. The effects on flowering, however, were only quantitative and were not particularly striking.

In the period 1891-1893, Bailey (3) conducted investigations with light from arc lamps used for a portion or all of the night as a supplement to daylight, particularly with the idea of forcing vegetables. The additional light hastened the growth of lettuce and induced early flowering in spinach. Rane (99), working along similar lines with the incandescent carbon filament lamp, obtained much the same results with lettuce and spinach and observed earlier blooming in certain flowering plants. Corbett (28) demonstrated that night illumination, as a supplement to daylight, markedly stimulated top growth at the expense of the roots, in sugar beets; and he observed stimulation of growth in several other plants.

According to H. A. Allard (1) an interesting reference to the photoperiod as effecting plants is given by A. Henfrey, in his book, "The Vegetation of Europe", 1852, where he proposes that the length of day is a factor in the natural distribution of plants.

Klebs also, as early as 1913, seems to have sensed the fact that photoperiod affected the time of flowering of some of his experimental plants. With _Sempervivium funkii_ he was not able to secure flower development in the winter by changes in temperature, nutrition, etc., so he exposed them to a few days of continuous electric illumination. They produced flowers in the same greenhouse in which other, non-lighted plants remained vegetative. Concluded Klebs (54):

"In der freier Natur wird sehr wehrscheinlich die Blutezeit dadurch bestimmt dars vonder Tag und nachtgleishe (21 Mar$_z$) ab die Lange des Tag es Zunimmt, die von einer gewissen Dauer ab die Aulagen der Blute veranlasst. Das licht wirkt wohl nicht als ernah render Factor, sondern mehr Katalytisch."

In the work of Tournois (120) with _Cannabis sativa_ and _Humulus japanicus_, published in 1912, there is found what is apparently the first definite suggestion that the attainment of the flowering stage may be hastened by a relatively short photoperiod. Tournois demonstrated that a precocious type of flowering which occurs in very early spring plantings of these species can be reproduced by allowing the plants to receive sunlight for only six hours daily. Apparently he did not extend his researches in this direction.

## DISCOVERY OF PHOTOPERIOD

To Garner and Allard, however, goes all the credit for demonstrating the fact that the length of day qualitatively controls the change from vegetative to reproductive growth in many plants (34). It was a big step, from the point of view of that time, to accept the fact that such a "dilute" factor as the length of daylight should have such a marked influence on development, but from the first report this conclusion was inescapable.

Their discovery came as a result of breeding experiments with tobacco, in which a new variety, "Maryland Mammoth", failed to flower during the summer months, and of soybean experiments in which successive plantings throughout the spring and early summer all tended to flower at the same time. In the case of the tobacco the investigators were at first misled by the fact that plants in small pots in the greenhouse during the winter and early spring flowered profusely, suggesting a nutrition angle. After a time, however, the critical observation showed that as spring approached, from the stumps of the flowering plants there arose new shoots of typical vegetative growth. From this it seemed quite clear that some seasonal variation was involved. Experiments soon showed that it was the duration of the light period which regulated the reproductive habit.

They made many subsequent studies (35, 36, 37) to determine the number of plants which are photoperiodically sensitive. Particular attention was paid to the reproductive response but the effects of photoperiod on tuberization, bulbing, character and extent of branching, root growth, pubescence, pigment formation, abscission and leaf fall, dormancy and death were also studied. They observed that other environmental factors, particularly temperature, modified the effects of photoperiod.

## CLASSIFICATION

Garner and Allard (34) proposed the classification of plants in relation to photoperiod essentially as it stands today, because they early recognized the importance of the length of the dark period. They called those plants "short-day plants" which have a maximum critical photoperiod, above which flower formation is inhibited (i.e. a minimum dark period requirement). Those plants which have a minimum critical light period requirement (limited dark period tolerance) were called "long-day plants"; while plants whose flowering response is not qualitatively affected by daylength were called "indeterminate". The parenthetical expressions above indicate more closely the present accepted definitions, especially for short-day plants. In the case of long-day plants a more complicated situation exists; they will flower on light periods greater than their critical, regardless of the length of the dark period, but they will also flower on short cycles of light providing the dark periods are also short (e.g. six hours light--six hours darkness; five seconds light--five seconds darkness) (37).

Within these categories there is the greatest possible variation. Some short-day plants may remain vegetative almost indefinitely if kept on a long-day, while others will eventually flower, and in some the short-day character is only indicated by the formation of flowers on nodes nearer the base of the plant (11). The long-day plants also intergrade imperceptibly into the indeterminate, there being plants in which a long-day may speed flower formation by only a few days.

## INDUCTION AND INHIBITION

Early in their studies Garner and Allard (35) observed that by exposing certain plants to the proper photoperiod for a comparatively short length of time (in Biloxi soybean for ten days) flower and seed production would subsequently occur. Since then numerous investigators have observed similar phenomena, although considerable variation is reported (18, 19, 31, 66, 67, 68, 98).

Rasumov studied after-effects in representative long- and short-day plants (100), and concluded that treatments early in the life of the plant would markedly effect the subsequent growth and development of the plant. In a rather striking demonstration, Murneek started plants of Rudbeckia in the greenhouse under natural light so that as the season progressed the daylength increased from ten to thirteen hours. At the longest daylength some of the plants were moved to a long-day bench where they formed stalks and flowered normally; of the others which at the same time were transferred to a short-day bench: some remained vegetative, some formed "vegetative flowers" and some formed true flowers on the rosettes. The different reactions, Murneek suggested, could be the result of different degrees of development in the individual plants at the time they were exposed to the long-day (13 hour) photoperiods. Those which became "ripe to flower" first received the longest induction, enough to induce flowering,

although not stem elongation; those that remained vegetative had received little or no induction; while those which produced "vegetative flowers" had been subjected to an intermediate induction period (75). A variation of this explanation is also possible: it may be that the critical period varies in the individual plant (suggested by work reported here, see RESULTS), then some would have received a longer induction period than others.

The phenomena of induction exhibits striking variations between species of plants, when viewed from the point of view of the stimulus produced. Induction is most striking in short-day plants; cocklebur requires only one short-day (at normal temperatures) for induction (48), and Biloxi soybean only two days (12). Long (65) has shown that in the soybean these two cycles must be consecutive, that regardless of the number of cycles given on alternate days no flowering would occur. Hamner has demonstrated that twenty hours of light between the subsequent dark periods is the maximum interruption which will still permit induction to occur in the soybean (44). In cocklebur the number of cycles required to induce flowering can be increased to about seven by lowering the temperature during the dark period to 40° F. In this case it has been shown that the stimulus will carry over one long-day after the first four induction cycles (44). This suggests that the stimulus produced during each dark period rapidly disappears, yet Hamner and Bonner (48) found that, in cocklebur, the stimulus continues to be produced after the leaf is transferred back to a long-day, although this is not true of Biloxi soybeans (44). Hamner and Bonner concluded that once the reaction had attained a certain rate, it became autocatylytic in cocklebur.

In an annual beet (a long-day plant), which has an induction period of about twenty days, it has been demonstrated that, after the first ten days, it is possible to interrupt the induction cycles with short-days for more than ten days without any apparent reduction in the quantity of the stimulus (80).

The destruction or accumulation of the stimulus has not yet been studied in other plants but it is already clear that a great diversity of response exists. It seems likely (but not certain) that this diversity represents differences in the rate of disappearance of the stimulus. It would seem possible to test the validity of this assumption, and if it is found to be true, to determine whether this represents a difference in the nature of the stimulus or differences in the internal physical and chemical environment of the plant to which the stimulus is exposed.

Murneek has repeated the demonstration of the induction of flower formation without stem elongation in Rudbeckia with high temperatures (78). He considers the effect of the short-day on long-day plants to be inhibitory. Greulach (43) confirmed the findings of Murneek, and he too considered the effect of short-day on the elongation and flowering of long-day plants to be an inhibition of flowering. Neither, apparently, seriously considered the alternative; that elongation and flowering take place only in the presence of some stimulus, and when this is withdrawn (as in short-day conditions) the plant reverts to vegetative growth. The question remains in doubt, although recent work on the action spectra of photoperiodism seems to support their conclusion (see LIGHT AND PERCEPTION).

## PHOTOPERIOD AND ANATOMY

In 1936, Roberts and Wilton (107) reported that plants induced to flower, in comparison to those not induced, showed more complete differentiation of the xylem and phloem, and assumed that this meant a cessation of cambial activity. However, Psarev reported an increase in stem diameter of the soybean at the time of flowering (97). Most of the subsequent work has been done by Roberts and his associates; they repeat and refine their previous observations.

The rosette type of growth, that is, the growth and development of many leaves with no corresponding elongation of the stem, is characteristic of many long-day plants when grown under short-day conditions. Upon exposure to a period of photoinductive cycles, the stem elongates and flowers are formed at the apex. In the case of Gypsophila elegans, reported in this paper, the elongation takes place almost equally at all internodes, so that little or no indication of the previous rosette condition remains. In other plants the same general habit may be observed or the stem may elongate only from the apex, giving rise to a scape with few or no leaves.

Roberts and his associates, in various papers (103, 105, 106, 107, 124, 125) have reported a cessation of cambial activity in all types of plants at the time of flower induction. They claim to have observed this phenomena in typical long-day plants, but nowhere do they mention or even imply that their non-flowering long-day plants were in the rosette condition. On the contrary, the only mention made of the rosette habit of growth, in Robert's papers, appeared when he stated (106): "An extensive study of the relation of stem anatomy to flowering of monocots has not been attempted as so few of the commonly available species have stems suitable for sampling when in a non-flowering condition. The grains and grasses as well as most other locally grown species of this group are in an induced state before stems long enough for sampling of internodes are produced."

This certainly implies that long-day plants with the rosette habit of growth either were not used or were used only after they had begun to elongate. A number of examples are given, including a few presumed long-day plants, but this writer has been unable, as yet, to determine whether any of those mentioned usually grow with the rosette habit under short-day conditions. Most of the "long-day plants" which they used do not show particularly striking long-day responses. While all long-day plants do not grow as rosettes and all plants with the rosette habit are not typical long-day plants, never-the-less the two characteristics are strongly correlated.

It is certainly reasonable that determinate annual plants, upon reaching a late stage of maturity and fruiting, would lose cambial activity. As the plant soon dies it is apparent that this must occur. In plants of a rosette habit, however, a great deal of cell elongation must occur; and preliminary observations by the author bear out the logical assumption that considerable cell division also occurs.

Even Robert's own evidence fails to bear out his conclusions. He shows many microphotographs comparing cross-sections of flowering and non-flowering stems (106, 107, 116), and these show clearly that in flowering stems differentiation of xylem and phloem cells occurs much more rapidly; lignification of the xylem can be observed immediately adjacent to the cambium region, while in non-flowering stems several differentiating cells separate the lignified xylem cells from the phloem. This, however, does not necessarily prove that cambial activity has ceased. It could be proposed rather that the subsequent differentiation was accelerated. In many of his photographs the number of vascular layers in the flowering stem appear to greatly exceed that of the non-flowering stems.

## PERCEPTION AND LIGHT

Garner and Allard (35) found, by exposing only a portion of the plant to an inductive photoperiod, that in Cosmos the effects are largely localized; that portion of the plant which received the inductive cycle responded typically but the remainder of the plant remained uneffected by the treatment. They pointed out that the inductive stimulus may, in some cases at least, be transported from one part of the plant to the other, for example, in the tuberization of potatoes. This was later verified with several other tuber producing plants by Rasumov (101, 102).

Knott (56), in extensive work with the spinach, was led to believe that the leaves in some way hasten the photoperiodic effect in plants. In 1936 Cailahjan (20), Moskov (72), and Psarev (96), apparently working independently, each came to the conclusion that the green leaves are the organs which receive the "photoperiodic stimulus", and that certain physiological processes arise there which act on the growing points, "directing" them either to further vegetative growth or to floral initiation. Cailahjan and Moskov proposed substances of a hormonal nature, which Cailahjan called "florigen". At the same time Kiupper and Weirsum (60) in Holland advanced the same idea. Later investigators, including Hamner and Bonner (48) and a great many others, have confirmed the fact that the leaf is the organ of perception of the photoperiodic stimulus.

Borthwick and Parker (13) found that the youngest fully expanded leaf was the most effective in the reception of the stimulus in Biloxi soybean and this has been found generally true of other species (46).

Considerable early work was attempted on the action spectrum of photoperiodism (126) but the technical problems involved were numerous and it was not until the work of Parker, Hendricks, Borthwick and Scully (14, 92, 93) that clear-cut results were obtained. By interrupting the dark period in short-day plants and extending the light period in long-day plants with light of very narrow wave lengths they were able to accurately determine the action spectrum for these plants. They found that the spectra for the two types is apparently identical, and that while the spectra generally resembled that of the chlorophylls, it seems quite clear that the differences found were real. This was further borne out when it was found that this spectrum coincided very closely with the spectrum for the so-called "etiolation effect" in peas (94). In both these responses there is a comparatively high effect in the red and low activity in the blue portion of the spectrum. A similar action spectrum has been obtained by Weintraub and Price (122) for the inhibition of elongation of the first internode of Avena. Goodwin and Owens (39) reported similar results and they attributed this to absorption by protochlorophyll masked in the blue by carotenoids. Parker and Borthwick (91) have pointed out that this action spectrum does not fit that for the transformation of protochlorophyll to chlorophyll, and they suggest instead the resemblance of these spectra to the absorption spectrum of the pigment phycocyanin. At this time, however, the receptor pigment remains unidentified.

This light effect is the one which interrupts the dark reaction in short-day plants, and apparently interrupts a dark reaction in long-day plants as well. It may be characterized as being fully effective even when of short duration (1-30 minutes) and at very low intensities (1-10 f.c.).

In both long-day and short-day plants light of "normal" intensities is required for at least a portion of the day. There is strong evidence that this high intensity light effect is involved in the production of the precursor of the stimulus (44) and it may play a significant role in transport also. In contrast to the low intensity light effect (discussed above) the high intensity light effect is apparently photosynthetic in nature (see THEORIES).

## NATURE AND TRANSLOCATION OF THE STIMULUS

It was pointed out earlier in this paper (see PERCEPTION AND LIGHT) that the leaf has been shown to be the organ of perception of the photoperiodic stimulus. It therefore seemed likely that a study of the translocation of the stimulus from the leaf to the growing point presented a point of attack in studying the nature of the stimulus.

Investigations in this direction have shown that the transmission of the stimulus apparently takes place only through living cells, as indicated by the fact that its movement out of the leaf can be inhibited by low temperatures (15, 24), scalding (127), girdling (23), and narcotics (24). Further work (20, 21, 22, 73) demonstrated that the stimulus could readily cross graft unions. This work showed that induced plants, when grafted to non-induced plants of the same species, could transmit the stimulus to the non-induced plants causing them to flower. It was also demonstrated that when short-day plants were grafted to indeterminate plants (both kept on a long-day) the short-day plants were induced to flower, indicating that the stimulus in these two types are similar. Some of these authors also reported the cross-transmission of the stimulus between short-day and long-day types but this has not been confirmed since the early work.

Hamner and Bonner (48) reported that the stimulus could cross a "diffusion contact" prepared by separating the graft partners with a piece of lens paper, and careful microscopic examination of the graft, made after the termination of contact, revealed no evidence of tissue contact. However, Withrow and Withrow (127) in attempting to repeat these experiments failed to effect transmission except in those cases when subsequent examination revealed tissue contact, if only a few cells. In another attempt to demonstrate transfer of the flowering stimulus across a non-living contact, Moskov (72) reported that by placing an induced leaf in close proximity to the cut petiole of a non-induced plant in a water medium, induction would occur. Galston attempted to repeat this experiment, but without success (33), and Melchers and Lang have also reported failure of the stimulus to cross a "diffusion contact" (70).

Hamner and Bonner (48) demonstrated that while an induction cycle of one day sufficed to induce flowering in the cocklebur, the leaf must remain on the plant for at least four days for flowering to subsequently occur. They also present evidence to show that the leaf continues the production of the flowering stimulus after it has been returned to a non-inductive cycle. Long (65), as was mentioned above, found that a short inductive cycle given to Biloxi soybean resulted in flowering at only a few nodes in the vicinity of the induced leaves. Hamner and Bonner (48) showed that the non-induced leaves influence the translocation of the stimulus; when ~~two~~-

branched cocklebur plants were treated so that one branch (the donor) received an inductive cycle while the other (the receptor) remained on a long-day the receptor flowered, but this could be prevented if the young leaves of the receptor were removed. They also demonstrated that if the older leaves of the receptor branch were also removed, flowering would occur.

This strongly suggests that the old leaves which are not induced exert an inhibitory effect on the translocation of the stimulus, while the young leaves promote this translocation. This was confirmed by Borthwick and Parker (11) who found that only by defoliating the leaves of the receptor branch of a two-branched soybean could the receptor be made to flower, and by Heinze, et al., (51) who showed that this is also true of grafted soybeans. Moskov (74) reported that if the old leaves are kept in complete darkness instead of on a long-day their inhibitory effect was decreased. Stout (115) has combined these effects in an experiment in which annual beets having three stems were treated so that one stem was on a long-day, another was on a short-day and the third was kept in continuous darkness. The stems in long-day and continuous darkness flowered (in that order), but the stem on a short-day remained vegetative. Stout concluded that the stimulus moves with the carbohydrates. Although a definite conclusion hardly seems warranted as yet, this remains one of the most likely possibilities. Recent experiments by Roberts

(104) in which he shows that, when the induced leaves of cocklebur are shaded, the plants respond much more slowly than the controls but sucrose sprayed on the leaves of the shaded plants will overcome this delay, lends further support to this theory, although Roberts draws an entirely different conclusion from his work. The buds also exert an effect on the translocation of the stimulus; if they are removed from the "donor" branch of cocklebur, the stimulus appears to reach the receptor with greater force (48).

Several possible explanations suggest themselves to account for these phenomena. If, as Stout has suggested, the stimulus is translocated with the carbohydrates it would be possible to visualize the effects exerted by the young and old leaves of the receptor. It has been demonstrated that the growth regulator 2,4D is translocated in this manner (71, 121). With this hypothesis it would be more difficult to explain the response arising from the removal of the buds, but it could be suggested that this would remove a demand for sugars on the donor side. The effect of the removal of most of the buds on the receptor side might furnish a clue to this question. The fact that these organs are the major centers of auxin production in the plant also suggests a possible relationship, although Bonner (6) has shown that the primary effect of applied auxin in inhibiting flowering of <u>Xanthium</u> is on the production or the translocation out of the leaves. It could also be suggested that the buds use up some of the stimulus which would otherwise be free to move to the receptor, and, not necessarily in conjunction, the old leaves might absorb or destroy that portion which, in some way, comes under their influence. It should be simple to resolve this problem.

## EFFECT OF TEMPERATURE

It was believed for a time by many workers that in photoperiodically responsive plants, the duration of light was the only factor which affected the flowering response. Soon, however, Thompson and others discovered that celery (118), beets (117,) lettuce (119), and stocks (95), for example, which give responses to photoperiod, may also be induced to flower by chilling.

Chroboczek (27) working with beets, showed that favorable conditions of light and temperature are essential to the development of a fertile inflorescence, as well as to initiate flower primordia. By regulating the temperature and the photoperiod, the time normally required for seedstalk formation could be greatly shortened or materially lengthened. Steinberg and Allard (114) reported that the critical period for flowering in soybean, Rudbeckia bicolor, and beet may be altered to a limited extent by temperature, and, conversely, the favorable temperature range for flowering may be shifted by the action of the photoperiod. Knott (57), studying the effect of temperature on the photoperiodic response of spinach, found that with photoperiod fixed at fifteen hours per day, seed-stalk elongation occured sooner if the temperature during the treatment was held at 60° to 70° F., than at a higher or lower temperature. Roberts and Struckmeyer found that the responses of many species of plants to photoperiod could be materially altered by varying the temperature above or below normal (105). Gilbert (38) studied the interrelation of length of day and temperature in *Xanthium pennsylvanicum*, and reported that temperature influenced the time of production of flower primordia. It is not clear in many of these studies whether the temperature exerts its primary effect on the initiation of flower primordia or on the subsequent visible flowering response of the plant.

In the strawberry, the production of flowers and runners are independent functions of photoperiod and temperature (30). Flowers, which are produced under short-day conditions, can be induced on a longer photoperiod at lower temperatures. The production of runners, a long-day response, is encouraged by high temperatures.

In the onion a very interesting situation exists (50). Bulbing and flower-formation are antagonistic. Bulbing is a response to long photoperiod and is irreversible after an adequate induction period, except that the tendency may be destroyed by high temperature over-winter storage. During the growing season, however, high temperature speeds up the bulbing response. Flower formation was, for a long time, believed to be the result of short-day photoperiods, but Heath and Holdsworth show that, at the low temperatures necessary to inhibit bulbing, flowering is actually stimulated by long-day conditions. They postulate a rather complicated balance between a bulbing hormone, a flowering hormone and auxin; together with their precursors and alternative, inactive end-products (see THEORIES). Murneek (75, 78), as has been previously pointed out, succeeded in partially bypassing the photoperiodic requirement of Rudbeckia by a high temperature treatment, and this has also been reported with China aster (5).

Borthwick and Parker (15) studied the effect of localized low temperature on flower initiation in Biloxi soybean, in which the petiole and growing point were cooled separately. They found that in cooling the petiole or the growing point to 10° C. there was only a relatively small effect on flower-bud formation, while their previous work (87) had shown that cooling the entire plant to 12.5° C. during the dark period, flower-bud formation could be almost entirely prevented. They concluded that the production of the flowering stimulus in the leaf was the temperature-sensitive reaction. In a further report (90), they confirmed this conclusion by cooling or heating the leaves while the rest of the plant remained at normal temperatures, demonstrating that the limits of photoperiodic induction in Biloxi soybean are narrower than the growth temperatures. Long (65) found that in cocklebur, lowering the temperature to 40° F. would increase the induction period from one day to about eight days, but it increased the critical period only slightly.

The effects of temperature on the photoperiodic reactions are still not well understood, but it seems quite probable that the effects are multiple. Temperature effects the rate of growth of plants and thus would influence the time of attainment of the "ripe to flower" condition, and the rate of the growth response to the stimulus. It has also been shown (above) that temperature has a specific effect on the "dark reaction" in short-day plants, confining the reaction to a comparatively narrow range of temperatures; and, within this range, regulating the critical period and the induction period, probably through its effect on the rate of chemical reactions. It might be suggested that this effect on the critical period and the induction period could be the result of : (1) the quantity of the stimulus produced in a single dark period, (2) the partial destruction or dissipation of that already produced, or (3) an effect on the effective distribution of the stimulus produced. It would seem feasible to test these and other possibilities. If, as seems quite clear (see THEORIES), two separate reactions take place during the dark period, it would be of interest to determine which of the reactions is the temperature sensitive. The author has attempted to study this question, but without definite results to date.

Action spectrum work (see LIGHT AND PERCEPTION) has produced evidence that the dark reactions necessary for flowering in short-day species may be similar in nature to a dark reaction in long-day plants which inhibits flowering. The action spectra for the effective interruption of the dark periods have been shown to be the same, thus it would seem logical to propose that the photochemical reaction involved in both cases could effect, so strikingly, only reactions which are similar in nature. It would be of interest to determine whether the effects of temperature on the dark reactions of the two types are comparable, as well as to investigate, by other means, the nature of the two reactions. The apparent demonstrations that some plants are long-day plants at some temperatures and indeterminate at other temperatures (105) might be explained by this phenomena. It might be proposed that within a relatively narrow range of temperatures the dark reaction could take place (as has been shown to be true of short-day plants, see above) resulting in an inhibition of flowering, while outside this range the inhibition would be prevented.

Vernalization, a prolonged chilling treatment required by some plants before flowering will occur, has been recently reviewed by Whyte (123). The biennial Hyoscyamus niger, apparently a normal vernalization requiring plant, has been shown by Melchers (69) to flower in the first year if a scion of a short-day plant (maryland Mammoth tobacco) is grafted to it; and the plants are kept on a short-day. He proposes a second hormone "vernalin" normally present in annual plants but requiring a cold treatment for activation in biennial species. These results do not contradict Hamner's hypothesis (see THEORIES) and may be considered to lend some support to it, although Hamner and Bonner (48) have shown that the dark "condition" in Xanthium is not transmissable. Stout (115) has shown that in beets, an annual beet grafted to a biennial will induce the biennial to flower, supporting the idea that the biennial is incapable of production of the flowering stimulus before cold treatment; and that the plant is capable of responding normally to the stimulus.

It has been shown (29) that the effect of the cold treatment can be localized in the growing point, which suggested that the effect was a modification of the potentialities of the embryonic region, but the more recent evidence (above) suggests that the effect is on the young leaves. Gregory and Purvis (42) have shown that, in winter wheat and rye, vernalization may take place in the seed of the subject plants before it has ripened, and they found that the effect was directly on the embryo, but it would not occur in vaccuo. It is not clear whether the cold treatments required by the grasses is comparable to the treatments required by the biennial dicotyledonous plants.

## EFFECT OF OTHER ENVIRONMENTAL FACTORS

Considerable early work attempted to relate photoperiod to the carbohydrate: nitrogen ratio (2, 83, 88), but no definite correlations have been found. In probably the most intensive work, Murneek (76, 79) found that upon induction, soybeans increased in nitrogen with respect to any form of carbohydrates. It has been abundantly shown that nutrients can markedly affect the number of flowers as well as the subsequent fruit-set (58, 81); but nutrition has never been proven to substantially affect the critical period or the induction period of photoperiodically sensitive plants.

As the experimental work in this paper apparently shows (see RESULTS), water-tension, or the effect of water tension on nutrition, seems to effect the rate and quantity of flower production but it has not been shown that it has any qualitative effect.

## THEORIES ON THE MECHANISM OF PHOTOPERIODISM

The theories of the mechanism of photoperiodism represent, to date, attempts to formulate, from the available data, the step-wise reactions taking place within the plant which result in the production of the flowering stimulus. The first real effort was that of Hamner (44) who, it appears, directed his experiments to that end. His hypothesis represents the best one to date, in the opinion of the author.

Hamner originally proposed a general scheme to assist in explaining the reactions occuring in cocklebur and Biloxi soybean. More recently he has expanded the concept to include all types of photoperiodically sensitive plants (47). This expanded hypothesis has not yet been published in full, but Snyder (113) has presented that portion which is applicable to long-day plants. In his hypothesis for short-day plants, Hamner has proposed to let the symbol "A" represent the result of the reactions taking place in the light period, and "B" the result of those taking place in darkness; then "C" would represent the summation of "A" and "B", the substance or condition which moves to the growing point and there induces the flowering response. By exposing cocklebur plants to cycles of three hours darkness followed by three minutes light for twenty-four hours or longer Hamner showed that flowering would not occur if this treatment was immediately followed by the usual sixteen hour dark period. It was apparent then, that some precursor of the stimulus, normally produced in the light period and utilized in the subsequent dark period, had been dissipated by the short cycles. This was confirmed when it was shown that, when the plants were given a relatively short, bright light period after the series of short cycles and before the long dark period, the plant would flower; and that the intensity of flowering was proportional to the length and intensity of the light given, up to a maximum. These experiments showed that "A" was produced in the light, and was an essential precursor, which must be present at the beginning of the dark period, in order that the dark reaction could occur. It appeared that "A" might be some product of photosynthesis, and this idea was given support when Borthwick and Parker (89) showed that, in Biloxi soybean, induction was directly proportional to photosynthesis. More recently Bonner has found, in unpublished experiments, that in the cocklebur the initial light period may be replaced by the infiltration of sugar or citrate into the leaf (7). In his early work (above) Hamner also found that if the initial light period was followed by a long exposure to very low intensity light, the plant would fail to flower. He assumed that "A" would gradually disappear under these conditions.

The substance or condition "B" is proposed to be the result of the dark reaction, and to act in a more or less catalytic manner after it has reached a threshold value (the critical period), resulting in a transformation of "A" into "C", the flowering stimulus. The "B" is proposed to be the light sensitive portion of the reaction, being negated by as little as one minute of low intensity light in the middle of the dark period.

The "C" is the final product of the reactions occuring in the leaf; it is the stimulus which moves to the growing point and there induces flowering.

In his more recent extension of the hypothesis to include other types of photoperiodic plants, Hamner uses the same symbols to represent the same reactions or conditions. In long-day plants he assumes that the "B" is light stable and always present, so that the limiting factor is the amount of "A", the product of photosynthesis, or the interaction resulting in the production of the stimulus, "C". Snyder, as mentioned above, has reported that plantain, a long-day plant, using light and dark cycles of other than twenty-four hours, will flower in long light periods regardless of the length of the dark period, but that with short light periods it will flower only if the accompanying dark periods are also short. This supports the hypothesis, for, if in short light periods something is produced which disappears when the succeeding dark period is too long ("A"); while in long light periods a stable product is formed ("C"), as the above experiment suggests, then this follows the proposed hypothesis. The only unexplained point would seem to be the disappearance of the "A" during the dark periods. It has been shown that the action spectra for both long- and short-day are identical. This must mean that the disappearance of the "A" in long-day plants is the result of a positive reaction, presumably the same reaction which results in the formation of "B" in the short-day types. This paradox has not as yet been clarified, although it is possible that the level of auxin in the plant may also play a role here (see below).

Hamner has also proposed that the hypothesis might also apply to those plants which require vernalization before they are capable of flowering. He suggests that in this case the formation of "B" may be dependent on the cold treatment, after which it is stable as in long-day plants. The plants would then react as long-day or day-neutral plants. It was pointed out earlier that Hamner has observed that, of those plants which require vernalization, some may subsequently behave as typical long-day plants and others as indeterminate, but none, so far as is known, show the characteristics of short-day plants. In further support of this idea Gregory and Purvis (42) have shown that in winter rye and wheat the cold treatment can be replaced by a treatment with short-day in rendering the plant capable of subsequent flowering, but these plants still required a subsequent long-day treatment before flowering would occur. One point, concerning both long-day and vernalization requiring plants which has not as yet been clarified is that if "A" is a typical product of photosynthesis, and it is limiting in long-day plants, then sugar-feeding should reduce the critical light requirement. This has been shown to be true only in Hyoscyamus, an apparently atypical long-day plant (65). It may be that in most long-day plants both sugar and auxin are deficient; this point should be investigated.

Gregory (41) has attempted to show that Hamner's scheme for short-day plants is untenable in its present form, but he begins by misinterpreting the scheme. He assumes that as "B" is formed during the dark period it immediately reacts with "A" to form "C". He goes on to point out that, if the scheme is as he interprets it, certain difficulties arise. If "B", the light sensitive reaction, reacts with "A" as fast as it is formed, then the sensitivity cannot be accounted for. If, on the other hand, the "B" accumulates during the dark period then the quantity of "A" would limit the rate of the reaction, and this is not true.

Gregory goes on to propose that the "A" is reversibly converted to "B", and the "B" is then moved out of the leaf during the dark period. At the critical period the quantity of "B" translocated out of the leaf is supposed to have reached the critical value required to induce flowering. However Hamner (48) has shown that it takes four days for the stimulus to move out of the leaf, and that this is apparently not a function of the quantity of the stimulus. Under the hypothesis as proposed by Hamner, Gregory's objections are no longer valid

Bunning (16, 17) proposes to explain the photoperiodic phenomena through a diurnal rhythm which he assumes to be present in all plants, and for which he has considerable experimental evidence. This rhythm is characterized by two phases which he has named the photophile phase and the skotophile phase. They represent quantitative and qualitative changes in the biochemistry of the plant. The photophile phase, as the name implies, is the light phase, characterized by a stimulation of flowering when light is applied; while light applied during the skotophile phase tends to inhibit flowering, especially in short-day plants. Light acts to start the rhythmic cycle and when further light is applied during the ensuing photophile phase it reinforces the momentum of the cycle and promotes flowering. In long-day plants, because the cycle does not start until several hours after the initial light stimulus, a small amount of light, offered after several hours of darkness, will be introduced at the time of the maximum of the photophile phase and thus promote flowering. If the light is offered later (after 12-15 hours in long-day plants) it will arrive at the time of the skotophile phase and the plant will not flower. The light effect in long-day plants is a reinforcement of the photophile phase rather than an inhibition of the skotophile, as shown by the fact that long-day plants flower readily in continuous light.

It would appear from this that when a plant is on one rhythm, the experimental dark period would have to coincide with the existing skotophile phase in order to be effective in promotion or inhibition of flowering (depending on the type of plant). For long-day plants the critical experiments have not been performed, while in the case of short-day types the existing evidence is conflicting. Cocklebur may be removed from the long-day cycle at any time during the light period (after a brief minimum, see above) and the critical period is found to be the same for the one day induction. Biloxi soybean, however, offers some support for the hypothesis, in that it has been shown that this plant has an optimum light requirement previous to the dark period, but this could also be explained by a gradual dissipation of the product of the light reaction, as has been shown to occur under low intensities of light (44). It may also be suggested that the stimulus which was produced during the last cycle was adversely affected by the continued light. On the basis of the present evidence this theory cannot be rejected but it seems rather fruitless as a working hypothesis. It suggests no new approaches to the ultimate problem, the biochemical reactions which result in flowering; instead it would seem to set us back another step: to the study of the rhythms which make possible the reactions which produce the flowering stimulus. While this would be an interesting and worthwhile problem, its solution would not be necessary in order to carry on the present work, and in fact, an elucidation of the reactions resulting in the production of the flowering stimulus might be of great value in the study of the proposed cycles. It should be kept in mind, however, that when the problem reaches the stage of identifying the basis for the specific reactions involved this idea may play a very important part.

Lang and Melchers (62) propose a general scheme to outline the reactions taking place in <u>Hyoscyamus</u> <u>niger</u> which results in either flower formation or further vegetative growth. They propose two separate reactions, one (the primary reaction) taking place independent of light, is that which directly promotes flower formation, after it has attained a critical threshold level. The second reaction (the secondary reaction) takes place only in darkness, and it acts to inhibit the primary, flowering reaction. The products of the primary reaction, presumably the "flowering hormone", may accumulate, thus producing the induction phenomena.

In this form the hypothesis fails to take into account the observed flowering by other long-day plants on short cycles of light and darkness (37) but it apparently holds true for <u>Hyoscyamus</u>. It should be pointed out that some of the other reactions of <u>Hyoscyamus</u> are apparently unique. Defoliation and sugar-infiltration of the leaves will cause flowering in _Hyoscyamus_ (61) but this does not seem to be true of other long-day species.

Cholodny (26) in 1939, suggested that the experimental evidence at that time did not preclude the possibility that auxin was the controlling mechanism of flowering. Experiments with auxin (Indol-3-acetic acid) have since shown that while it is not the flowering hormone, it may play an important part in the production of the hormone (10). Recent experiments have shown that when cocklebur is kept very near the critical period, the application of 2,3,5 Triiodobenzoic acid, an "antiauxin", will result in the initiation of flower-like forms at the growing point (6). This seems to be further indication that the dark reaction in short-day plants may be, in part at least, a reduction of the auxin concentration at the site of production of the stimulus. Unpublished experiments by the author suggest that applied auxin stimulates and accelerates stem elongation in certain long-day plants (especially _Raphanus sativus_), but the effect on flowering itself has not been determined. Leopold and Thimann (63), in investigating the effects of applied auxin on the formation of flower primordia in long-day and short-day plants, found that applied auxin at any concentration would inhibit flowering in short-day plants, but in the case of the long-day species low concentrations of auxin increased the number of flower primordia markedly. This increase in flower primordia was directly correlated with the growth rate of the plant, as measured by weight. At higher concentrations of auxin the production of flower primordia was suppressed also in the long-day type; and in no case was flowering induced when the plant was not on an inductive cycle. These results may be fitted into the hypothesis of Hamner, if it is assumed that in long-day plants auxin is limiting the production of the stimulus, while in the short-day types the concentration of auxin is greater than the optimum and is inhibitory. The effect of the long-dark period in both types may represent a reduction in active auxin, in the later case to a level which permits the production of the stimulus and in the former causing an even greater deficiency. This idea has not yet been critically tested, but it would seem feasible to do so.

Heath and Holdsworth (50) as already mentioned, (see TEMPERATURE) have done a great deal of work with the onion and its peculiar reactions to various environmental stimuli. They have found that bulbing is the result of a long-day stimulus in the presence of high temperatures. The stimulus was found to be perceived by the youngest emerged leaf blade, and it was irreversible after an adequate induction period.

They propose a novel explanation for the critical period required to induce bulbing, but unfortunately it is apparently not applicable to other long-day phenomena. They propose that under the influence of "B" (their bulbing hormone) the linkages of the molecules or micelles of the cellulose making up the cell walls are broken and, given sufficient time, new cellulose is deposited between the old. If the length of the light period is insufficient, the "B" is not longer present to deposit the new cellulose, and the cell wall returns to its original dimensions. The "B" is produced from a precursor "A" which is formed in light. The "A" is transformed to "B" as it is formed, under high temperatures, in a reversible reaction; at lower temperatures the "A" is irreversibly changed to "C", considered a leaf-growth hormone. Experimental evidence has shown that the critical period is shorter at high temperatures and also decreased with age. They explain this by proposing that the higher the temperature, the more "A" goes to "B" instead of to "C". In older plants the greater leaf surface results in the production of more "B" during the light period which persists longer into the succeeding dark period and the greater quantity of "B" should result in more rapid deposition of new cellulose.

Heath and Holdsworth have found that the induction period for bulbing of the onion is independent of noticable swelling, which seems rather difficult to reconcile with the above hormonal mechanism. They suggest, however, that the induction period may be associated with the failure of the plant to produce new roots, although they admit that this has not yet been subjected to experimentation.

The proposed substance "C" is produced from "A", in mature leaf bases, apparently quite independent of the temperature, and it may also be formed in the growing point in storage, but this reaction takes place only at high temperatures. "C" is proposed to be essential for flowering, by inducing auxin formation in the elongating scape.

To account for the flowering response of the onion, Heath and Holdsworth propose another hormone system, the two forms of which they call "E" and "F". These two related hormones are considered to be in equilibrium, and both are destroyed by high temperature. They suggest that either "E" or "F" can promote inflorescence initiation but "E" is necessary to induce floral initiation and hence scape elongation. By removing the swollen leaf bases before storage in the fall or in the spring and comparing the flowering response, they have been able to show that the flowering stimulus is present in these swollen bases in the fall and gradually diffuses into the growing point during the storage period. During the winter, according to their hypothesis, the "E" is gradually changed to "F". Sometime in the spring the threshold value of "F" is reached to permit flower initiation, the length of time required depending on the size of the set. The "F" initiates the inflorescence toward spring; then, when growth resumes, the now active root system permits the change of "F" back to "E", which induces scape elongation. As both of these hormones are destroyed by high temperatures, flowering is prevented by a very warm spring or delayed planting until the weather is warm.

Of the schemes which have been discussed here, the only one which seems applicable to an entire group of plants is that of Hamner. There appears to be no valid experimental evidence which would render this scheme untenable in any short-day plant. This may be due, in part, to its apparent simplicity, yet it represents a significant advance, in that it provides a basis for further elucidation of the mechanism of the photoperiodic response, and experiments to this end are now in progress. With regard to the extension of Hamner's hypothesis to include long-day and vernalization requiring plants the experimental evidence is not so clear, as has already been pointed out; and further work will be required to settle this question.

## III METHODS AND MATERIALS

The experiments described were performed in the horticultural greenhouses at Michigan State College between March and September of 1949. The greenhouses were of the conventional type, steam heated when the temperature was low and ventilated when it became too warm. As these experiments were conducted from early spring and continued throughout the summer a wide range of temperatures were encountered, ranging from the lowest night temperatures of 50° F. to an occasional high of 105° F. The consistency of the experimental results and the uniform behavior of the controls provide strong evidence that temperature within the extremes cited has little or no qualitative effect on the flowering response of these plants.

To regulate the photoperiod two frames were constructed over which double thicknesses of black cloth could be drawn (Fig. 2). One was normally closed at four P.M. and opened at eight the following morning. The other was equipped with two pairs of white florescent lights attached through a General Electric time clock so that the photoperiod in this frame could be regulated.

For the determination of the critical period these lights were regulated to turn on at four A.M. and go off after eight A.M., and to be on again from four P.M. to eight P.M. In later experiments they were on from sunset to midnight and from three A.M. until sunrise, thus giving an adequate long-day.

Near the experimental set-up another florescent light was installed and kept burning throughout the night to provide a twenty-four hour photoperiod when required.

Seeds of Gypsophila elegans, var. New York Garden Market, were obtained from commercial sources; and the cocklebur fruit was kindly provided by Dr. Beale of the University of Chicago.

## EXPERIMENT 1, A Study of the Critical Period and the Induction Period in Gypsophila Elegans.

Preliminary observations indicated that Gypsophila elegans, a long-day plant, was sensitive to extremely weak intensities of light supplementing the day light of the winter months.

The first experiment was designed to find the approximate critical period and to investigate the extent to which induction would take place in this plant.

Two flats of Gypsophila elegans were sown on March twenty-second and immediately placed on an eight hour day in the greenhouse. On April tenth, uniform sized seedlings were selected and transplanted to one hundred twenty 4½" unglazed clay pots in good greenhouse soil, three plants per pot.

On May sixth, after the seedlings had resumed vigorous growth, they were placed under the experimental light periods of eight, ten, twelve, fourteen, sixteen, and twenty-four hours, six pots in each period. An extra twenty-four pots were moved to the sixteen hour period and at the end of two days, six of these pots were moved back to the eight hour photoperiod. At the end of five, ten, and twenty days other groups of six pots were returned from the long-day to the short-day bench. The remaining six pots were left on the sixteen hour day for the duration of the experiments.

In order to expose the plants to the varying day lengths, the pots for the ten, twelve, and fourteen hour days were set in wood flats to facilitate handling, and were moved from one frame to the other according to the following schedule:

During the day: 10 and 12 hour pots moved to the long-day bench
At 6 P.M.: 10 and 14 hour pots moved to the short-day bench
After 8 P.M.: 12 and 14 hour pots moved to the long-day bench

Thus all plants were exposed to eight hours of sunlight and varying periods of artificial illumination each day. It may be assumed that the quantity of photosynthesis taking place in all plants was essentially equal.

The results were recorded on June twenty-first when most of the plants on long-day were flowering profusely. Measurements were made from soil level to the tip of the stem or to the uppermost flowers, when these were present. The plants were continued on the various day lengths for another two weeks and no qualitative variations from these results were observed; and, in addition, the eight hour controls were allowed to remain for another six weeks.

## EXPERIMENT 2, Attempts to Transfer the Flowering Stimulus in Gypsophila Elegans.

At the same time that experiment 1 was run, a second experiment was attempted to discover if Gypsophila elegans could be induced to flower by the injection of extracts of flowering plants.

The general method of Grainger (40) was used. Plants beginning to elongate were selected for extraction, cut into small pieces and ground in a mortar. An amount of distilled water was added to approximately double the volume and the mixture was filtered through a coarse cloth. The mixture was injected by means of a hypodermic needle and syringe into several of the leaves and petioles of each of the ten test plants. It was estimated that approximately 1.0 c.c. was injected into each plant at each application.

Sixteen applications were made beginning on May twenty-fourth and continuing until June eighteenth, covering a period of twenty-five days. On June eighteenth the injections were discontinued and the plants remained on an eight hour day for the duration of the experiment, a period in excess of two months.

It should be noted that the injections, while not administered daily, covered a period exceeding the time of visible response of plants put on a long-day.

## EXPERIMENT 3, Attempts to Transfer the Flowering Stimulus in Xanthium Pennsylvanicum.

This experiment was conducted to combine the application of 2, 3, 5 triiodobenzoic acid, shown by Bonner (6) to effect a "partial initiation of the inflorescence" in Xanthium, with the here-to-fore unsuccessful injection technique in an attempt to induce floral initiation in cocklebur.

One hundred Xanthium fruit were soaked twenty-four hours in tap water and planted one in each 2" pot on July eighth. They were immediately placed on a long-day (twenty-one hours). On July twenty-eighth they were transplanted to 3½" pots and on August second, after they had recovered from the shock of transplanting, treatments were started (Table 2). The short-day treatment was provided by placing the plants on the regular short-day bench with eight hours of light and a sixteen hour dark period.

Extracts were made from complete Xanthium plants which had been placed on short-day and were showing macroscopic flower formation, in the same manner as described in experiment 2. The extracts were injected into the petioles of 2-3 of the youngest fully expanded leaves of each test plant. Again a total of approximately 1.0 c.c. of extract was injected into each test plant.

The treatments were continued every day for six days, at which time they were discontinued and all plants were returned to the long-day bench. The results were recorded fourteen days later, at which time the plants were dissected and examined as described by Hamner and Bonner (48). The results appear in Table 2. There was no effect by the treatments.

## IV RESULTS

It has been shown (Table 1) that, under the conditions employed for these experiments, the average critical period for Gypsophila elegans is approximately eleven hours. Those plants whose stems were less than one centimeter long were still rosettes, and had shown no visible indication of future flowering.

As has been previously mentioned, those pots which were exposed to the ten, twelve, and fourteen hour photoperiods were set in flats to facilitate transfer; but those pots kept on the eight, sixteen, and twenty-four hour photoperiods were set in sand and were not disturbed during the course of the experiment. It was noted during the course of the experiment that the fourteen hour pots showed the first visible response, followed by the twenty-four, sixteen, and twelve hour pots respectively. It can be seen that this order follows that of the ultimate degree of response. If it is assumed that, on daylengths greater than the critical, the degree of response is correlated with the length of day; and that a variation in water-tension and mechanical disturbances accelerate the rate of response, then the observed results can be quantitatively accounted for (Figure 1). That the response is related to the length of day is shown for those plants in which the other treatments are comparable, so that the only assumption which need be made is that mechanical disturbances accelerate the rate of response in this plant; as van Overbeek (86) has shown to be true with the pineapple.

In the second part of this experiment, where an attempt was made to determine whether induction could be observed in this plant, it was found that no induction would occur. No elongation was apparent in any of the induction series, except for those plants which had received a long-day treatment for twenty days. Five of these plants showed some elongation (up to eight centimeters) before being transferred back to the short-day. Upon transfer to the short-day the elongation immediately ceased. On two of the plants which had flower-buds at the time of the transfer, the flowers opened and appeared normal but they were without pedicels (Figure 5).

Some of the plants of Gypsophila elegans were allowed to remain on an eight hour day for a total of five months. By the end of this time many of these plants had died while others showed the development of one or many lateral buds, producing "secondary rosettes". Throughout the entire course of the work no plant was ever observed to begin stem elongation or flowering while on the short-day.

The injection experiments showed no promise of success.

Table 1. Length of stems of _Gypsophila_ _elegans_ exposed to varying daylengths for forty-five days, as an indication of the flowering response.

| Hours of Light per Day | | | | | |
|---|---|---|---|---|---|
| 8 | 10 | 12 | 14 | 16 | 24 |
| 0.5 | 0.5 | 0.8 | 0.5 | 1.0 | 3.0 |
| 0.5 | 0.5 | 0.8 | 14.0 | 1.0 | 12.0 |
| 0.5 | 0.5 | 2.5 | 15.0 | 10.0 | 14.0 |
| 0.5 | 0.5 | 1.0 | 17.0 | 0.5 | 1.0 |
| 0.5 | 0.5 | 7.0 | 24.0 | 1.5 | 8.0 |
| 0.5 | 0.5 | 8.0 | 11.0 | 3.0 | 17.0 |
| 0.5 | 0.5 | 0.5 | 14.0 | 0.5 | 8.0 |
| 0.5 | 0.5 | 2.0 | 16.0 | 0.5 | 9.0 |
| 0.5 | 0.5 | 14.0 | 3.0 | 12.0 | 14.0 |
| 0.5 | 0.5 | 0.5 | 13.0 | 0.5 | 15.0 |
| 0.5 | 0.5 | 1.0 | 20.0 | 0.8 | 18.0 |
| 0.5 | 0.5 | 1.0 | 15.0 | 9.5 | 19.0 |
| 0.5 | 0.5 | 17.0 | 17.0 | 0.5 | 2.0 |
| 0.5 | 0.5 | 4.0 | 24.0 | 2.0 | 10.0 |
| 0.5 | 0.5 | 14.0 | 14.0 | 2.0 | 18.0 |
| 0.5 | 0.5 | 0.8 | 15.0 | 0.5 | 14.0 |
| 0.5 | 0.5 | 0.8 | 15.0 | 0.5 | 17.0 |
| 0.5 | 0.5 | 4.0 | (dead) | 1.0 | 17.0 |
| | | | | 0.5 | |
| | | | | 1.5 | |
| | | | | 4.0 | |
| | | | | 0.5 | |
| | | | | 14.0 | |
| | | | | 18.0 | |
| | | | | 1.0 | |
| | | | | 15.0 | |
| | | | | 20.0 | |
| 0.5 | 0.5 | 4.4±1.3 | 14.6±1.4 | 4.7±1.4 | 12.0±1.4* |

Average Length of Stems (in centimeters)

* S.D.= $\sqrt{\frac{\Sigma(x)2 - \frac{(\Sigma x2)}{n}}{n(n-1)}}$

Table 2. The effect of plant extract injections and 2,3,5 triiodobenzoic acid on the flowering response of _Xanthium_ _pennsylvanicum_.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| SHORT-DAY | Control | f | f | f | f | f | f | f | f* |
| | Water Control | f | f | f | f | f | f | f | f |
| | Extract Injection | f | f | f | f | f | f | f | f |
| | TIBA | f | f | f | f | f | f | f | f |
| | Injection & TIBA | f | f | f | f | f | f | f | f |
| LONG-DAY | Control | v | v | v | v | v | v | v | v |
| | Water Control | v | v | v | v | v | v | v | v |
| | Extract Injection | v | v | v | v | v | v | v | v |
| | TIBA | v | v | v | v | v | v | v | v |
| | Injection & TIBA | v | v | v | v | v | v | v | v |

* f - flowering; v - vegetative

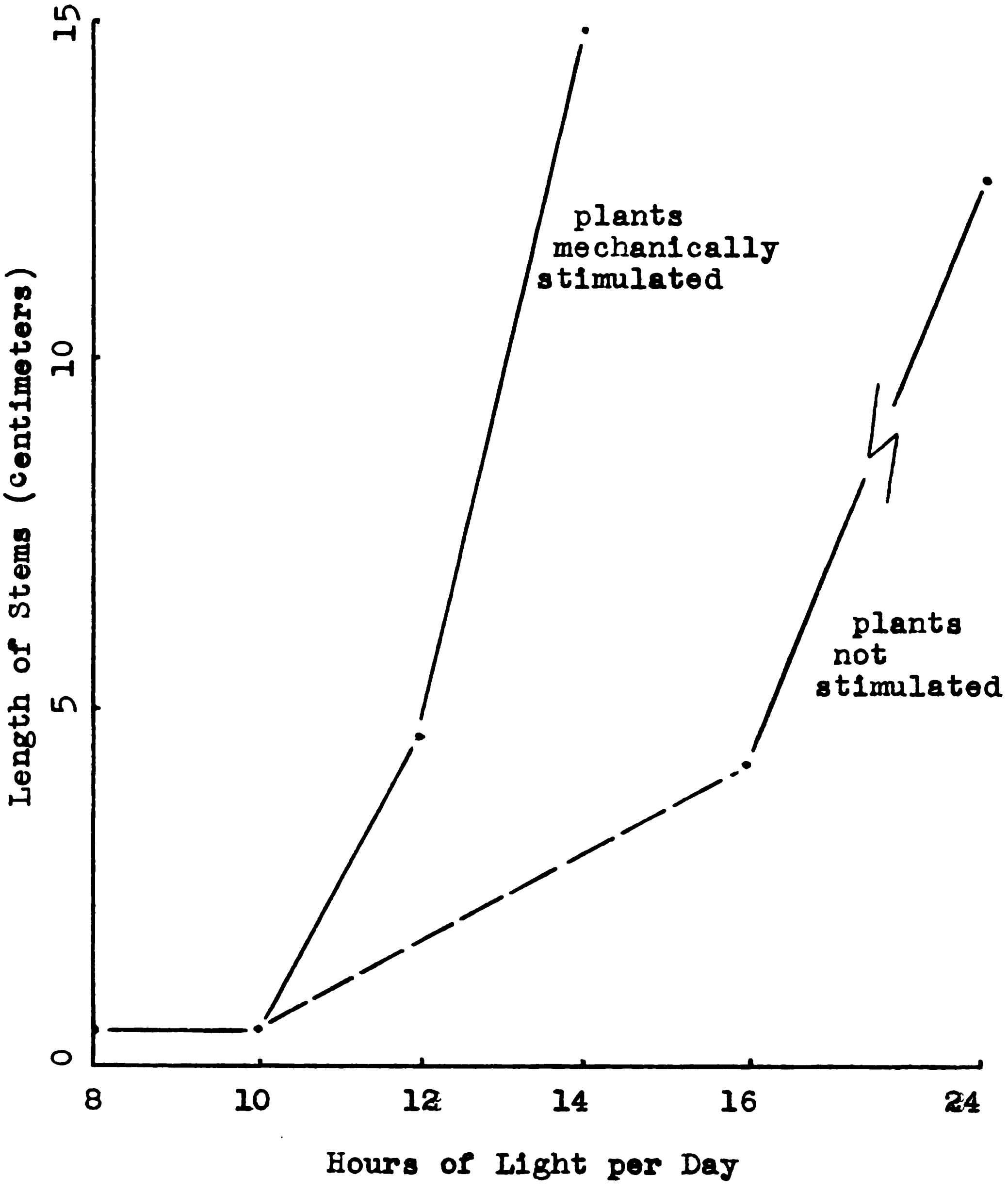

Figure 1. The relationship of the length of the light period and mechanical stimulation to the flowering response (as indicated by stem elongation) in *Gypsophila elegans*.

Figure 2. Experimental bench used for regulating the photoperiod.

Figure 3. Typical plants from each of the six photoperiods. Left to right: 8, 10, 12, 14, 16, 24 hours of light per day, after forty-five days.

Figure 4. Comparison of plants on the fourteen hour photoperiod (left) with those on the sixteen hour photoperiod (right), forty-five days after treatments were started.

Figure 5. Gypsophila elegans which had previously been exposed to a twenty day induction period, after forty-five days.

## V DISCUSSION AND CONCLUSION

A number of plants extremely sensitive to photoperiodic stimuli have been described and studied in the past few years. The study of these plants has furnished most of the present knowledge of photoperiodism. The discovery of other plants also sensitive could conceivably result in a significant increase in the knowledge of photoperiodism.

One of the most significant advances that could be made toward the isolation and identification of the flowering hormone would be the successful extraction of an active principle from an induced plant, which upon application to a vegetative plant would induce flowering.

It may be found that the plants in which photoperiodic induction is the most pronounced are not necessarily those which may prove most successful for extractions. In fact thousands of extraction and injection attempts have been made (9) especially with cocklebur and other very sensitive photoperiodic plants, but no successful results have ever been substantiated. In the attempts of Bonner and Hamner (9) a few apparently successful results were observed but they could not be consistantly reproduced. Several reports have been made of varying degrees of success using plant extracts (4, 8, 69, 103) but all are without confirmation.

However, it would seem that further attempts to extract the active principle are not likely to prove fruitful until more can be discovered regarding the nature of the stimulus through other techniques, or unless some new combination of known facts can give specific suggestions for experimental techniques.

For example, Bonner (7) points out the essential similarity between the nature of the stimulus, so far as it is known, and the nature of certain of the graft-transmissable viruses. Should this comparison prove to be valid it would suggest the great difficulties involved in any attempted extraction and re-introduction.

Galston (33) points out the possible reasons for failure: (1) unsuccessful extraction, (2) inadequate concentration, (3) unsuccessful injection, or (4) lack of suitable bioassay. This assumes that the active principle can exist independent of living cells, an assumption which remains, at the moment, open to question.

In the experiments described herein it was, of necessity, tentatively assumed that by its nature extraction and reinjection is possible. Attempts were made to take into consideration the four factors listed above in various ways as described.

The experiments were two fold in their aim. In experiment 2 typical injections were carried out in an effort to discover a plant which might react where numerous others had failed; while in experiment 3 reference was made to the recent work of Zimmerman and Hitchcock (128) and Galston (32) in which they found 2, 3, 5, triiodobenzoic acid when applied to plants acted as an inhibitor of auxin activity and that of Bonner (56) where he applied TIBA to cocklebur held very near the critical period and observed the formation of the inflorescence-like primordia at the apex, but no flowers.

This suggested that perhaps the partial stimulation of the TIBA could be combined with an extraction and injection technique to induce flowering, and this was attempted.

Since these experiments were performed, Bonner (7) has apparently induced flowering in cocklebur with 2, 3, 5 triiodobenzoic acid when the plants were exposed to conditions very close to the critical period by giving an adequate dark period but interrupting it in the middle with a "spot" of light. This would seem further indication that one of the reactions taking place in the dark period, apparently the one most light sensitive, is the lowering of the auxin level.

## VI SUMMARY

1. The literature relating to photoperiodism is discussed from the point of view of possible explanations of the photoperiodic phenomena.
2. The critical period of *Gypsophila elegans* was determined and found to be between ten and twelve hours of light.
3. Environmental factors other than photoperiod were found to affect the rate of response of *Gypsophila elegans* to photoperiod.
4. *Gypsophila elegans* was found to be incapable of induction.
5. Attempts were made to induce flowering in *Gypsophila elegans* by injecting the brei of flowering specimens into vegetative plants of the same species, without success.
6. Attempts were made to induce flowering in *Xanthium pennsylvanicum* by injecting brei made of flowering specimens into vegetative plants of the same species and in addition treating a portion of the injected plants with 2, 3, 5 Triiodobenzoic acid, without success.

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