KINETIC STUDIES ON THE PHYSIOLOGY OF FLOWERING

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ABSTRACT

Xanthium pennsylvanicum is induced to flower by exposure to a single uninterrupted dark period equal to or exceeding some minimum duration (ca. 9 hours). A system of stages of floral bud development is described, which gives a quantitative measurement of the degree of induction caused by various treatments. A number of factors which may affect the degree of induction are investigated, and it is concluded that age of the leaf and light intensity before and after induction strongly influence the effects of a given inductive dark period, while other factors are less important in this respect. Methods in the use of auxin are also described.

Auxin is shown to inhibit induction in the leaf, but to promote floral bud development after induction is complete. Applied auxin will replace a requirement for the presence of active buds after and just before induction.

The induced state is discussed, and it is proposed that this condition consists of a given concentration of florigen in the plant which is maintained at the level brought about by the act of induction.

Concentration curves indicate that auxin acts in floral inhibition much as it does in other auxin-induced phenomena. Applied auxin has little effect upon the critical night length, but inhibits the rate of florigen synthesis which follows. Auxin is most effective when applied two to three hours after the beginning of the dark period, and its effectiveness decreases up until translocation of florigen out of the leaf is complete. Light interruption of the dark period is most effective after 8 hours. Light interruption and auxin are additive in their inhibitory effect.

It is suggested that the act of induction consists of at least three phases: transformation of photo-receptor pigment, preparatory reaction, and hormone synthesis. A possible mechanism of auxin action through destruction of florigen is discussed.

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INTRODUCTION AND STATEMENT OF PROBLEMS

The Physiology of Flowering

In the higher plant, as well as some other organisms, Weismann's concept of the continuity of germ-plasm does not hold strictly true. There is no particular group of cells set aside after the first divisions of the zygote, which has the single function of producing reproductive cells. Rather the zygote produces primary meristems--groups of cells which divide and grow and produce new cells which may differentiate into either vegetative or reproductive tissues and organs. Thus the meristematic tissue in the growing point of the stem, for a substantial portion of the life of the plant, produces organs which are purely vegetative--the stem with its complex vascular and supporting tissues, and leaves with their intricate structure so well suited to effective photosynthesis. At some time, however, this pattern of growth abruptly changes, and these same meristematic tissues produce cells which will differentiate into the reproductive organs. In nearly every instance this is an irreversible change, and the meristematic cells themselves, after being spent in the production of reproductive organs, are no longer able to reproduce and form vegetative tissues. If all its apical meristems are so utilized, the plant dies (annuals, biennials); otherwise other meristems must assume the job of vegetative development

(perennials).

The study of the physiology of flowering has been primarily concerned with an investigation of this change in the primary meristems from a vegetative to a reproductive condition, and with the forces internal and external to the plant which may bring about this change. Many other aspects of the physiology of flowering might also be studied, such as the differentiation of the individual flower parts, floral maturation, anthesis, etc. The present study, however, is concerned primarily with the problem of floral initiation.

One may envisage two possible situations in regard to the initiation of floral primordia: (1) The plant responds to its genetic constitution in such a way that it flowers at a certain age or stage (e.g. a certain number of nodes), independently of its external environment. The so-called day-neutral plants approach this condition. (2) The plant is genetically so constituted that it will flower in response to certain changes in the external environment. If these factors of the environment can be recognized and controlled, flowering can be controlled. The discovery that certain plants flower or remain vegetative depending upon the environmental conditions to which they are exposed, has opened many interesting avenues of research into the physiology of flowering.

The following environmental factors are often correlated with flowering in nature, and have been experimentally investigated from the standpoint of their effect upon flowering:

1. Temperature
2. Moisture: in the soil and the atmosphere.
3. Level of soil nutrients.
4. Light: intensity and duration.

It has been found that the growing points of biennial, winter annual, and some other plants respond to low temperature treatment by subsequently initiating floral primordia. The process of exposing such plants to low temperatures to bring about flower production is known as vernalization, and has been extensively studied (Reviewed by: Murneek and Whyte, 1948; Melchers and Lang, 1948; Lang, 1952; Melchers, 1952.). Although this thesis is not concerned directly with the problem of vernalization, a number of the findings of workers in this field will be presented here as background for future discussion.

The shoot apex itself must receive the cold treatment, while leaves, storage organs, etc. are insensitive to vernalization. Dormant plants cannot be vernalized. Seeds may often by vernalized, but at least a small amount of water must have previously been supplied. Seeds have been vernalized on the parent plant by applying the cold before the seeds have become mature. Usually vernalization exhibits a broad temperature optimum--often from 1° to 7° C. The degree of vernalization reaches an optimum with time, and after this optimum time, extent of vernalization may decrease (over-vernalization). Vernalization may be reversed by high temperatures (devernalization), but only within a few days after cold treatment. Vernalization requires the presence of O_2, but devernalization

can occur under anaerobic conditions. Although the excised embryo can be vernalized, the presence of the endosperm promotes the process. If a plant which has been vernalized is grafted to a non-vernalized plant near the growing point, the non-vernalized plant will flower, indicating that vernalization produces a substance which can be transmitted across a graft union.

Many plants flower towards the end of the growing season when both soil moisture and soil nutrients, especially nitrogen, are present in relatively small amounts. It was long thought that these factors were the cause of flowering. Klebs (1896 and 1918) studied numerous plants over a twenty year period and concluded that plant nutrients, especially nitrogen, and total carbohydrate within the plant were of importance in the control of flowering. The relation of light intensity and CO_2 content of the air to carbohydrate production was also thought to be important. Fischer (1905 and 1916) from his studies concurred in these conclusions, and proposed that a high carbon/nitrogen ratio resulted in flowering, while plants remained vegetative when this ratio was low. Overwhelming evidence against this hypothesis has since accumulated. Kraus and Kraybill (1918) pointed out, however, that carbon-nitrogen changes in plants are of considerable importance in flower development, fruit set, and growth of fruits and seeds.

Studies have been made relating nutritional factors to photoperiodism. In 1939 Neidle studied the effect of nitro-

gen nutrition upon the flowering of Xanthium, a short-day plant which is caused to flower by dark periods longer than ca. nine hours. She reported that response to photoperiodic induction (see below) was somewhat promoted by high nitrogen concentrations in the culture solutions. Von Denffer in 1940 studied a number of plants and concluded that the flowering of short-day plants was promoted by a high nitrogen level, while the flowering of long-day plants was promoted by a low nitrogen level. Naylor (1941) found that nutritional conditions affected the flowering response of Xanthium only to a slight extent. At present nutritional factors are considered to be at most, of secondary importance in flowering. Since these factors may influence slightly the flowering responses of photoperiodically sensitive plants, however, they should not be allowed to become limiting in normal experimental work.

Photoperiodism

Historical. In 1920 Garner and Allard discovered that the flowering of Maryland Mammoth tobacco and other plants could be controlled by adjusting the relative length of day and night (the photoperiod) to which these plants were exposed. Thus the daily photoperiod was recognized as being an important factor in the initiation of floral primordia, and that part of plant physiology dealing with the study of photoperiodism came into being. Other workers before Garner and Allard had come close to the discovery of photoperiodism. Henfrey in 1852 suggested the possibility that the natural

distribution of plants is at least partly tied to the duration of the light period during the growing season. In 1913 Klebs came very close to the discovery of photoperiodism with his observation that Sempervivum (now recognized as a long-day plant) could be caused to flower in winter if it was kept under long photoperiods or continuous light. An attempt was made to explain these results on the basis of nutritional factors (Klebs, 1918), which may explain the failure to recognize the principles of photoperiodism.

Garner and Allard in their paper (1920) divided plants into three groups according to the manner in which they respond to photoperiod: (1) day-neutral plants which flower when they reach a certain age or stage, more or less independently of environmental conditions; (2) short-day plants which flower when kept on short photoperiods; (3) and long-day plants which flower when kept on long photoperiods. It is now known that short-day plants flower when exposed to a period of uninterrupted darkness exceeding some minimum length (the critical dark period). Long-day plants will flower if kept on continuous light, but require photoperiods of shorter duration than usual if the dark period is briefly interrupted by light of low intensity. Thus the dark period appears to be of critical importance to both types of plants.

A complete review of the work following that of Garner and Allard is beyond the scope of this discussion, and a number of excellent reviews have been written in recent years which cover all but the most recent work. These reviews will

be mentioned here, and in the short description of photoperiodism which follows, only occasional reference will be made to papers of particular interest, or which form a foundation for the actual experimental phase of this thesis.

Most of the facts of photoperiodism presented here were assembled from the following reviews, with further reference to original papers. A review by Lang published in 1952 is probably the most comprehensive of the recent reviews, and contains extensive references to much of the older literature as well as to recent papers. Melchers and Lang wrote an extensive and complete review in the German language in 1948. This review covers most of the work on vernalization as well as photoperiodism. In a series of lectures given at the University of London in 1952 Melchers presents a very clear exposition of the status of photoperiodism at that time. Two reviews by Naylor (1952 and 1953) have been referred to frequently. A recent review by Bonner and Liverman (1953) outlines the partial processes concept which will be discussed below, and reviews the work on auxin which is the principle problem of this thesis. The actual foundation for this thesis consists of work by Bonner and Thurlow (1949) on auxin inhibition of flowering in Xanthium and subsequent work by Liverman contained in his thesis (1952) as well as in two publications (Liverman and Bonner, 1953 a and b). Many of the basic photoperiodic properties of Xanthium were originally studied and described by Hamner and Bonner (1938). A number of other reviews have also been published, and these may be found by con-

sulting the papers listed above.

Before further discussion of the special properties of long-day and short-day plants--the partial processes of photoperiodic induction--a number of general points will be discussed.

Sensitivity to Photoperiodic Induction. All plants must attain a certain ripeness to flower before floral buds will be initiated by photoperiodic treatment. Usually a minimum number of leaves must be produced before ripeness to flower is attained. Xanthium, the plant studied exclusively in this thesis, becomes ripe to flower at a very early age. Indeed, it is reported that the cotyledonary leaves will respond to photoperiodic induction (Hamner and Bonner, 1938). Sensitivity of these plants to photoperiod remains constant over a long period of time (Chapter I). Xanthium maintained on long days has remained vegetative for more than a year without any sign of loss of sensitivity to photoperiod (Naylor, 1953).

It has been reported in the literature (Lang, 1952, p. 283; Naylor, 1953, p. 161; Hamner and Bonner, 1938) that in Xanthium the leaf most sensitive to photoperiodic induction is the first fully expanded one. Experiments to be presented later indicate that this statement is in error (in Xanthium), and that the most sensitive leaf is the one which is most rapidly expanding (the half expanded leaf--page 36).

Experiments indicate (Lincoln, 1954, Carr, 1953) that Xanthium, and other plants, will not become induced to flower unless there are actively growing buds on the plant. This

will also be discussed later, in the light of experiments presented in this thesis (Chapter II).

The Flowering Hormone. A number of experiments indicate that during photoperiodic induction certain substances are produced in the leaves, which are transported to the tip to initiate floral differentiation. It has been shown that the leaves are the only organs which perceive the photoperiodic stimulus. In Xanthium a single leaf maintained on short day will induce the entire plant to flower (Hamner and Bonner, 1938). A plant which has been photoperiodically induced will cause flowering in a vegetative receptor to which it is grafted, indicating that some substance or condition has been transmitted across the graft union.* Such a transmission of the flowering stimulus will only take place through living tissue. The stimulus will not go through a liquid filled tube, tissue diffusion contact, etc.

The term hormone has been applied to any substance which is produced in one place and translocated in small amounts to another part of the organism where it has a controlling influence upon some phase of the metabolism or growth of this organism. The experiments described above (as well as others--see Lang, 1952, p. 287) indicate that the flowering stimulus fits

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*A number of unsuccessful attempts have been made by the author, to transmit the flowering stimulus across a graft union (320 grafts). Although the unions appeared perfect, the receptors always remained vegetative. A number of variations in amount of induction, number of leaves on the plants, etc. were tried, all without success.

such a definition, and hence the term flowering hormone has been applied to this stimulus. This stimulus or hormone has been called florigen by Cajlachjan (1936), anthocalin by Went (1938), and anthesin by Cholodny (1939). The term florigen, although considered by many to be poor terminology, has been widely used. In this thesis the terms florigen or the flowering hormone or stimulus will be used.

Attempts have been made to isolate and characterize the flowering hormone since evidence for its existence was first recognized. Bonner reports that in his laboratory alone, approximately two thousand different kinds of extracts of flowering Xanthium plants have been made over a period of 12 years (Bonner and Liverman, 1953), and in no instance would any of these extracts induce flowering in vegetative plants. Many other workers have had the same experience. Some published reports may be found in the literature of substances extracted from plants which will induce flowering (Bonner and Bonner, 1948; Roberts, 1951), but in no case have the results been repeatable, and in no instance does it appear that florigen itself has been isolated.

The difficulties encountered in extracting the hormone and using it to induce flowers in vegetative plants might arise from one or more of the following situations: (1) The proper method of extraction and/or reapplication has not yet been tried. (2) Florigen is translocated within the plant only in the phloem (there is considerable evidence in support of this idea), and extraction and reapplication so dilutes it

with liquid from the other tissues of the plant, that its concentration within the sieve tubes of the vegetative test plant is too low to induce flowering. Another version of this general idea is that florigen is destroyed by tissues other than phloem. Neither version has been tested. (3) The hormone consists of some highly complex molecule such as protein or nucleoprotein. Attempts to extract it may result in denaturation and attempts to reintroduce it would be expected to be futile. If this is the case, its failure to pass through a liquid junction may be because it is so unstable that the liquid denatures it, or that it is so large and complex that the tissue on the receiving end of the junction is unable to absorb it. Certainly the failure to isolate florigen is not convincing evidence that it does not exist.

Studies have been made of the translocation and graft transmission properties of florigen (Lang, 1952; Naylor, 1953). It will pass both up and down a stem and in chain fashion through a series of two-branched plants which have been grafted together. Almost all combinations of reciprocal grafts have been made between long-day, short-day, and day-neutral plants (Lang, 1952). Both short-day and long-day plants, when induced, will cause graft partners of either type to flower. This is strong evidence that the flowering hormone is at least physiologically identical in the different types of plants.

Within some species there are varieties which respond differently to photoperiod. Few genetic studies have been

made, however. Those that have been made indicate that difference in response to photoperiod may be due only to a single gene pair (tobacco), or to a number of genes even on different chromosomes (Solidago sempervirens, Milo grain sorghum, etc.). In almost every instance modifier genes also occur (Lang, 1952; Naylor, 1953).

Bonner and Liverman (1953) state that the ability of florigen to pass through living tissues only, resist all attempts at extraction, and pass across a graft union from plant to plant without any considerable dimunition of activity, suggest that the flowering stimulus, in Xanthium at least, possesses certain of the characteristics of a virus disease. They suggest that florigen may be a "hormone entirely different from those with which we have been acquainted in the past; that it may be a more complex hormone, perhaps of a protein nature rather than a small molecular organic substance such as those which have heretofore been extracted and characterized."

Induction. The term induction has already been used a number of times. Although Chapter III is devoted to a discussion of the nature of induction, some definition of the term is in order. In the strictest sense, the term induction refers to the ability of some photoperiodically sensitive plants to continue the production and development of flowers after being exposed to the proper photoperiod, even though the actual development (or even initiation) of flowers takes place under conditions which themselves would not cause

the initiation of floral primordia. (This definition also applies to plants capable of vernalization.) Thus Xanthium plants grown on long-day remain vegetative indefinitely, but three to five days after a single short day, developing flower primordia may be detected, and these continue to develop until ripe seeds are produced, even though the plants remain on long-day. This induced condition, or ability to produce flowers under an unfavorable photoperiod after being exposed to the proper photoperiod, may be transmitted by grafting through a number of generations. This could be understood on the basis that florigen, once made, is continually reproduced in plants which are capable of being induced.

Some ambiguity arises in the use of the term induction. The term is used in two ways: as an act and as a condition. The act of induction refers to the transformation of the plant from the non-induced to the induced condition. In our present understanding of the processes of photoperiodism, the act of induction--to induce a plant--refers to the processes involved in hormone synthesis. The condition or state of induction refers to the plant after hormone synthesis has taken place, providing the plant is one which is capable of the induced state as described above. The condition of induction is imperfectly understood, but may mean that the plant is capable of maintaining a given concentration of hormone in its tissues (Chapter III). Plants which cease development of flowers when removed from the favorable photoperiod (e.g. Chrysanthemums), are not capable of attaining the induced state, and

hence the term should not be used in reference to these plants.

The Partial Processes. A clearer understanding of photoperiodism has been obtained by dividing this complex of phenomena into a number of partial processes (Bonner and Liverman, 1953). This has had the advantages associated with organization and classification, and also has provided a basis for theoretical considerations of the photoperiodic reactions. These partial processes have been worked out for both long-day and short-day plants, and in Figure 1 they are schematically represented for both types of plants, but in this discussion only short-day plants will be considered. Bonner and Liverman (1953) divide the photoperiodic reactions into (1) a high intensity light process, (2) a dark process, and (3) a low intensity light process. They further mention hormone synthesis, translocation, and differentiation as partial processes. On the basis of further work completed since the above classification, and other special considerations, the following proposed revision of this classification is presented:

I. Hormone Synthesis
    1. The High Intensity Light Process
    2. Reactions of the Dark Period
        a. The Dark Process
        b. The Low Intensity Light Process
    3. The Second High Intensity Light Process
II. Translocation of the Flowering Stimulus
III. Differentiation of the Growing Point
IV. Development of the Flower and Fruit

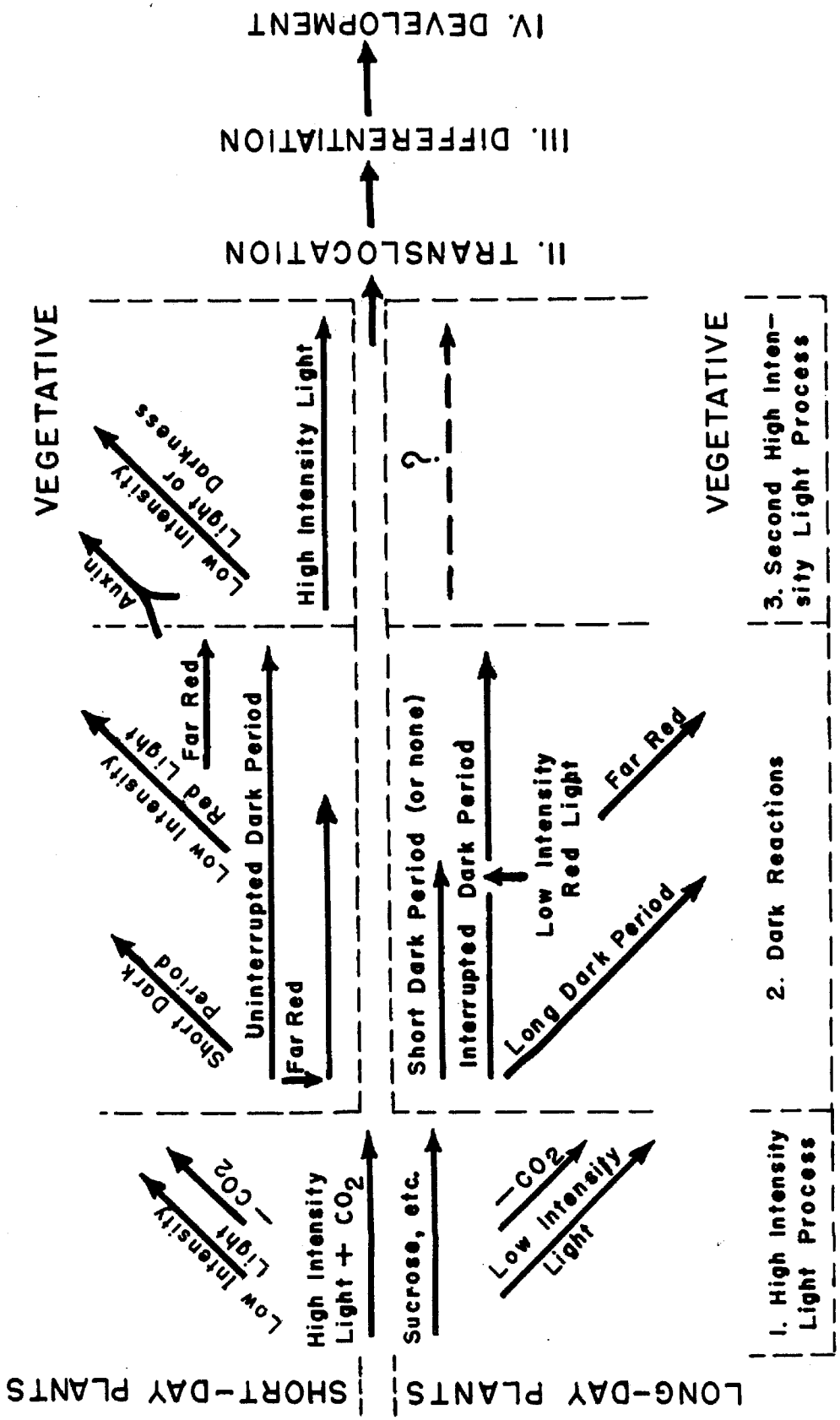

Figure 1. The partial processes of photoperiodism. Slanted arrows indicate treatments which tend to act against flower initiation, while horizontal arrows indicate those which, when occurring in proper sequence, result in flower production. The processes shown are discussed in the text for short-day plants.

Only the various phases of hormone synthesis will be discussed here, although translocation, differentiation, and development are important phases which have bearing upon the work in this thesis, and will be mentioned later.

1. The High Intensity Light Process. Hamner (1940) was able to demonstrate in an ingenious experiment, that Xanthium requires high intensity light before the inductive dark period in order for the latter to be effective. He subjected plants to darkness interrupted at three hour intervals by three minutes of light, and after a number of these cycles, allowed the plants to remain in uninterrupted darkness for a period long enough to normally insure flowering. When plants are so treated they fail to become induced. If, however, at the end of the three hour cycles of light interruption, the plants are exposed to bright sunlight for one to three hours, and then subjected to an inductive dark period, induction will occur. Thus the inductive dark period must be preceeded by light of high intensity, in the neighborhood of 3×10^4 foot-candle-minutes. Low intensity light, of the order of 100 to 200 foot-candles, for several hours before induction, will also materially decrease the extent of flowering (Mann, 1940).

It appears at present from two lines of evidence that the high intensity light process is a photosynthetic one. This reaction will proceed only in the presence of CO_2, and it has been demonstrated with Xanthium that the high intensity light process may be replaced by treatment of the leaves with sugars or other energy producing compounds such as Krebs

cycle acids (Liverman, 1953 a). It appears, then, that the function of the high intensity light process is to produce, by photosynthesis, precursors or an energy source for the processes which are to follow.

2. Reactions of the Dark Period.

a. The Dark Process. In the short-day plants it has been shown that a long, uninterrupted dark period is required for induction. The minimum length of this dark period which will result in flowering is known as the critical night length. The dark process refers to the reaction or reactions which take place during this dark period. It is felt by most workers in the field that an understanding of the biochemistry of the dark process would lead to an understanding of photoperiodism, and perhaps many other light controlled biological phenomena as well, yet to date very little is understood about this process.

The dark process seems to be somewhat temperature dependent. Low temperatures tend to increase the critical night length. Except for single inductive cycles, however, this temperature dependence is not very great, and a change in temperature of a number of degrees results in a change of only a few percent in the critical night length.

It has recently been demonstrated that the critical night length may be shortened to a certain extent by exposing Xanthium plants to far red radiation just before the inductive dark period (Borthwick, Hendricks and Parker, 1952).

Applied auxin inhibits the effects of the dark process (Bonner and Liverman, 1953). Whether this inhibition is directly upon the dark process itself or upon some later phase of induction has been a principle problem of investigation in this thesis, and will be discussed later in that respect.

b. The Low Intensity Light Process. Some implications about the nature of the dark process can be drawn from data obtained in the study of the low intensity light process. If, in a short-day plant, the inductive dark period is interrupted by a brief exposure to light of relatively low intensity, induction will not occur. The reaction or reactions which take place within the plant in response to this exposure to low intensity light, that is, the reactions resulting in halting or reversing the dark process, are known collectively as the low intensity light process. The quantity of light required to bring about this reversal of the dark process is very small, on the order of 200 foot-candle-minutes or only about 1% that required in the high intensity light process. The time of effectiveness of the light interruption has been studied, and it has been shown that light is most effective near the middle of a dark period which is from 12 to 16 hours in length (Liverman, 1952). The exact relationship is shown in Figures 35 and 36.

The quality of light effective in reversal of the dark process has been studied extensively at the Plant Industry Station, Beltsville, Maryland, where the equipment has been

assembled for such studies (Parker, Hendricks, Borthwick, and Scully, 1941; Parker and Borthwick, 1950). The inductive dark period was interrupted near the middle by light of various wavelengths, and the amount of light needed to bring about inhibition of flowering was determined. Plotting this amount of light against the wavelength, an action spectrum could be obtained. The action spectrum should be a function of the absorption spectrum of the pigment which absorbs the light in the low intensity light process. The action spectrum shows a pronounced maximum effectiveness in the red region, with a smaller maximum in the blue. Green is least effective in the low intensity light process. The action spectrum suggests that some pigment not greatly unlike chlorophyll in its absorption properties might be responsible for the light inhibition. The workers at Beltsville point out that the pigment is probably not chlorophyll, and suggest that it may be an open-chain tetrapyrrol. The action spectrum is much like that found for other light related phenomena in plants, such as etiolation, leaf expansion, lettuce seed germination, growth of the Avena coleoptile, etc. Recent work indicates that the pigment may be a pheophorbide, which has been isolated from light sensitive plants (Todd and Galston, 1954).

The workers at Beltsville have recently reported that the effect of red light in the low intensity light process may be reversed by far red radiation (Borthwick, Hendricks, and Parker, 1952). A number of plant growth phenomena gov-

erned by light follow this pattern. Thus these workers propose a reversible photoreaction which controls the dark process, and hence the induction of flowering in Xanthium.

3. The Second High Intensity Light Process. It has recently been demonstrated by Lockhart and Hamner (1954) using Xanthium that high intensity light is required following the dark period as well as preceding it. If plants are subjected to a light interruption during an inductive dark period at some time after the critical night length, and then returned to darkness for a period of time, they will flower to a lesser degree than controls which are returned to high intensity light at the time the test plants are returned to darkness. The deduction has been made that a short dark period (e.g. four hours), following an inductive dark period, caused a destruction of flowering stimulus (or some precursor) produced by the dark process. Total darkness is not required to bring about this destruction. Mann (1940) reports that Xanthium is inhibited in its flowering if, after a single inductive dark period, the plants are returned to light of low intensity. This has been repeated by the author, and will be discussed later (page 44).

One may conclude that high intensity light following an inductive dark period promotes flowering, probably by stabilizing the products of the dark reaction. It should be mentioned, however, that high intensity light is not absolutely required, as plants will flower if left in total darkness as shown by Leopold (1949), and by Lang. The author has also seen flower

primordia on Xanthium plants left in total darkness two weeks.

The Effect of Applied Auxin on Short-Day Plants. The background for the work on auxin described in the following sections may be summed up by the following points (Lang, 1952; Bonner and Liverman, 1953; Liverman, 1952):

1. Auxin applied to short-day plants results in an inhibition of flowering.

2. This inhibition has the kinetic characteristics of a saturation phenomenon, approaching a maximum with increase in concentration. This will be further discussed in Chapter IV, but here it may be said that a plot of the response for floral inhibition against concentration of applied auxin yields a curve very much like the curves obtained for growth of Avena coleoptiles, and other auxin responses.

3. In the auxin inhibition of flowering in Xanthium, auxin acts in its normal capacity as an auxin. Only auxins are effective in this reaction, and antiauxins will overcome this auxin inhibition of flowering as they will reverse other auxin responses.

4. The effect of auxin is specifically upon floral induction and not upon growth of the plants. This has been shown by studies of growth after auxin application, and is confirmed by experiments reported here showing the effective time of auxin application (Chapter IV).

5. The inhibitory effect is in the leaf and upon hormone synthesis. This has been concluded from the results of earlier work, and is confirmed in greater detail by the work in this

thesis (Chapter II).

6. Under certain conditions the concentrations of auxin effective in the inhibition of flowering may be very low, approaching so called physiological concentration. This is also discussed later (Chapter I).

7. It has recently been shown that auxin applied in very low concentrations, and at low temperatures, promotes flowering in many plants (Leopold and Guernsey, 1953 a,b,c). This will be discussed later and a possible explanation suggested (Chapter II).

Statement of Problems

The principal problem of research in this investigation has been to attempt to better understand the role of auxin in flowering. Often however, the course of investigation has deviated from this purpose, although the principal deviations have been in the setting up of methods for efficient experimentation with *Xanthium*. The principal approach to the problem has been through kinetic studies, and after some deliberation it was concluded that only *Xanthium pennsylvanicum** would be used in these studies.

Kinetic studies may be defined as those which study the dynamic aspects of a given process. In most of the experi-

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*Some confusion exists regarding the nomenclature of *Xanthium*. *Xanthium pen(n)sylvanicum* Wallr. (cocklebur) is synonymous with *X. saccharatum*, the term used by various other workers in photoperiodism. Specimens of the type of plants used in these studies have been filed at the U.C.L.A. Herbarium (Lockhart and Hamner, 1954).

ments reported here, the results may be illustrated graphically by plotting the flowering response against either the time of treatment or the concentration of an applied chemical, given at some particular time. The stage system described in the first chapter is designed to measure the initial rate of development of the floral bud, and as a first approximation this initial rate of development will be considered an indication of the concentration of florigen produced by a given inductive treatment. Hence most of these experiments are designed to study the change in final concentration of florigen produced by a given induction when the plants are treated in various ways at different times and with different concentrations of chemicals. Such studies will be referred to as kinetic studies.

In a field as broad as flowering, it would seem logical to investigate all possible aspects through the use of as many different kinds of plants as possible. This has been done by many workers for a number of years. Comparison of results obtained using different plants is, however, often misleading. Furthermore, work with many species often leads to a mass of descriptive facts, which are difficult or impossible to apply to general theory. It would seem, that if the process of induction could be completely elucidated for a single plant, precedents in methods as well as the actual knowledge gained, might open the way for elucidation of the process in other plants, and finally, perhaps, in all plants. In view of these considerations only Xanthium was used in the experiments reported in this thesis.

There is also considerable advantage to the use of a particular test plant in the study of a given physiological phenomenon. The Avena is a well known example. It would appear that Xanthium is also a well suited test plant, in this instance for the study of the physiology of flowering in short-day plants. Since Xanthium will respond to a single inductive dark period, experiments may be performed in which the time of application of a particular chemical (or time of any treatment) may be studied in relation to the dark period, etc. With continuous inductive cycles, a given treatment at the end of the dark period might influence the process of induction which takes place in the following dark period. An attempt has been made in this thesis (Chapter I) to characterize the variables in the environment which effect the response of Xanthium to photoperiodic induction, and describe them so that the results of experiments done under different environmental conditions may be compared on the same basis. Although this has perhaps been only partially successful, a sufficient start has been made to justify further study along these lines. This attempt to refine the techniques of experimentation with Xanthium was required to allow the use of a quantitative measurement of the degree of induction caused by a given treatment. Unfortunately this refinement came only gradually throughout the course of other experiments, rather than at the beginning as background for these experiments.

The refinement in techniques, in turn, required a suit-

able measurement of the degree of induction; one which had quantitative meaning in the study of the physiology of flowering. It is believed that the system of floral stages presented in Chapter I meets these requirements.

Thus the aims of this investigation have become at least two fold: first, to study the environmental factors and experimental techniques which will make Xanthium an effective test plant in the quantitative study of the physiology of flowering of short-day plants; and second, to study the role of auxin in the flowering of this plant. It is further hoped, that through a study of the role of auxin, insight might be gained into the processes which occur during the inductive dark period, and hence insight into the actual nature of the act of photoperiodic induction.

Chapter I

METHODS OF EXPERIMENTATION WITH *XANTHIUM* AND OTHER SPECIAL INVESTIGATIONS

Method of Growing Plants

The plants (*Xanthium pennsylvanicum* - see page 22) were grown essentially in the manner described by Liverman (1952) and by Bonner and Thurlow (1949). The strain used was originally derived from burs collected in nature in the Chicago area, but all plants used in the experiments reported in this thesis were grown from seed produced at the California Institute of Technology. The burs were planted, after soaking overnight in water, in washed river sand. For the first year of the two years spent on the experiments, the ends were clipped from the burs in an attempt to increase the degree and uniformity of germination (Bonner and Thurlow, 1949). It was found, however, that with burs older than one year, unclipped burs germinated to as great an extent and with as much uniformity as those which had been clipped. The younger burs also germinated fairly well without clipping, and the clipping process was therefore discontinued completely. Ten to twenty days after planting (depending on the size) the plants were transplanted into four inch clay pots or plastic containers (4 in^2 by 6 3/4 inches high) filled with a mixture

of equal parts of sand, loam and leaf-mold. Nitrogen, added occasionally in the form of $(NH_4)_2SO_4$, aided by producing greener, more thrifty plants, and probably also by increasing somewhat the sensitivity to photoperiodic induction (Neidle, 1939). The plants were used in the experiments reported below four to ten weeks after planting of the seed.

The plants were grown in a greenhouse maintained at a minimum of 20° C., and were kept in a vegetative condition until time of treatment by supplementary light from incandescent filament lamps arranged to achieve a light intensity of at least 100 foot-candles at the leaf surface and a total photoperiod of 20 hours. At the time of photoperiodic induction the plants were moved into dark rooms in which the temperature was controlled within one or two degrees of 23° C. In most experiments the plants were prepared and transported into dark rooms, etc. with the aid of trucks which hold 16 of the clay pots or 25 of the plastic containers. In some instances the plants were carried in flats to basement darkrooms, which could be entered through a darkened hallway, permitting treatments during the dark period (flash of light, auxin application, etc.). After one or more inductive cycles, plants were returned to long-day on the greenhouse benches.

The Stage System

Most of the previous results of experiments with Xanthium have been reported on the basis of the percent of plants within a given treatment which produce flower primordia (re-

ferred to here as percent flowering). This method gives qualitative results which separate the act of induction and the processes of development taking place after induction. A clear distinction is drawn between treatments which are able to bring about flowering and those which are not. Semi-quantitative results have also been obtained with this system (e.g. curves of percent inhibition with varying auxin concentrations--Liverman, 1952).

If, however, it is desired to study the quantitative aspects of flowering (as in the kinetics of flowering), the percent flowering is unsatisfactory for the following reasons.

(1) The system assumes variability in the plant material. If there were no variability, all plants within a treatment would respond alike, either flowering or remaining vegetative. Thus the variability within each treatment must represent the variability of all the plants within the experiment.

(2) Error is often introduced into percentage data by border-line plants--that is, those plants whose growing points show a degree of differentiation so slight that the worker cannot be positive whether they are in a flowering or a vegetative condition. On a percentage basis, their weight is equal to plants which are clearly in one condition or the other. To omit them completely from the final calculation seems hardly satisfactory, although this is often done.

(3) The percent flowering system fails to measure visible differences between treatments which flower one hundred percent. This is probably the most serious drawback of percent flowering

against quantitative studies on the physiology of flowering. Treatments must be confined near the threshold range of flowering to obtain even semi-quantitative results expressed this way.

The use of the degree of development of the floral bud as a measure of floral induction has been utilized by various workers in various ways (e.g. Leopold and Guernsey, 1953; Carr, 1953; Lockhart and Hamner, 1954; Khudairi and Hamner, 1954). Such a system, assigning numerical values to various stages of floral bud development, gives a measurement of flowering which may be used in quantitative studies. Variability within treatments is not required to obtain quantitative data with the stage system; very little weight is given to border-line plants; and differences between treatments may be measured even when all plants within a treatment flower.

A system of stages of floral development has been designed by the present author, which possesses the above advantages. It is easy to use, well defined, and experimental data resulting from its use lend credence to the system as a valid technique in studying quantitative aspects of the physiology of flowering. As will be shown below, the system, although arbitrarily defined, has been found to possess some interesting physiological corollaries.

The objection has been raised (e.g. Parker, et al., 1939) that a stage system introduces the problem of environmental conditions during development, into the interpretation of ex-

perimental results. This objection is minimized by maintaining equal conditions for all treatments from the time of induction until dissection of the growing points. Only the experimental treatment during induction is varied. In addition, studies of the effects of the environment upon development have been made by the present author (see below), and others (e.g. Roberts, 1951). It should also be noted that percent flowering can always be obtained from stage data, if one wishes to consider only the qualitative aspects of flowering.

Figure 2 shows in a series of drawings the stages of floral bud development. The drawings are made to scale, but size is of secondary importance in classifying the stages. Often axillary buds will be at the same stage as the primary bud (Table 4), although they may be much smaller. Weak plants in a particular treatment may have buds smaller than those of the other plants, but in the same floral stage of development. Figure 3 shows photographs of some of these stages. Although the various details may be discerned in the photographs, the drawings show clearly the criteria used in classifying buds into particular stage groups. Table 1 lists the anatomical criteria used in the classification, and it may be seen that these criteria are simple and easy to apply.

The stages as defined are related numerically in a linear fashion to the rate of development of the floral bud. Figure 4 shows the degree of development (as stage) of the bud plotted against time after induction. It may be seen that, beginning about $2\frac{1}{2}$ days after induction, the rate of development in

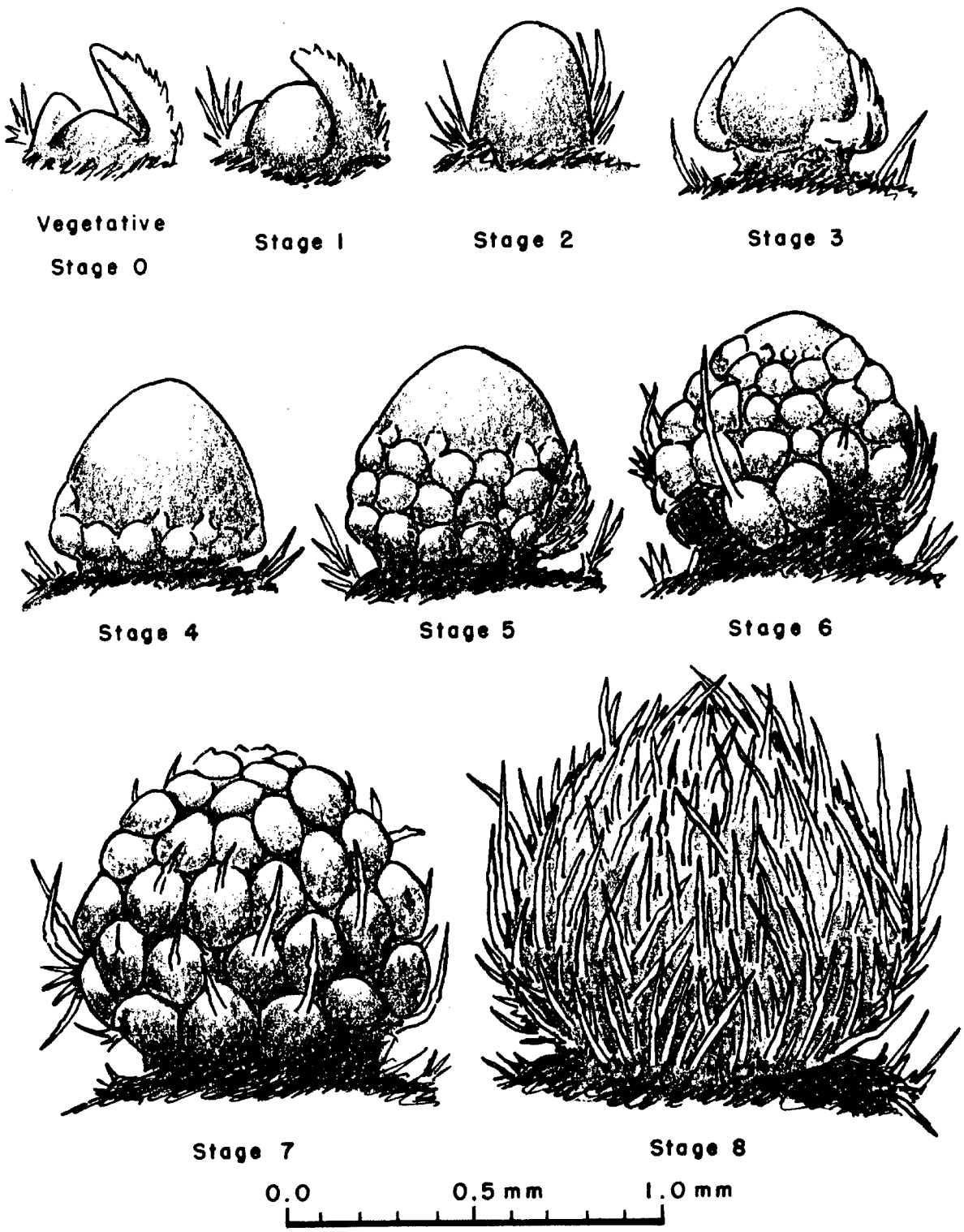

Figure 2. Stages of development of the staminate inflorescence primordium of Xanthium (See Table I). The degree of pubescence of the bud varies somewhat, depending upon the experimental treatment.

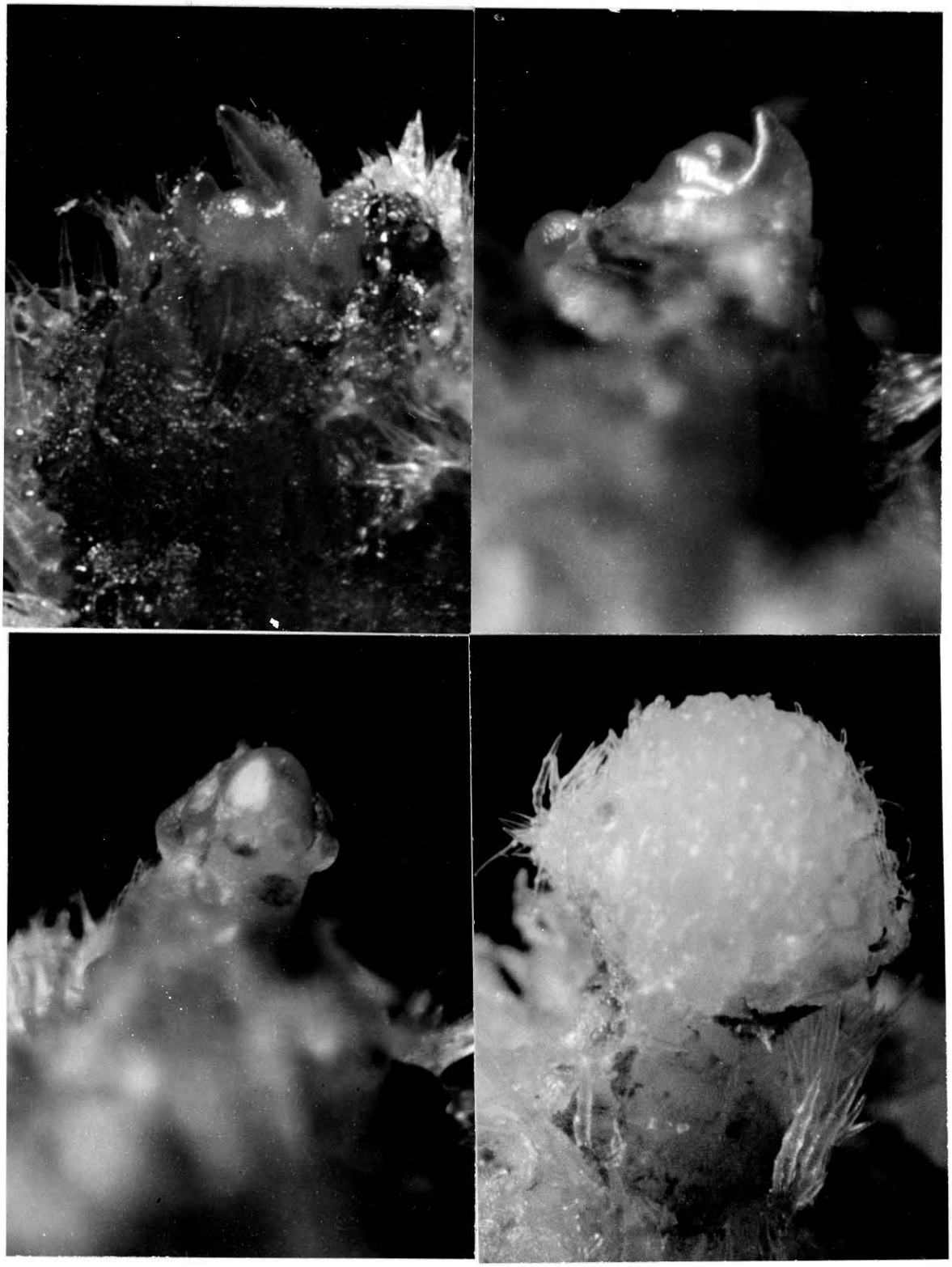

Figure 3. Microphotographs of some stages of development of the floral bud. Upper left, vegetative; upper right, stage 1; lower left, stage 3; lower right, stage 7.

Table 1

CRITERIA USED IN CLASSIFYING BUDS ACCORDING TO FLORAL STAGE

Stage 0.	Vegetative. Growing point relatively flat and small, as shown in Figures 2 and 3.
Stage 1.*	First clearly visible swelling of the growing point--hemispherical shape.
Stage 2.*	Inflorescence primordium at least as high as broad, but not yet constricted at the base.
Stage 3.	Inflorescence primordium constricted at the base, but no flower primordia yet visible (often some leaf primordia at the base).
Stage 4.	First visible flower primordia, covering no more than the lower one quarter of the inflorescence primordium.
Stage 5.	Flower primordia covering from one to three quarters of the inflorescence primordium.
Stage 6.	Flower primordia covering all but the upper tip of the inflorescence primordium.
Stage 7.	Inflorescence primordium completely covered by flower primordia. Slightly to moderately pubescent.
Stage 8.	Very pubescent and usually larger than stage 7 (diameter equal to or greater than 1 mm.)

*Stages 1 and 2 have been observed to revert to stage 0 under some conditions. Hence they are ignored or referred to as intermediates by some authors. Some induction is required, however, for the growing point to differentiate to any extent at all, and the numerals assigned to these stages have been found to be a suitable measure of this induction.

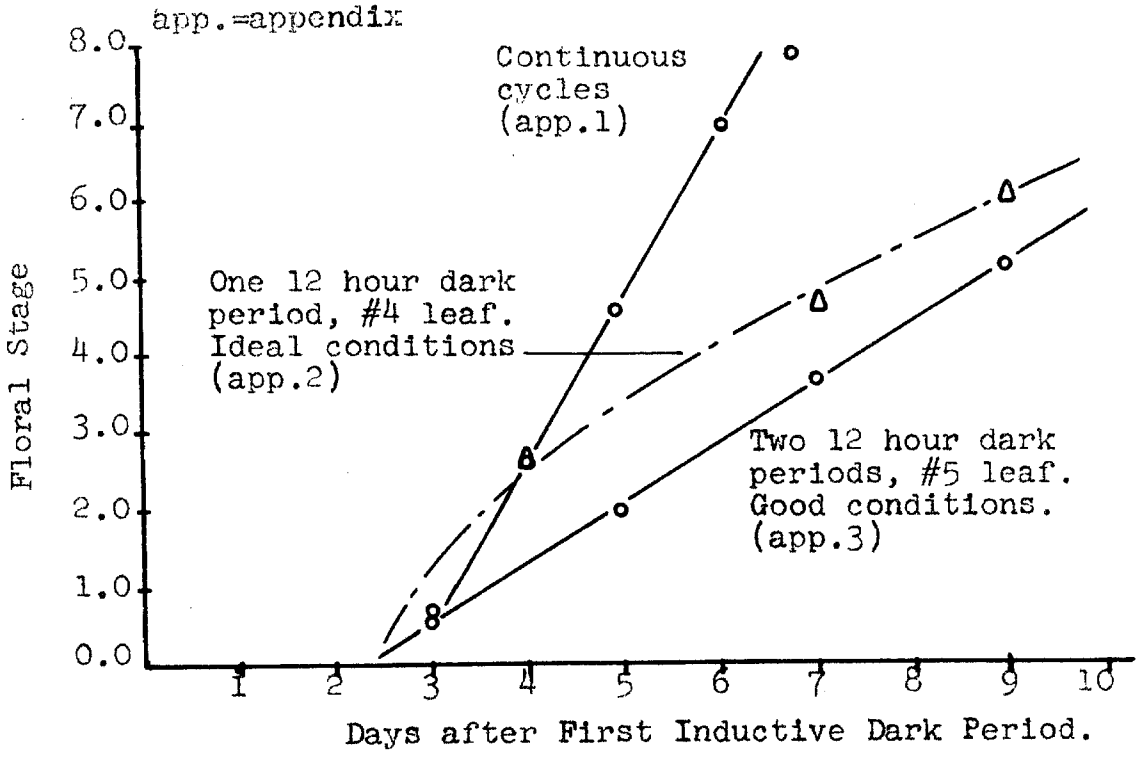

Figure 4. Relation of floral stage to time after beginning of induction under various conditions.

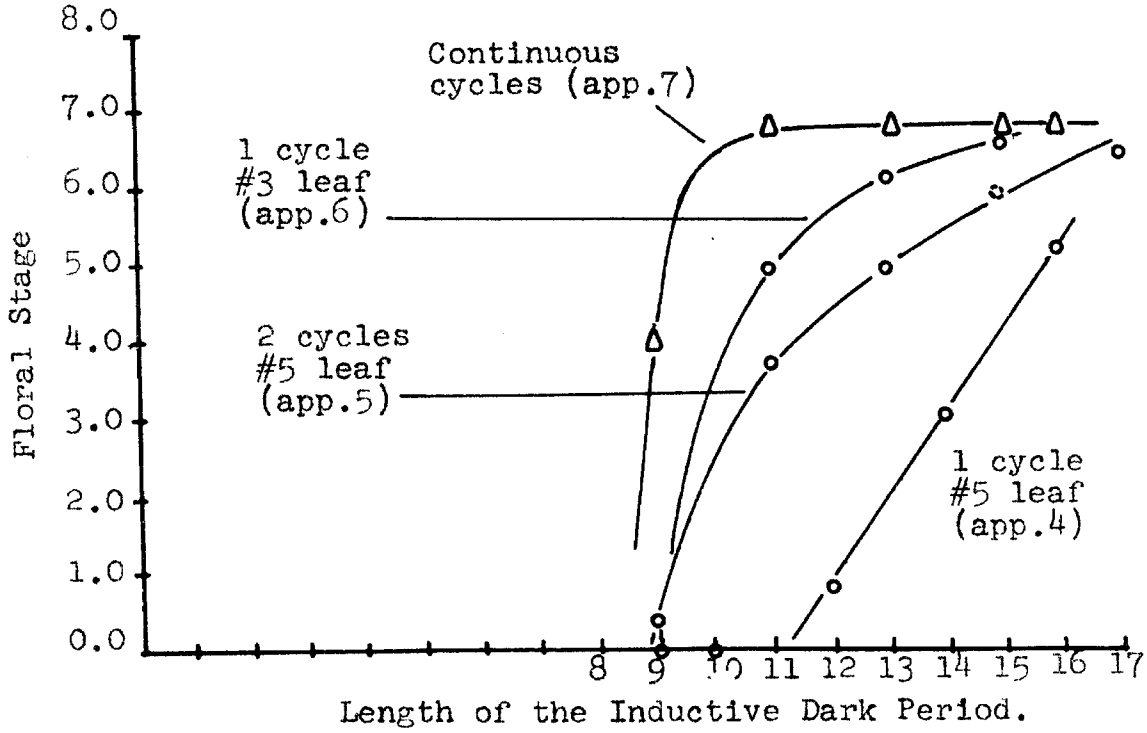

Figure 5. Relation of floral stage to length of the inductive cycle under various conditions.

numerical stage under optimal light conditions, with two or more inductive cycles, is directly proportional to time. Under poor light conditions, the final rate is lowered but the initial rate is the same as under high light intensity (Figure 20). A single inductive cycle often causes a decreasing rate of development. To compare treatments in an experiment, plants must be dissected while development is still proportional to time. During this period, the exact time of examination of the buds is of secondary importance, and stages can be adjusted to any desired time after induction for comparison between different experiments.

Figure 5 shows the results of a number of experiments relating floral stage to length of the inductive dark period. It may be seen that with sub-maximal induction the stage of flowering is proportional to the number of hours by which the dark period exceeds the critical night length. The curves tend to reach a maximum after which increase in night length no longer results in an increase in flowering (or only in slight increase). The exact time at which this maximum is reached depends on various conditions, some of which are discussed below. With continuous inductive cycles the buds develop at a maximum rate (Figure 4), and stage is virtually independent of night length (Figure 5).

The relation between stage and night length is of importance. The stage system indicates that in this instance there are different degrees of induction, and appears to be a good measurement of this phenomenon. As such it can be applied to

other experiments to measure their effect upon the degree of induction.

Factors of Importance in Experimentation with Xanthium

Leaf Size. In experiments involving the treatment of leaves with auxin and/or light, it is desirable to utilize plants which have been defoliated to a single leaf. Such a procedure saves time in the application of chemicals and insures good control of light intensity at the leaf surface (Parker, Hendricks, Borthwick, and Scully, 1946). As will be pointed out below, this method also increases the degree of control over the response of the plants. The response to photoperiodic induction of the various leaves on a Xanthium plant has been studied recently (Khudairi and Hamner, 1954), and it is reported that the half expanded leaf is the one most sensitive to an inductive dark period. To study in more detail the response of different leaves, further experiments were conducted.

In order to establish arbitrary size limits for the various leaves, the length of the midribs of the leaves on 100 plants was measured. The smallest leaf measuring longer than 1 cm. was referred to as leaf #1, the next leaf down the stem as #2, and so on (Figure 6). The length of the various leaves, plotted against the leaf number, is shown in Figure 7. From the smooth curve drawn between the points, arbitrary limits for the length of the various leaves can be established, as shown in Table 2. These sizes were arbitrarily used in all

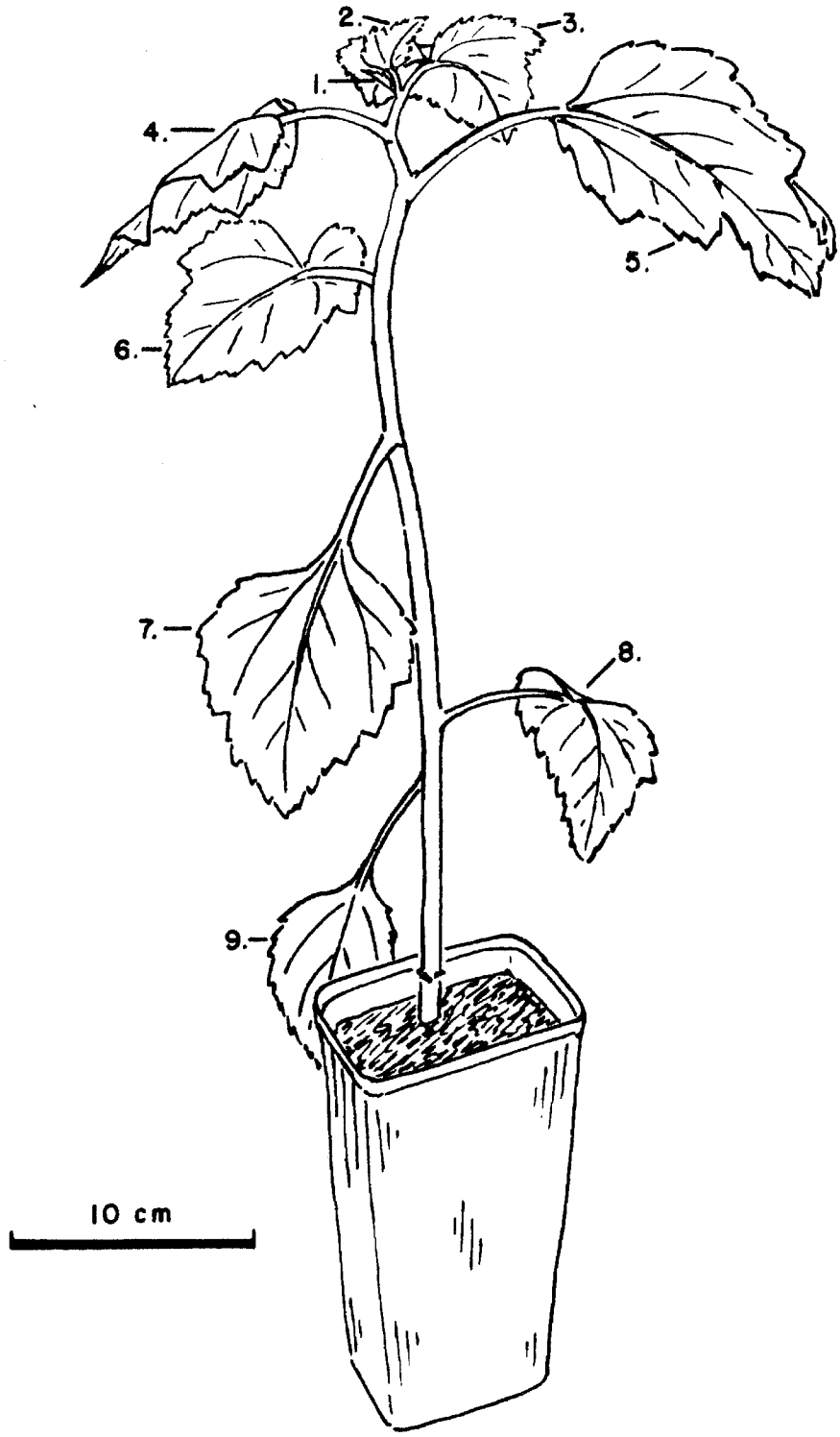

Figure 6. A mature plant of Xanthium pennsylvanicum Wallr., showing method of numbering the leaves according to physiological age.

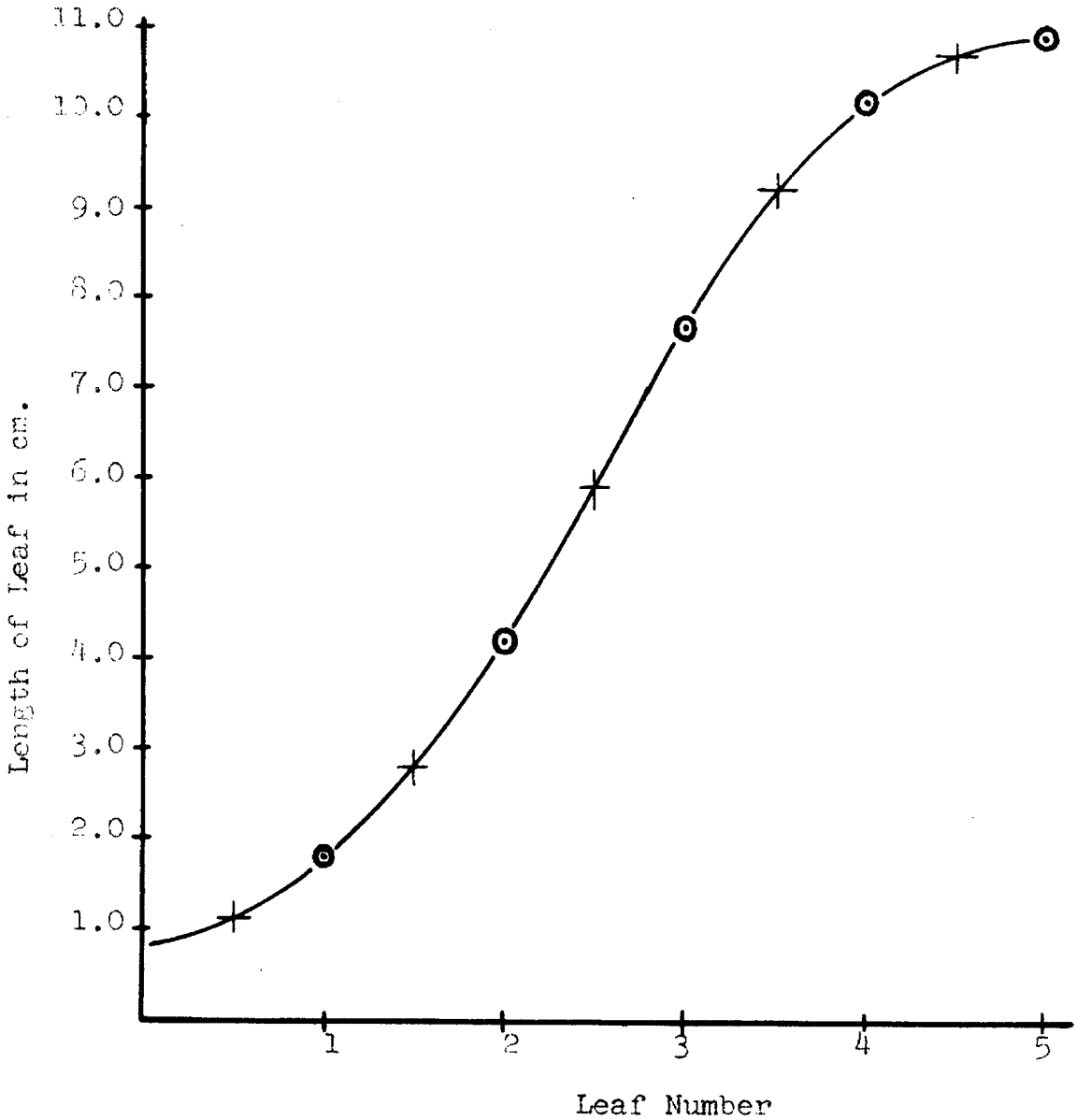

Figure 7. The relation of the length of the leaf to the leaf number. This curve, resulting from the measurement of the leaves on 100 plants, was used as the arbitrary standard for the definition of leaf number according to size. The crosses on the curve indicate the size limits as shown in Table 2.

Table 2

ARBITRARY SIZES ASSIGNED TO VARIOUS LEAF NUMBERS

Leaf number	Length of the midrib in cm.
Young #1	1.2 to 1.8
Old #1	1.8 to 2.8
Young #2	2.8 to 4.2
Old #2	4.2 to 5.9
Young #3	5.9 to 7.7*
Old #3	7.7 to 9.2*
Young #4	9.2 to 10.2
Old #4	10.2 to 10.7
Young #5	10.7 to 10.9

*Used to sort plants according to leaf size in subsequent experiments.

subsequent experiments even though environmental conditions sometimes altered the curve of Figure 7 slightly. A transparent plastic measuring device was made, with which plants could be classified into three groups on the basis of the length of the #3 leaf: young #3, old #3, and those plants whose #3 leaf did not fit the dimensions in Table 2 (from 5% to 25% of the plants).

In an experiment to test the sensitivity of the various leaves, plants were divided into the three groups described above, the third (non-conforming) group discarded, and the plants defoliated except for one leaf. The plants were divided into groups of 16, each group consisting of plants with old or young leaves of each number from 1 to 7. The plants were given two 16 hour dark periods, and the apical buds examined after 13 days. The data are plotted as leaf number vs. stage in curve A of Figure 8. From the curve it can be seen that the #3 leaf is the most sensitive to photoperiodic induction.

The lengths of the leaves were determined, and the averages are plotted in curve A, Figure 9. If, as a first approximation, the assumption is made that the leaves are produced at a constant rate and grow to a constant maximum size, the leaf number scale may be replaced by a time scale (by measurement, about four days per leaf). The curve then expresses the elongation of the leaf as a function of time. If the assumption is also made that the square of the length of the leaf is proportional to its area, then the curve relating the

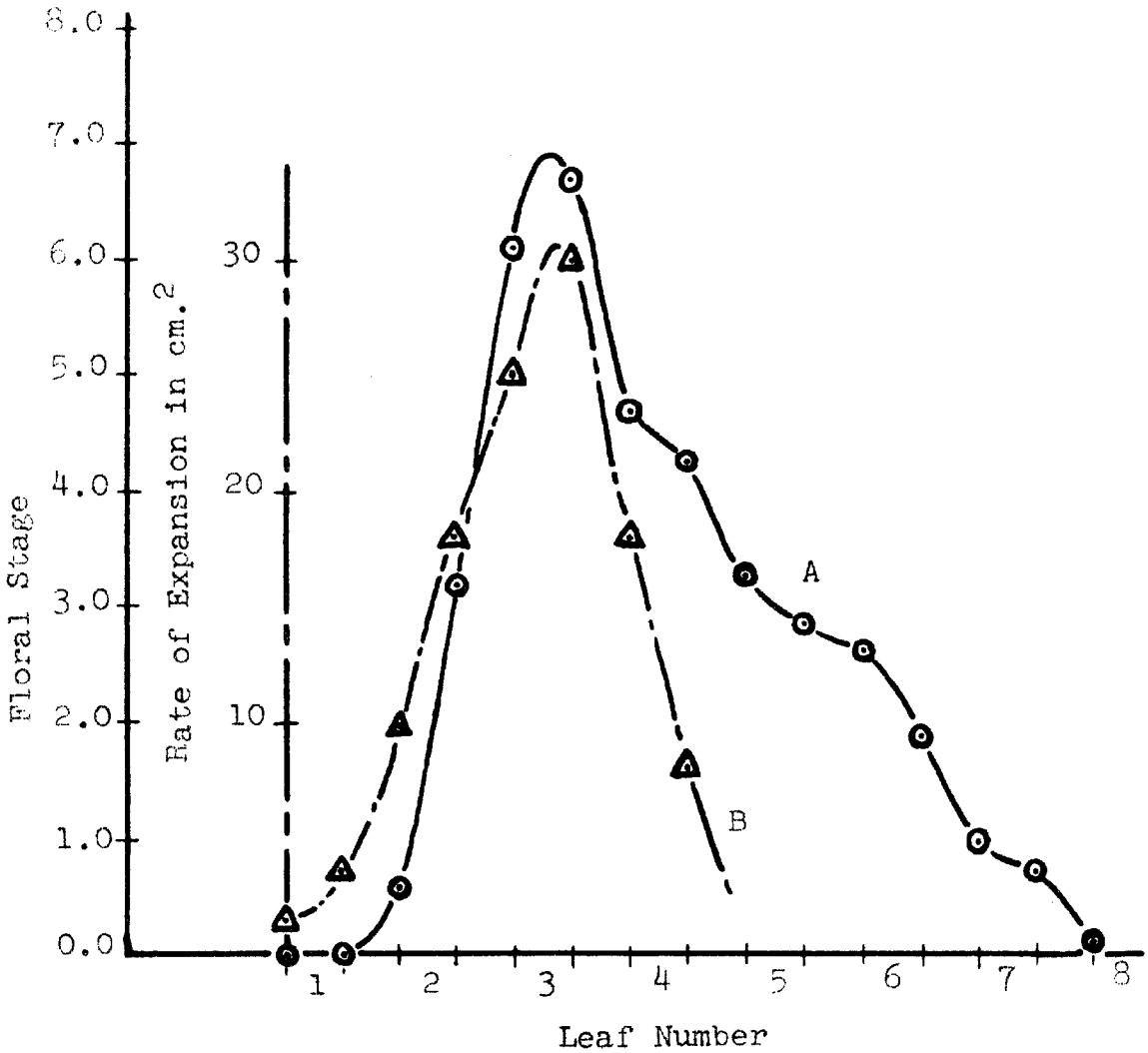

Figure 8. Showing the correlation between leaf sensitivity to photoperiodic induction and rate of expansion of the leaf (app.8). Leaf sensitivity is indicated by curve A, showing the floral stage 13 days after induction (two 16 hour dark periods), of plants which had been defoliated to a single leaf, as explained in the text. The method of obtaining the rate of expansion, is explained in the text. Leaf sensitivity experiments have been repeated in less detail ca. 10 times.

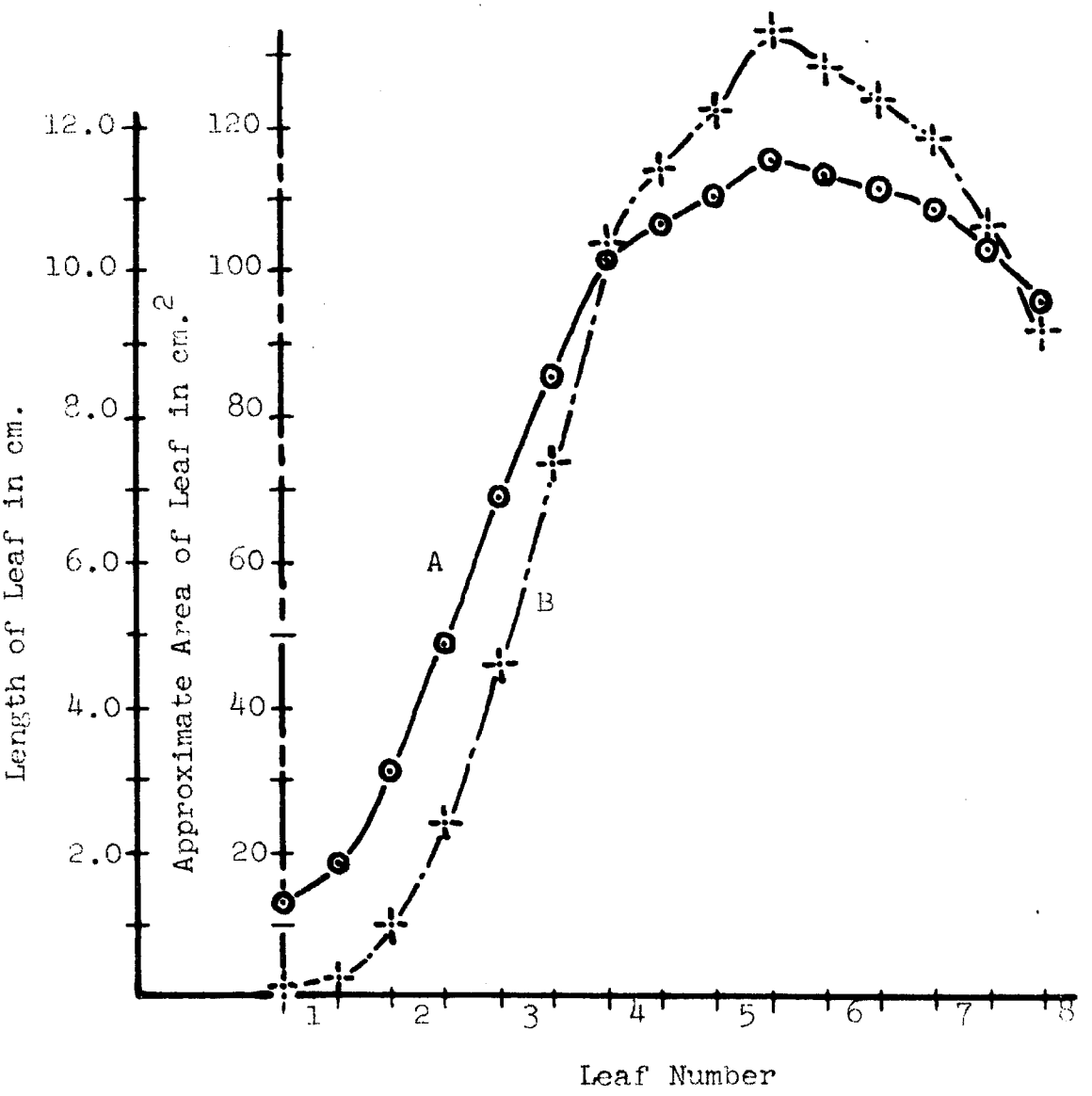

Figure 9. Curve A shows the relation of leaf length to leaf number, of the plants used in the experiment illustrated in Figure 8. Curve B shows the approximate area of these leaves, as a function of leaf number.

square of the length of the leaf to the leaf number (time) is a curve showing the expansion of the leaf as a function of time (Curve B, Figure 9). The slope of this curve (sq. cm. per day) gives the rate of expansion of the leaf. The slopes were measured and are shown in Curve B, Figure 8. The correlation between rate of expansion of the leaf and sensitivity to photoperiodic induction is evident. Thus it may be concluded that the most rapidly expanding leaf is the one most sensitive to an inductive dark period.

The fact that the curve of rate of expansion vs. time (Curve B, Figure 8) does not match the sensitivity curve (Curve A, Figure 8) for leaves older than #5 is owing to the fact that the assumptions made above are not completely valid for all leaf sizes. (1) Leaves are probably not produced at an exactly constant rate. The shoulders in curves A and B of Figure 8 (leaf #4) indicate that some environmental factor had affected the growth rate of this particular leaf. (2) It is obvious from Figure 9 that all leaves do not attain the same maximum size. The older leaves, produced when the plant is quite small, do not reach as great a size as the younger leaves. This fact itself is sufficient to explain why the two curves in Figure 8 do not match for the older leaves. (3) The area of the leaf is probably a function of the square of the length multiplied by some constant expressing the exact shape. The introduction of a constant into the calculation of the leaf area from leaf length would not affect the general shape of the rate of expansion curve (B, Figure 8).

Although more detailed work would be required (actual measurement of growth rate of the various leaves) to obtain the exact shape of the growth rate curve, the assumptions are probably valid well within experimental error for the first four leaves, and it is here that the greatest differences in both sensitivity and growth rate are encountered. It would appear to be a valid conclusion that the sensitivity of the various leaves to photoperiodic induction is correlated with rate of leaf expansion. Bonde (1953) has shown that the concentration of an auxin antagonist in the leaves of Xanthium is also proportional to their photoperiodic sensitivity (and hence to the rate of leaf expansion).

From the fairly large differences in sensitivity of the various leaves, it is obvious that in experiments utilizing plants which have been defoliated to a single leaf, it is of the greatest importance that the leaves be chosen according to size.

Time of Year. It was noticed in a number of experiments, that the degree of response varies considerably with time of year. This has been observed by a number of authors (e.g. Mann, 1940). In Figure 10 rate of floral development is plotted against the time of year, for a series of comparable experiments. Data are plotted as floral stage nine days after a single 16 hour inductive dark period, for plants defoliated to the #3 leaf. The months of the year are arranged so that days of approximately equal length in the spring and the autumn, fall on the same place on the abscissa.

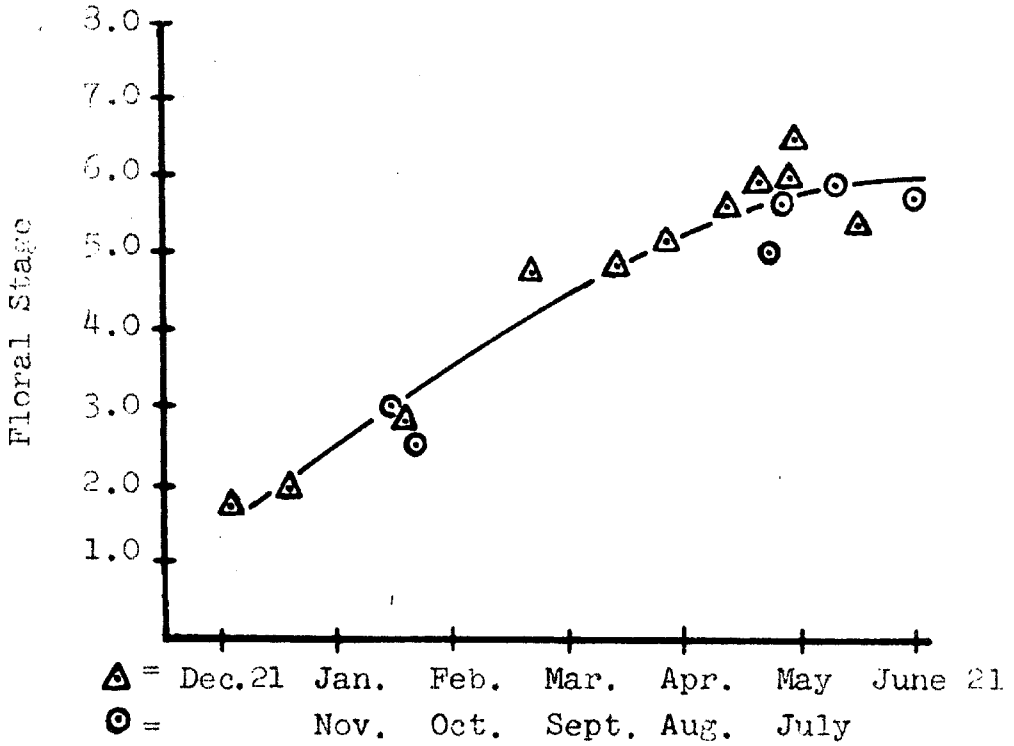

Figure 10. Floral stage of the apical bud 9 days after a single 16 hour inductive dark period, utilizing plants defoliated to the #3 leaf.

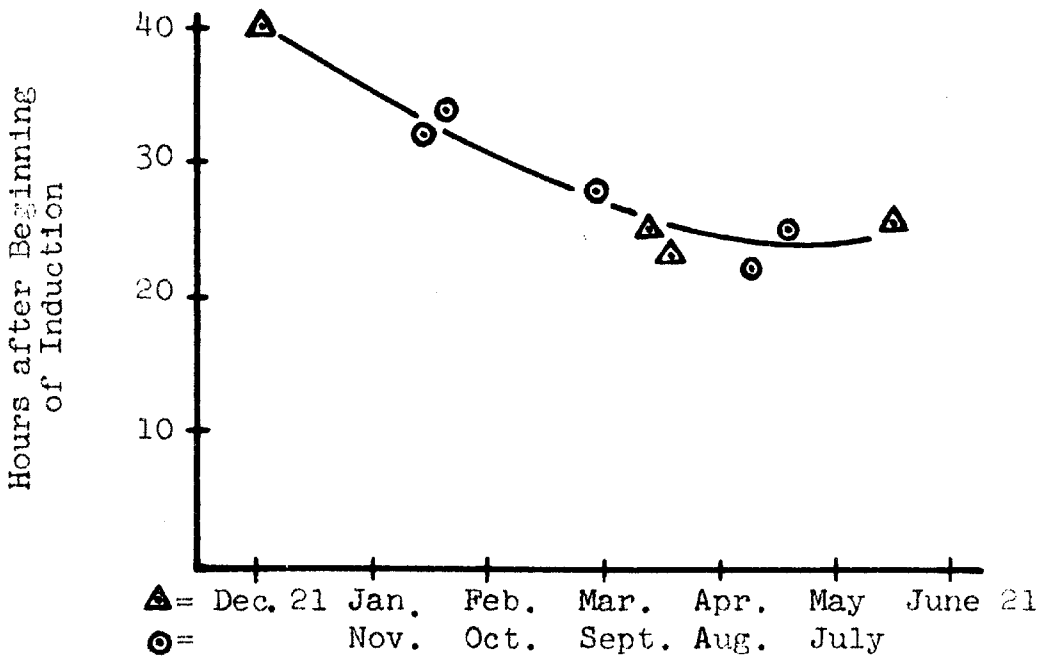

Figure 11. The relation of time of year to time of translocation of florigen out of the leaf, as explained in the text.

It may be seen that a given inductive cycle is much more effective in the summer than in the winter.

The time at which the flowering stimulus is translocated out of the leaf may be measured by defoliating groups of test plants at different times after induction (Khudairi and Hamner, 1954). If the flowering hormone has been translocated completely out of the leaf, the leaf may be removed and the floral bud will develop almost as rapidly as if the leaf is left on the plant. If translocation has not occurred, removal of the leaf results in the plant remaining vegetative. Thus the degree of flowering in groups of plants which have been defoliated at various times after induction gives a measure of the rate of translocation of the stimulus out of the leaf, as shown in Figures 19, 32, 33 and 34. The rate of translocation of flowering stimulus out of the leaf is also a function of time of year, as is shown in Figure 11, Curve A. The number of hours needed to translocate half the stimulus out of the leaf is plotted against time of year. It may be seen that translocation takes almost twice as long in the winter as in the summer.

It will be shown later (Chapters II and IV, Figures 19, 32, 33 and 34), that the time at which applied auxin becomes ineffective in floral initiation is related to the time at which florigen is translocated out of the leaf. Hence it is also related to time of year.

It has been known for some time that light intensity influences floral induction in Xanthium (pp. 16 and 20). One

might consider other factors as being responsible for the time of year effect (e.g. temperature, humidity), but this effect has been observed with plants grown in the Earhart Plant Research Laboratory, with all factors of the environment except light intensity controlled. In this greenhouse the plants are subject to the seasonal variation in light intensity, as in other greenhouses. Thus it appears that light intensity is the most important single factor in the effect of time of year upon flowering response, modified only slightly, if at all, by other environmental factors.

An illustration of the effect of light intensity for a number of days before induction and during development of the bud upon flowering, is given in Table 3. Plants were grown in Earhart Laboratory with all environmental conditions held constant except light intensity. After two weeks all plants were induced with a single 16 hour night, and then returned to their original conditions of varying light intensity. As shown in the table, the degree of flowering (after 10 days) was greatly influenced by light intensity.

The requirement for high intensity light before induction has been discussed in the introduction (page 16). It was concluded that the high intensity light process is probably a photosynthetic one.

A requirement for high intensity light following induction was also discussed in the introduction (page 20). A short period of light followed by a short dark period after induction brings about destruction of the flowering stimulus

Table 3

THE EFFECT OF LIGHT INTENSITY FOR TWO WEEKS BEFORE INDUCTION AND DURING DEVELOPMENT (10 DAYS) UPON FLOWERING (APP. 10)

Light Intensity (foot candles)				
8:00 to 16:00	16:00 to 8:00	% Flowering	Floral Stage	Number of Plants
400	400	10%	0.02	20
1500	1500	50%	1.95	20
Sunlight (5000)		100%	6.81	11

Maintained at 23° C. day temp., 17° C. night temp.

(or some precursor) produced during the inductive dark period. This effect may also be obtained by returning plants to low intensity light (100 foot candles) for four to six hours following induction. The requirement for high intensity light following the dark period is not absolute, however, as plants will flower if left in total darkness.

In preliminary experiments by the present author it has been shown that neither sucrose nor anti-auxin applications overcome the requirement for high intensity light following the dark period, and that the inhibitory effect of low intensity light or darkness occurs specifically in the leaf. Data for these experiments are summarized in Table 4.

An effect of light intensity upon rate of development of the floral bud is discussed in the following chapter (Figure 20). If plants are shaded beginning a few days after induction, the rate of development of the bud is slowed to

Table 4

SOME EXPERIMENTS ON THE SECOND HIGH INTENSITY LIGHT PROCESS

1. Application of sucrose (app. 50)

Single 12 hour night followed by	10% Sucrose applied to leaves	% Flowering	Floral stage	Number of Plants
High intensity light (Sunlight)	-	70%	2.9	27
	+	89%	4.8	27
Low intensity light (85 foot candles--8 hours)	-	15%	0.15	26
	+	11%	0.11	26

2. Application of antiauxin (app. 51, 52)

Length of dark period	Followed by	Antiauxin Applied	%Flowering	Floral stage	Number of Plants
14 hrs.	Sunlight	None	100%	3.8	16
		5.0×10^{-4} M p.chlorophenoxy-isobutyric acid	100%	3.7	16
	20 minutes sunlight	None	81%	1.6	16
	9 hours 60 foot candles	antiauxin (as above)	88%	2.3	16
14 hrs.	Sunlight	None	100%	5.7	15
		8.0×10^{-4} M antiauxin	100%	6.1	15
	20 minutes sunlight	None	100%	4.0	15
	6 hours darkness	8.0×10^{-4} M antiauxin	100%	3.9	15

3. Second High Intensity Light Process Occurs in Leaf (app. 53)

Plants induced with a single 16 hour dark period, followed by 30 minutes of sunlight. Following this, either the tip was covered with a black paper bag (6 hours), the leaf was covered (4 hours), both were covered, or neither were covered, as shown. Eight plants per treatment.

Treatment			
Leaf	Tip(Post induction)	% Flowering	Floral Stage
Not covered	Not covered	100%	5.6
Not covered	Covered	100%	5.6
Covered	Not covered	65%	1.1
Covered	Covered	35%	0.6

a considerable extent.

Thus it has been shown that light intensity just before and just after induction, as well as during development of the floral bud, influences to a great extent the flowering of induced Xanthium plants. It is highly probable that the light intensity for a number of weeks preceding induction, through its effect upon the general condition of the plants, may affect the flowering process. The difference in elevation of the sun above the southern horizon affects the duration and intensity of sunlight throughout the year. Not only are the days shorter in the winter, but position and construction of Dolk Greenhouse, where most of the experiments were carried out, is such that shading from street trees is much more extensive in winter than in summer. The number of cloudy days is also related to the time of year.

Thus time of year and weather conditions must be taken into account and reported with the results of experiments concerning the flowering of Xanthium, and if necessary for comparison, corrections utilizing the curves of Figures 10 and 11 may be applied to experiments conducted at different times of the year. Ideally, plants should be grown under high intensity artificial light, with all environmental conditions controlled.

Other Factors which Effect the Induction of Xanthium. Ir addition to the size of the leaf and the light intensity before and after induction, there are a number of other factors

which influence the results of experimentation with Xanthium. A number of these factors have been investigated by Long (1939), who reports that the age of the plant, the humidity of the atmosphere, and the temperature, all affect induction, although the effects are slight. Other workers have reported like results (Snyder, 1940; Roberts and Struckmeyer, 1938).

The influence of age of plant has been investigated by a number of workers (Naylor, 1953, p. 154, 1941) as well as by the present author. Xanthium seeds were planted at one week intervals. Sixty nine days after the first seeds were planted, half of the plants in each group were defoliated to the #3 leaf, the other plants were left undefoliated, and all plants were induced with three 16 hour dark periods. The results are shown in Figure 12. It may be seen that the response of all plants older than ca. 32 days is very nearly the same. Plants with all leaves present responded only slightly better than plants defoliated except for the #3 leaf. Thus it is apparent that the age of the plant is at most only a minor factor in the induction of flowering in Xanthium provided that plants older than about one month are used.

It seemed possible that the stage of bud development for a given treatment might depend upon the number of buds present upon the plant (if, perhaps, each bud consumes a given amount of florigen to arrive at a particular stage). This idea was tested in the following manner: Plants were defoliated to a single leaf. The terminal buds of half of the plants were removed. In each group (with or without terminal buds), half

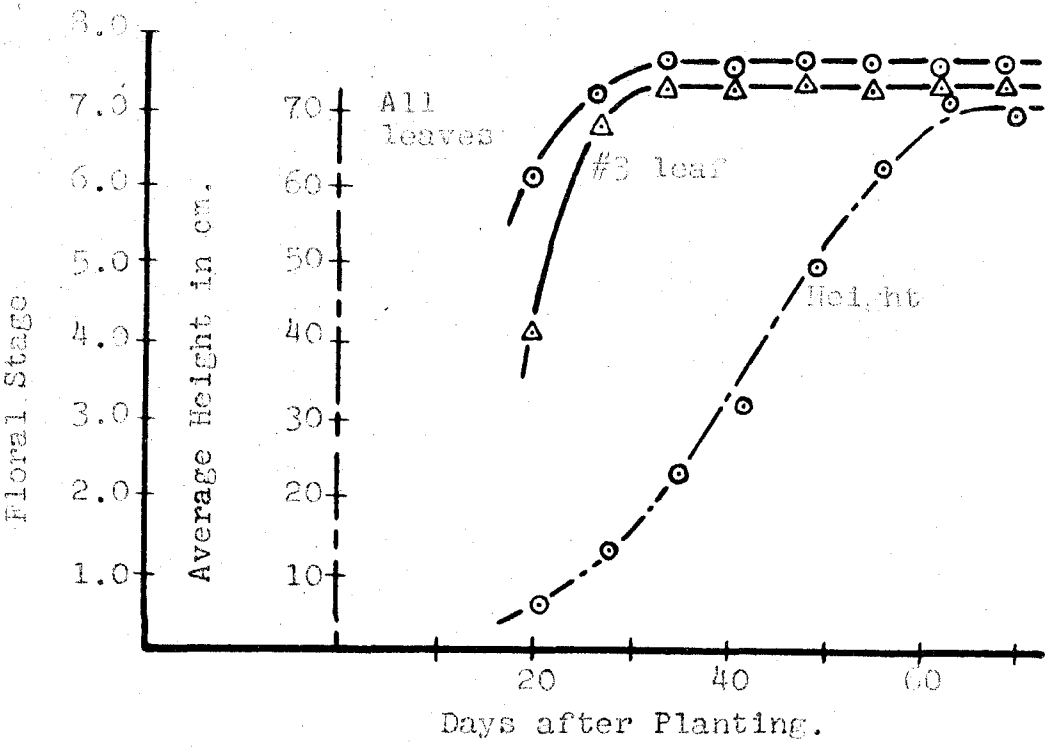

Figure 12. Relation of age of plant to photoperiodic sensitivity (app.9). Plants induced with three 16 hour dark periods.

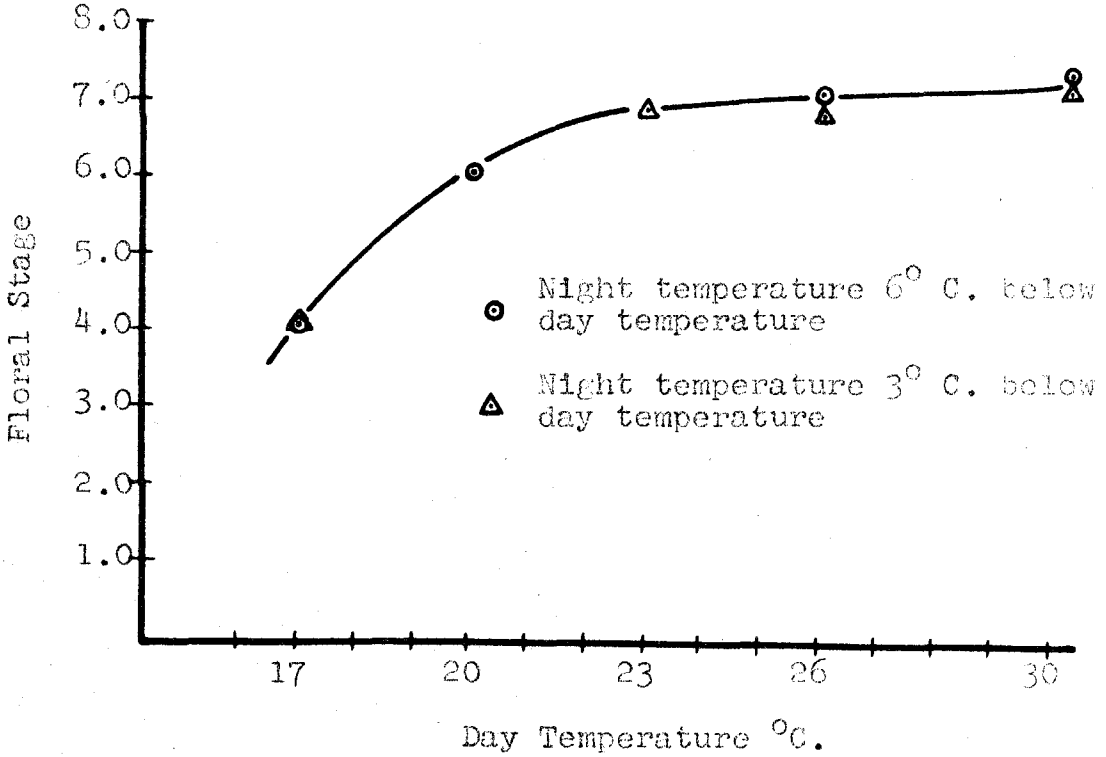

Figure 13. Relation of temperature to photoperiodic sensitivity. Plants grown before and after induction at temperature shown, induced with single 16 hr. night at 23° C. Plants grown at constant temperature yield an identical curve (app. 10).

of the plants were debudded so that only one bud was present on each. Three days were allowed for the axillary bud (or buds) of the group with the terminal bud removed, to become active, and then all were induced with a single 16 hour dark period. The condition of the buds after nine days is shown in Table 5. It may be seen that the stage of the terminal bud is only slightly dependent upon the number of buds present on the plant.

In the preparation of plants for each experiment, axillary buds are routinely removed at the time of defoliation. It is evident from Table 5 that if one or two of these buds are overlooked, it will not greatly affect the final result.

To investigate the importance of temperature during growth and development of plants before and after induction, plants were grown under various conditions in the Earhart Laboratories and then induced with a single 16 hour dark period at 23° C. The results are shown in Figure 13 in which floral stage is plotted against day temperature. It may be seen that above 23° C., day temperature during growth has very little effect upon flowering either before or after induction. This is in agreement with other work (Snyder, 1940) and is of considerable importance, since the temperature in most greenhouses, while not accurately controlled, is kept above 23° C. day temperature.

Cold temperatures during a single inductive cycle (and to a lesser extent, during continuous cycles) tend to reduce the degree of flowering and increase the critical night length

Table 5

EFFECT OF THE NUMBER OF BUDS ON THE PLANT, UPON THE FLORAL STAGE OF ALL BUDS AND OF THE TERMINAL BUDS (APP. 54)

Condition of Plants*		Number** of buds	Average stage of all buds	Product of number of buds times average stage	Floral stage of terminal buds
Tips left on plants	Buds left on plant	118	4.5	537	5.8
	All buds removed but one (terminal)	85	5.3	450	5.7
Tips removed three days before induction	Buds left on plant	219	4.4	958	6.0
	All buds removed but one (axillary)	64	6.1	392	6.8

*All leaves removed except one (#3).
**Very small buds near tip not examined. Sixteen plants per treatment.

(Long, 1939, Hamner, 1954). To investigate the time during induction when cold treatment is most effective in inhibition of flowering, plants were given a cold treatment (1° C. for the first or second half of a 16 hour dark period, the entire dark period, or not at all. The results are shown in Table 6. It may be seen that low temperature during the inductive dark period inhibits flowering, but that the timing of the treatment is relatively unimportant.

Thus it appears that temperature during the inductive dark period should be controlled, at least within broad limits, but that temperature during growth and development (on the greenhouse bench) is of relatively minor importance. In the experiments reported here, temperature during induction was controlled.

In a number of experiments with Xanthium, manipulation of plants during the inductive dark period has been done under a green light of low intensity. To test the effect of the green light itself upon induction, two groups of plants were placed directly under the light. One lot was covered and the other not. In another experiment, one group of plants was exposed to the green safelight (placed closer than normally) for ten minutes out of every two hours of a 16 hour night, and a control group was shaded from the light. The results of these two experiments are shown in Table 7. It may be seen from the table that short exposure to the green-light, every two hours, has a negligible effect upon flowering.

Table 6

EFFECT OF TEMPERATURE DURING THE INDUCTIVE DARK PERIOD

(APP. 55)

Temperature			
First Half of Dark Period	Second Half of Dark Period	% Flowering	Floral Stage
23° C.	23° C.	100%	7.3
23° C.	1° C.	100%	3.6
1° C.	1° C.	50%	0.7
1° C.	23° C.	80%	2.2

Plants trimmed to #3 leaf, induced with a single 16 hour dark period. Grown at 23° C. day temperature and 17° C. night temperature in the Earhart Laboratories.

Table 7

EFFECT OF GREEN LIGHT DURING DARK PERIOD

UPON FLOWERING

	Treatment	Floral Stage
First experiment (app. 12)	Green light on during entire dark period (29 ergs/cm^2/sec.)	5.2
	No green light	6.1
Second experiment (app. 56)	Green light on 10 minutes out of every two hours during dark period (Slightly higher intensity than above)	6.8
	No green light	7.0

Plants induced with a single 16 hour dark period, defoliated to the #3 leaf. Plants flowered 100 percent.

Methods in the Use of Auxin

Application. Since the principal problem of this thesis concerns the effect of auxin upon the flowering of *Xanthium*, the methods for its use have been investigated. The most important problem involves the method of application. In Table 8 the effectiveness of various methods of auxin application are summarized. The data are in the form of concentration needed to inhibit flowering by 50% when applied at the beginning of a single 16 hour inductive dark period by the methods indicated. Since a multitude of factors may influence the effectiveness of applied auxin, the figures in the table are correct only as to order of magnitude. It is clear, however, that the concentration of auxin which inhibits flowering to a particular degree is a function of the method of application. It should not, therefore, be compared with the concentration of auxin effective in other physiological responses such as the *Avena* test. Each of the methods has various advantages and disadvantages which are discussed in the following paragraphs.

1. Application to soil. If auxin is applied to the soil, very high concentrations are required to inhibit flowering. As a method this is very poor, but the high concentration required for effect indicates that small amounts of auxin accidently spilled on the soil may safely be ignored.

2. Lanolin Paste Applied to the Stem. This method may be used when the effectiveness of various points of applica-

Table 8

METHODS OF APPLICATION OF AUXIN

	Method	Auxin	Concentration which Inhibits 50%*
1.	Pour on soil (4 inductive cycles)	NAA	80.0×10^{-4} M**
2.	Lanolin paste (applied to stem) (one 16 hour night)	NAA	0.15%
3.	Leaves submerged in auxin solution at night (4 inductive cycles)	NAA IAA	0.03×10^{-4} M**
4.	Cuttings (4 inductive cycles)	NAA	0.3×10^{-4} M**
5.	Sprayed on leaves (one 16 hour night)	NAA IAA	3.0×10^{-4} M 4.0×10^{-4} M
6.	Leaves dipped in solution (one 16 hour night)	NAA IAA	2.0×10^{-4} M 3.0×10^{-4} M
7.	Submerged in auxin for short time (10 minutes) (one 16 hour night)	NAA	30.0×10^{-4} M
8.	Dipped and rinsed afterwards (one 16 hour night)	NAA IAA	30.0×10^{-4} M 40.0×10^{-4} M

*All values are approximate.
**Bonner and Thurlow, 1949.

tion is being studied, when plants have been defoliated, or when a continuous supply of auxin over a period of time is desired.

3. Leaves Submerged in Auxin Solution at Night. This method has been used to demonstrate that very low concentrations (i.e. within the physiological range) are effective in the inhibition of flowering. Low concentrations are indeed effective, but control plants submerged in pure water without auxin are also inhibited to a certain extent, and hence the results are difficult to interpret. Mechanical difficulties make the method highly impractical for normal experimentation with Xanthium.

4. Auxin Taken up in Solution by Cuttings. Xanthium cuttings may be placed in Hoagland's solution in test tubes shortly before induction. Auxin is then added alone in solution or an as additive to Hoagland's solution. Again low concentrations are effective, and a continual supply of auxin over a long period is maintained. It is difficult, however, to keep the plants healthy after treatment, as they tend to wilt easily. Some plants fail to survive. Shading to prevent wilting affects the rate of development of the floral bud. The method is well suited for certain experiments, but is impractical for normal usage.

5. Auxin Sprayed on Leaves. 6. Leaves Dipped in Auxin Solution. These two methods are simple to use, and quite effective for most purposes. Dipping is slightly more effective than spraying, probably because both surfaces of the

leaf are wetted. In both instances addition of a wetting agent (e.g. Tween 80) to the solution considerably increases the effectiveness of the auxin. Dipping seems to be the simpler of the two methods. Either the stem with all its leaves is dipped in auxin solution, or the plants are defoliated to a single leaf, which is dipped in the solution. From 500 to 1000 ml. of solution is usually made up, detergent is added in the proportion of two drops of Tween 80 per liter of solution, the leaves of all plants in the treatment are dipped in the solution, and the plants are shaken to remove excess solution from the leaves.

The method is rapid, effective and yields reproducible results. It has been utilized in many of the experiments reported in this thesis. The effectiveness of auxin applied in this manner seems to depend, however, to a great extent upon the humidity of the atmosphere. Auxin applied in the middle of the day in bright sunlight, when temperature is high and humidity low, is much less effective than auxin applied to plants just before they are placed in a cool, humid, dark-room--other conditions being equal. Under conditions of low humidity the plants dry off rapidly (20 to 30 minutes) after treatment with auxin. Under conditions of high humidity the solution remains on the leaves for long periods (up to two or three hours). Apparently auxin penetrates the leaf cells only when it is present <u>in solution</u> outside these cells. Hence the longer the leaves remain wet, the more auxin is able to penetrate, and the greater is the

inhibition of flowering. The differences could be reduced by artificially raising the humidity in the greenhouse, but it was impossible to raise the humidity to the level of that in a cool, closed, dark room with damp floors.

Unless temperature and humidity can be controlled exactly, it is not possible to compare treatments where auxin is applied by dipping at different times or in different places. A method was sought which would overcome this difficulty and allow a given amount of auxin to penetrate under all environmental conditions. Two such methods were devised.

7. Leaves Submerged in Auxin for Short Periods. To overcome the problem of varying humidity, auxin was applied in the following manner: Plants were well watered before treatment to relieve any existing water stress. Groups of 16 plants were defoliated to a single leaf per plant. The petioles of the leaves of eight plants were inserted between two glass rods, which were held together by rubber bands at the ends. Thus each treatment consisted of two groups of eight plants with the leaves held in more or less of a plane. The two groups were placed one on each side of a small white enamel tray (one liter capacity) so that the leaves could lie in the tray. The tray was supported by a ring stand and could be raised or lowered. Tap water was poured into the tray and allowed to cover the leaves for five minutes in order to wet the surfaces evenly. The tray was lowered and the water poured out, after which it was raised and filled with auxin solution of the desired concentration (comparatively high).

After ten minutes the auxin was removed and the leaves were rinsed with two changes of fresh water. The higher the auxin concentration, the shorter the time of submergence needed to bring about a given floral inhibition.

This method allowed the auxin to be absorbed under conditions of defined humidity, and time of auxin absorption into the leaf is confined to the ten minute period. The actual mechanics of the method introduce a number of problems, however. It is difficult to keep the leaves submerged, time the process accurately, prepare the plants for treatment, do a number of treatments at the same time, etc.

8. Leaves Dipped and Rinsed. To produce an easy, yet efficient method of auxin application the above two methods were combined. Plants are defoliated to a single leaf as usual, and the leaves are dipped in tap water five minutes before auxin application. The leaves are then dipped in a high concentration auxin solution. Some specific pattern is followed in dipping the leaves of a given treatment (starting at one end, working across, etc.), and then after a measured interval (5 or 10 minutes) the same pattern is followed in rinsing the plants first with tap water and then with distilled water. Plants which are in the greenhouse at the time of treatment may be moved to the dark room for the short period during which the auxin solution remains on the leaves, insuring standard conditions regardless of the time of treatment.

With respect to the concentration of applied auxin,

this method is diametrically opposed to the submergence of the leaves in low auxin concentrations during the entire night. Certainly the high concentrations required (25.0 x 10^{-4} M) are far from physiological. But this method was not devised to investigate physiological concentrations. The auxin must penetrate in a very short period of time (which is of considerable importance in some experiments), and the plants are not damaged in any way. Controls treated with water alone (or water and detergent) flower as well as untreated plants. Since fresh solution must be used for each treatment (the solution is diluted by the water present on the leaves from the pre-rinse), and the concentration of the solution is quite high, a relatively large amount of auxin must be used in each experiment. The synthetic auxins such as alpha-naphthalene acetic acid, however, are quite inexpensive, and even the relatively large amounts required for an experiment seldom cost more than a few cents.

Kind of Auxin. Various auxins have been used by different investigators. Li (1950) reports that indole acetic acid, alpha-naphthaleneacetic acid, 2,4-dichlorophenoxyacetic acid, and 2,4,5-trichlorophenoxyacetic acid are highly active in the inhibition of flowering, while 2,4-dimethylphenoxyacetic acid, 2,4-dichlorophenylglycine, 4-fluorophenoxyacetic and 2,4,5-triiodophenoxyacetic acids are only slightly active. Thus the specificity of the inhibitory reaction is quite similar to that of the auxin responses. The most commonly used auxins are indole acetic acid (IAA), alpha naphthalene acetic

acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D). IAA is probably the native auxin of plants, and hence it would be desirable to use this compound exclusively. NAA, however, is cheaper, and much more stable in solution. From experiments performed by the present author and others it is apparent that the action of IAA and NAA is very much the same, although NAA is more effective in the inhibition of flowering. Figure 14 shows the rate of development of floral buds after application of IAA or NAA. Again it may be seen that the response is essentially the same regardless of the auxin used. In the light of these results, NAA has been used almost exclusively in the experiments reported in this thesis.

The pH of the Auxin Solution. In classical auxin work, the pH of the solution is thought to influence the effectiveness of auxin by controlling the amount of auxin which penetrates the cells (Bonner, 1934). Auxin molecules penetrate best in the nonionized form, and hence the auxin is most effective when applied in acid solution. On the assumption that the same situation holds for Xanthium leaves, many experiments have been carried out by this author and others utilizing auxin solutions of about pH 5.0 or lower. Finally two experiments were run to test the effectiveness of auxin in the inhibition of flowering in Xanthium when the auxin is applied in solutions of different pH. In one experiment the auxin solutions were adjusted to a given pH with HCl or KOH in the absence of buffer, and in another experiment the pH was more carefully maintained by the use of citric acid buffer.

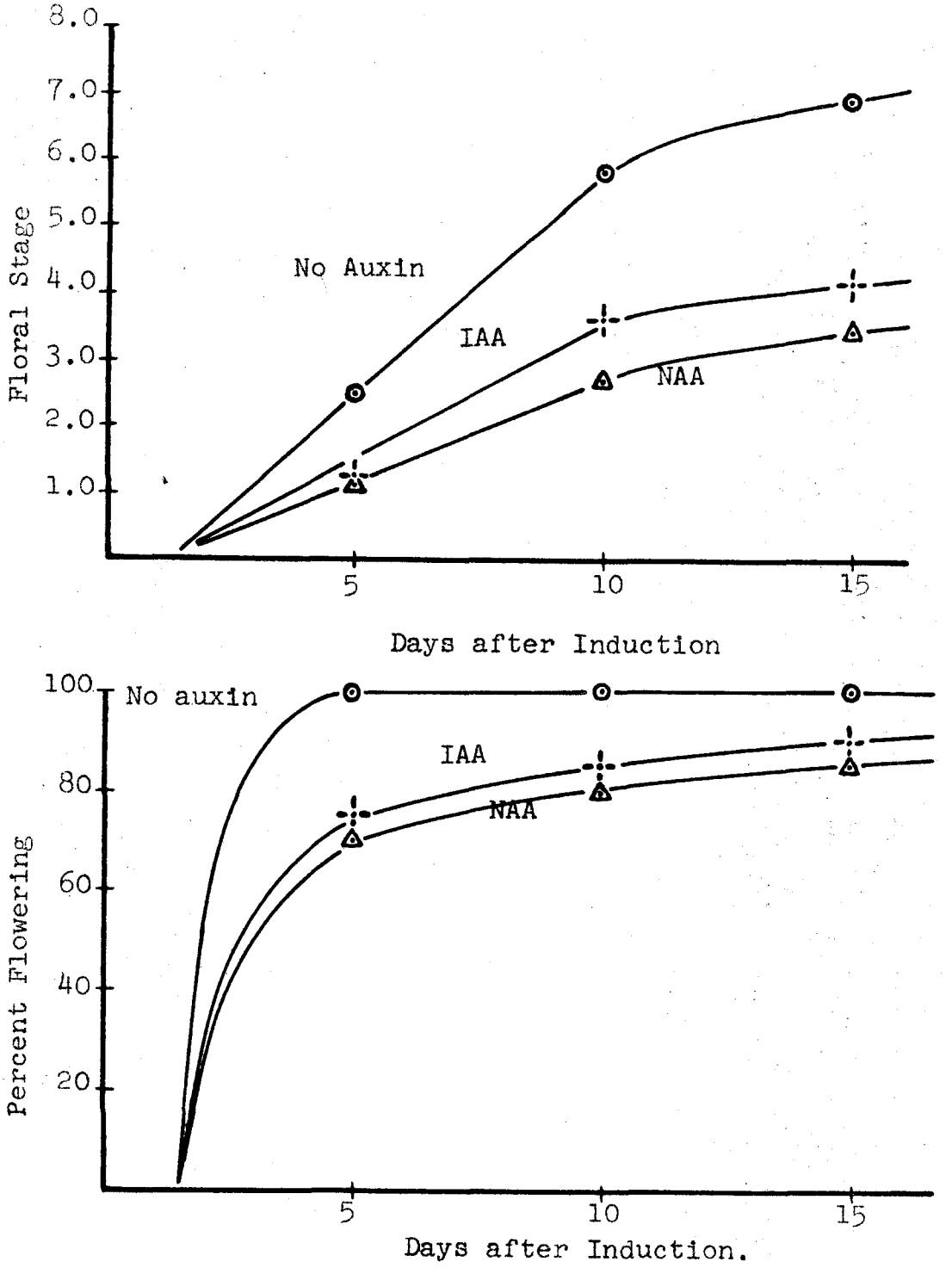

Figure 14. Rate of development of plants treated with auxin (IAA or NAA). Plants induced with two 12 hour dark periods, and dissected on the days shown (app.11). Sprayed just before the dark periods with 2.0×10^{-4} M auxin solution.

The results are shown in Figure 15. Contrary to the results expected on the basis of other auxin work, applied auxin was most effective at pH near neutral, and least effective in acid solution. In both experiments the auxin appeared to be more effective at pH 4.0 than at pH 3.0 or 5.0.

The explanation of these results is probably complex. It was noticed during application of auxin in the experiment utilizing buffer, that the solutions near pH 7.0 seemed to wet the leaves more thoroughly than the acid solutions. Detergent (Tween 80) was used in these experiments as a wetting agent, and it seemed possible that the effect of pH might be upon the wetting agent rather than directly upon the auxin. Ionization of acidic groups of the detergent molecule would increase its polarity, and presumably also increase its effectiveness as a wetting agent. Thus one might postulate that at neutral pH, the wetting agent would provide a greater surface area of contact between auxin solution and leaf cells, allowing more auxin to penetrate. Possibly the increase in effectiveness at pH 4.0 is a manifestation of the usual property of increased auxin penetration from acid solution, while further decrease in pH makes the wetting agent so inefficient that net effectiveness of the auxin again drops off.

The detergent used, Tween 80, is an ester and possesses but few free acidic groups. Samples of Tween 80 as well as of some other detergents were titrated with acid, and it was found that the detergents are buffered against addition of acid around pH 6.5, which is in agreement with the idea that

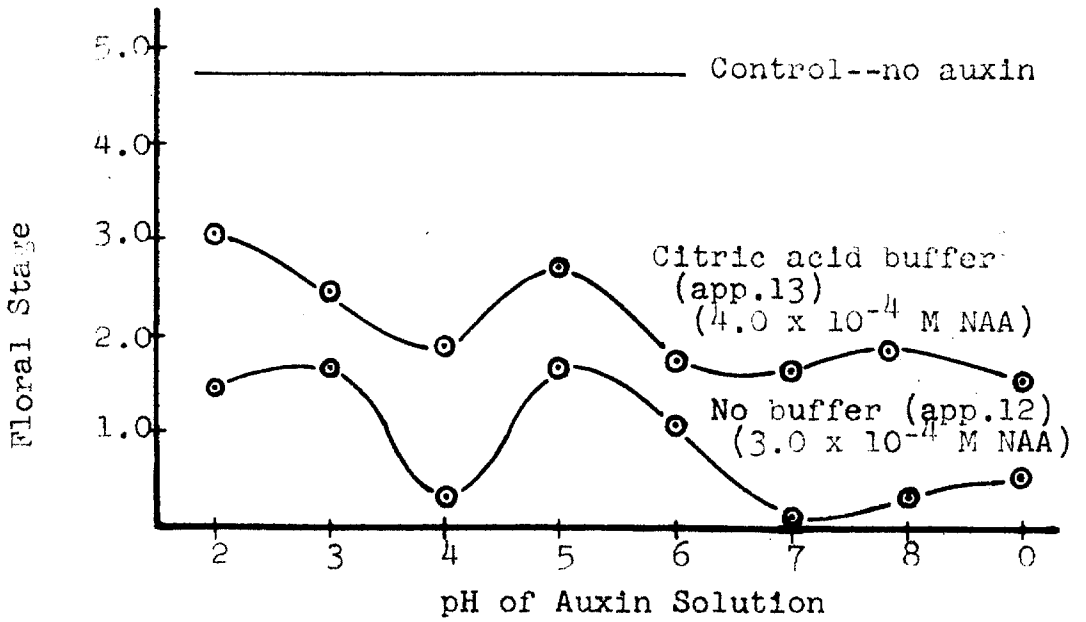

Figure 15. The relation of flowering (floral stage) to the pH of applied auxin solution. Induction by two dark periods. Auxin applied by dipping only before first dark period. The two curves represent two experiments. The floral stages of the "buffer" curve were adjusted so that control of both experiments were at the same floral stage.

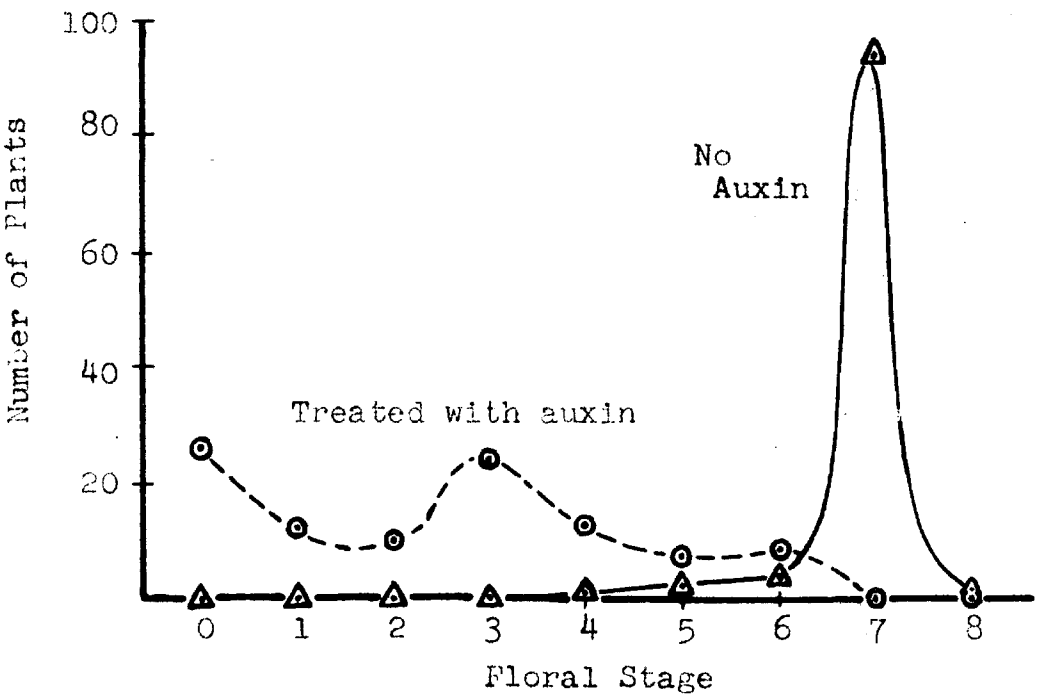

Figure 16. The variability in response to induction, which is introduced by auxin application (app. 14).

the effect of pH may be primarily upon the wetting agent.

Regardless of the explanation (further tests are in order), it is clear that pH of the auxin solution is of paramount importance in experiments involving the application of auxin to Xanthium, and that pH should be carefully controlled. The agreement between the buffered and non-buffered curves in Figure 15 indicates, however, that the addition of buffer is probably not necessary. Unfortunately many of the experiments presented in this thesis were done before the effects of pH were investigated.

Preparation of Auxin Solutions--the Presence of Salt. Un-ionized auxin molecules are only slightly soluble in water. There are, however, a number of different ways in which auxin may be brought into solution. A common method is to dissolve the auxin in a small amount of alcohol, and then add this gradually with stirring to a large volume of water. This is a simple method, but the objection may be raised that the alcohol in the solution may adversely affect the plants (Liverman, 1952).

The method used at the California Institute of Technology by earlier workers and continued by this author, consists of bringing the auxin (or other acidic substances) into solution by the addition of base. The material goes into solution much faster if the concentration of OH^- ions is very high. Under these conditions, up to a gram of NAA will dissolve in 10 to 20 ml. of water. The following procedure is used for the mixing of auxin solutions: The dry auxin is weighed

out and placed in a small beaker. A calculation is made to determine the amount of dry KOH which is required to neutralize the H^+ ions of the auxin. The calculation is made on the basis of the specified purity of the KOH, and will probably still result in a slight shortage of OH^- ions, as the distilled water contains dissolved CO_2 which neutralized some of the OH^- ions. The dry KOH is added to a small amount of distilled water (ca. 20 to 30 ml. per gm. of auxin), dissolved, and the solution added to the dry auxin. With some stirring most of the auxin will go into solution in the small amount of water. The solution is then decanted off into a large beaker, and a few drops of KOH solution are added to the auxin remaining in the beaker. When the remaining particles of auxin have gone into solution, distilled water is added, the solution is poured into the large beaker, and the small beaker is rinsed a number of times into the large beaker. Distilled water is then added to the large beaker until the final volume is almost reached. The pH is measured with the glass electrode (if the procedure has been followed carefully, the pH upon dilution will usually be around 5.5), and adjusted to the desired value with KOH or HCl. Only the minimum amounts of acid or base should be used in adjusting the pH. The solution is poured into a volumetric flask and made up to volume. Concentrated stock solutions (e.g. .005 M) may be made up, from which other solutions may be mixed.

This method is simple and rapid, and may be used for a wide variety of materials which are active as auxins or

antiauxins. Yet there is one possible criticism, and that is the presence of salt or at least free K^+ ions in the auxin solution. Do they have an effect upon flowering? As in the problem of pH, a large number of experiments had been carried out by this author and other workers before the problem was investigated in a preliminary way. Plants were treated just before induction with KCl solutions, auxin prepared in the usual manner (containing a small amount of K^+), and auxin plus KCl. The concentrations used and the results are shown in Table 9. The results indicate that KCl alone has little or no effect upon the flowering of Xanthium, but that KCl in the presence of auxin does seem to inhibit flowering to a greater extent than the auxin alone. Although the results are only preliminary, they indicate that great care should be used in making up auxin solutions to control the KCl concentration.

The increased inhibition of auxin in the presence of K^+ would appear to be in line with the work of Cooil (1952) on K^+ stimulation of auxin induced growth in the Avena coleoptile. It is quite possible that the interaction between auxin and K^+ ions could be of fundamental interest in itself, and a study of the phenomenon might yield knowledge which could be applied to our understanding of the physiology and biochemistry of flowering.

Table 9

EFFECT OF K^+ IONS IN THE PRESENCE OF AUXIN UPON INHIBITION OF FLOWERING

Treatment	% Flowering	Floral Stage
No auxin or KCl	94%	4.7
10. x 10^{-4} M KCl pH 6.0	94%	4.1
3.0 x 10^{-4} M NAA pH 6.0	44%	1.0
KCl + NAA pH 6.0 (concentration as above)	18%	0.2

16 plants per treatment. Two 16 hour nights, #1, #2 and #3 leaves on each plant (app.12)

Treatment	% Flowering	Floral Stage
No auxin or KCl	100%	5.4
10. x 10^{-4} M KCl pH 7.0	100%	5.4
4.0 x 10^{-4} M NAA pH 7.0	40%	0.6
KCl + auxin	27%	0.5

15 plants per treatment, one 19 hour night, #3 leaf (app. 57)

Variation in Flowering Response of Auxin-Treated Plants

In all experiments involving the application of auxin, one may note a great variation in flowering response within the plants of a given treatment. This is best illustrated by the following experiment. Two groups of 100 plants each were given three inductive dark periods. One group was treated with auxin just before the first two inductive dark periods. After 9 days (no auxin) and 13 days (auxin treated) the apical buds of the plants were examined, and in Figure 16 the number of plants in each stage is shown. The non-auxin-treated plants responded with great uniformity. Almost all of the apical buds were in the same floral stage at the time of dissection. The auxin-treated plants, however, were very non-uniform in their response. Some plants were only slightly inhibited by auxin, while others remained completely vegetative.

This increase in variability of response is found in all experiments with added auxin. It is obviously important in the carrying out of experiments, and may well be of fundamental importance to our understanding of the role of auxin in the physiology of flowering. There are two possible explanations for the variation in response of auxin treated plants. (1) The variation may be due to the technique of application. If this is the case, plants within a given treatment, treated as nearly identical as possible, must receive total amounts of auxin very greatly different from one plant to another. This, in view of the techniques of auxin application used, seems at best unlikely, and is fairly

difficult to test. (2) It is also possible that the plants may differ genetically in their response to applied auxin. Xanthium plants in nature have been continuously selected for their response to night length, but have had no selection pressure with respect to their response to artificially applied auxin. The differences in response might be due to differences in amount of auxin which penetrates the cells, or to differences in the biochemical mechanisms through which the auxin inhibits floral induction in Xanthium. In order to establish whether variation in response to auxin is genetically controlled, a number of clones were produced by making cuttings of individual plants. From three to five cuttings were made from each plant. A number of the cuttings failed to root (auxin was not used in rooting, as it may have had an effect upon the subsequent flowering), and the plants which did survive were quite non-uniform in appearance. None the less, after the plants reached a suitable size, four groups were treated with auxin, one group was left as a control, and all were induced with two 14 hour nights. The results are shown in Table 10. The stage of each plant in each clone is indicated. It may be seen that some clones do appear to be highly auxin-sensitive or highly auxin-resistant, but that a large number of clones show considerable variation within the clone. No positive conclusions can be drawn from this experiment, but some support for the idea of genetic control of auxin variability seems to be evident.

Whatever the theoretical significance of the variation

Table 10

THE RESPONSE OF PLANTS WITHIN A CLONE TO INDUCTION AFTER AUXIN APPLICATION

Treatment	Clone Number	Floral Stages (showing no. plants) 0	1	2	3	4	5	6	7	8
No auxin	21							2	2	
	35						1		3	
	60								1	3
	70								2	3
	85						1	2	1	1
	89								4	1
	92								2	3
	95								4	
	97							1	2	2
	98							2	2	1
	99							1		3
Sprayed with 2.0×10^{-4} M NAA	32				2		1	1		
	41					1		1		
	68						2		1	
	75				1				1	3
	76					1	1	1		
	78							1	3	
	81									3
	83						2	1	2	
	96							1	1	2
	90					1	3	1		
	100								2	3
Dipped in 6.0×10^{-4} M NAA	33					2	2	1		
	24	1			2					
	26		1	1	1		1			
	29		2	1						
	34		1	1						
	38	4								
	39		2	2	1					
	67	2			3					
	72				1	1				

Plants induced with two 14 hour nights. (August 19 and 20, 1953) Defoliated as nearly as possible to the #4 leaf. Dissected August 27, 8 days after induction. Auxin applied before induction, both nights.

in flowering response of plants treated with auxin, it is obvious from the extent of the variation shown in Figure 19 that it is a major problem in conducting experiments. There is no known way to classify plants as to auxin sensitivity except by observation after treatment with auxin and induction. Since the variation caused by applied auxin is so great, it seems quite probable that the plants making up different treatments in an experiment, might differ significantly in their average sensitivity to applied auxin. This is born out by examination of the experiments in this thesis utilizing auxin. Experiments involving auxin treatment seldom yield uniform and consistent data, or data which may be plotted to yield smooth curves, (e.g. Figures 25, 32, 33, 38). On the other hand, experiments which do not involve auxin often give points which fall on or very near to a smooth curve (e.g. Figures 4, 5, and 8).

Two ways may be envisaged to overcome the experimental problem presented by variation in response due to applied auxin.

(1) If the genetic explanation for the variation is valid, plants could be selected according to auxin sensitivity. A population might be built up of plants homozygous for either sensitivity or resistance to auxin. This would require some time, however, and it seems possible that other characters which might eventually be of value to us in the study of the physiology of flowering might also be eliminated by the selection process. Often the exceptions in plant

behavior have provided information about the rules. Still, for certain purposes, this technique might be desirable.

(2) At present the only thing which can be done about the auxin-induced variability is to use large numbers of plants in each experimental treatment so that the mean values of the stage of development have greater statistical significance. Here one is limited by the number of plants available, size of the proposed experiment, etc., and it is usually impossible to use the same number of plants per treatment in all experiments. The author has found that twenty plants per treatment is ideal with regard to handling, etc. When the size of the experiment dictates that fewer plants per treatment and more treatments be used, the author has often used 16 plants per treatment. Certainly if less than ten plants are used in a treatment receiving auxin, the results have only qualitative significance.

Some Statistical Considerations

As mentioned above, plants which have not been treated with auxin show considerable uniformity of response if conditions are sufficiently well controlled (Figure 16). The stage system provides an excellent tool for the measurement of the variation among plants which all flower. Of the factors discussed above, two appear to be responsible for considerable variation if they are not controlled (leaf size, light intensity or time of year), while other factors are of slight or no importance (age of plant, number of buds,

temperature, green safelight, etc.).

Since it is often impossible to obtain a uniform leaf size and light intensity for all plants in an experiment, the next best procedure is to distribute the variation in leaf size and light intensity among the plants of any single treatment. A procedure for this has been described above for leaf size. Plants are sorted into three groups according to the size of the #3 leaf. Each treatment is then made up by choosing a certain number of plants from each of the three groups. For instance, each treatment might consist of 9 plants from the group having "young #3 leaves," 9 plants from the group having "old #3 leaves," and 2 plants from the group which did not fit into the arbitrary classification of leaf size described above (page 39), making a total of 20 plants per treatment. To insure adequate randomization of plants exposed to different growing conditions prior to induction, plants are chosen at random from each of these groups. To insure randomization of conditions for post-induction development, the plants are set out on the bench after induction with each treatment placed along diagonal rows of a block design. This system is easy to set up, and it is easy to find and cut down each treatment at the end of the experiment (much more so, than if the plants are simply placed out at random).

With this method of experimentation a certain amount of variation within each treatment is introduced, but the range of variation should be the same in all treatments. In the

more basic forms of statistical treatment, the variation within treatments yields the measure of the variation to be expected by chance between treatments. With the experimental procedure described here, this is not justified. Such a calculation would indicate that the mean is less significant than it actually is. If the variation within a treatment can be understood and controlled on the basis of environmental factors, leaf size, etc., this variation is not due to chance and is not an indication of the deviation of the mean due to chance. The variation of the means due to chance must be studied in experiments designed to study it, as in Table 11 below.

The situation becomes complicated, however, when the variation within a treatment is not understood and controlled, as is the case with applied auxin (or threshhold induction). Again experiments must be designed to study this variation (Figure 16).

To determine the variability between treatments, five groups of 20 plants each were prepared as described above and induced with a single 16 hour dark period. After induction they were placed out on the greenhouse bench as described above. This procedure was followed in virtually all experiments reported in this thesis. The condition of the apical buds 10 days after induction is shown in Table 11 and Figure 17. It may be seen that the agreement between treatments is excellent. The variation within plants of a given leaf size may be due to light intensity variation before and

Table 11

AGREEMENT BETWEEN GROUPS OF PLANTS

INDUCED IN THE SAME MANNER

	Group Number				
	1	2	3	4	5
Floral Stage	6.15	6.16	6.10	6.10	6.20

Plants prepared as described in text, defoliated to #3 leaf, induced with a single 16 hour dark period (July 13, 1954), dissected after 10 days, twenty plants in each group. (See Figure 17)

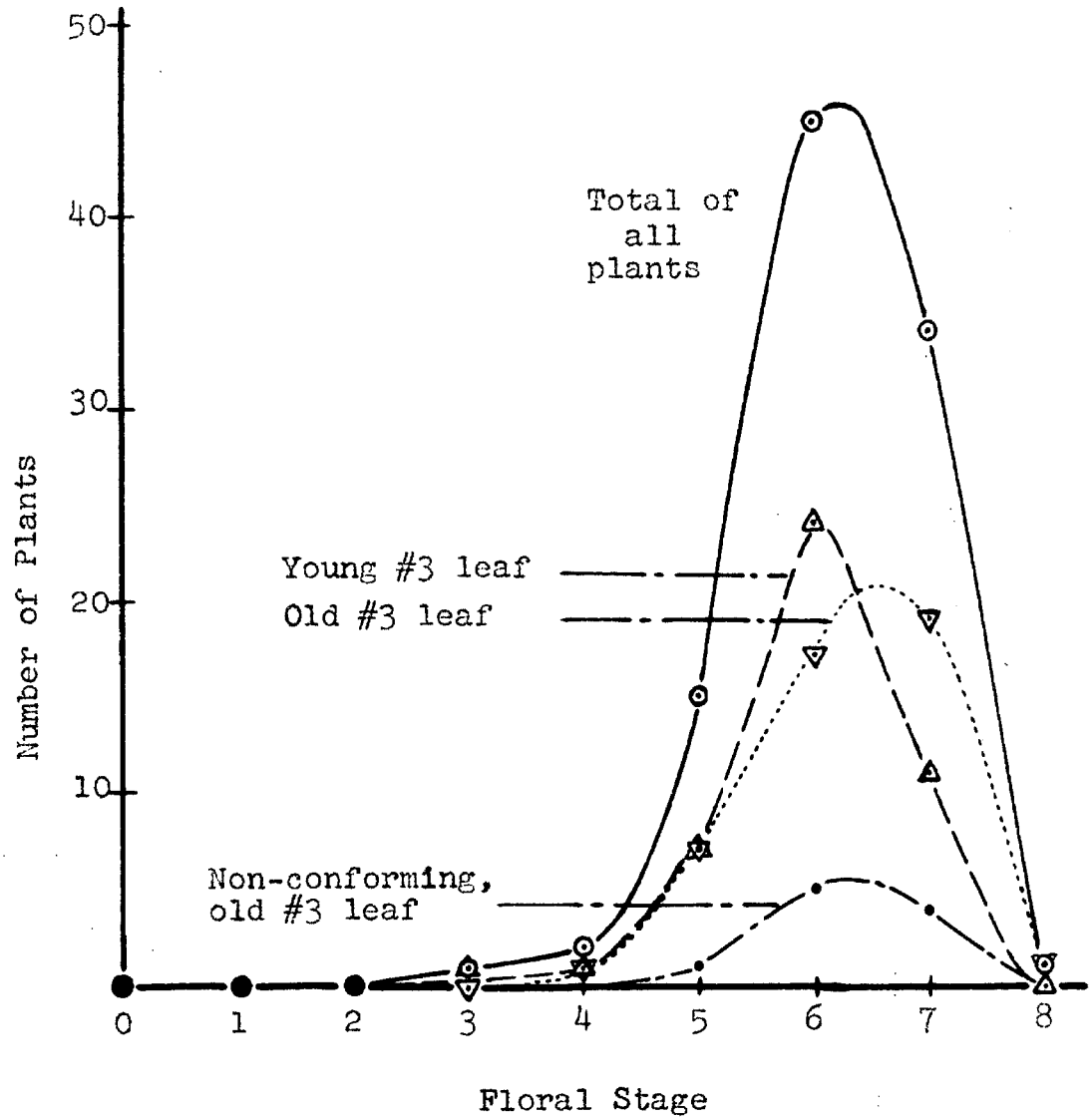

Figure 17. The variation in floral stage obtained when 100 plants are induced with a 10 hour dark period, following the standard procedure. These plants were divided into groups of twenty, each group being chosen at random as explained in the text. The floral stage of each group is shown in Table 11.

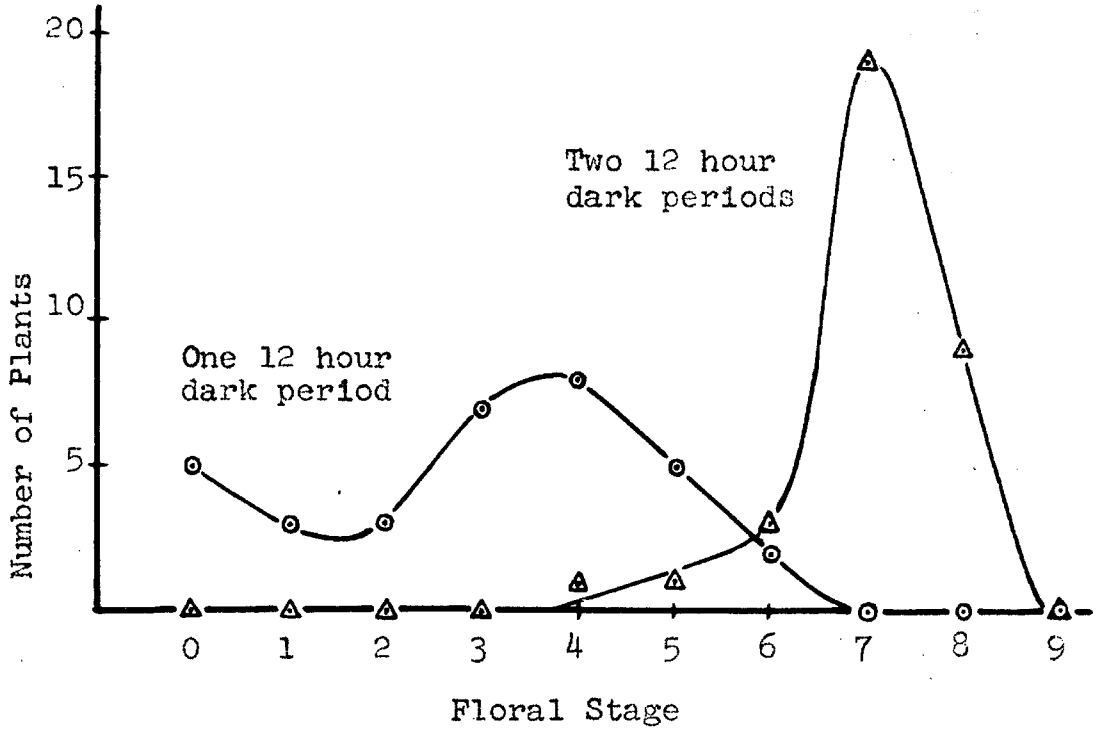

Figure 18. The relation between degree of induction and variability in floral stage. As induction increases, variability decreases (app.15). See also Figure 16--three inductive cycles.

after treatment, but this variation is apparently almost the same in all treatments, as described above. In this experiment the #3 leaf was used, and the average response for old and young leaf was very nearly the same (see Figure 8). With older of younger leaves (#2, #4, #5, etc.), the separation into old and young leaves is more important.

Another factor affecting the degree of variation is the amount of induction. This is illustrated by Figure 18, showing the condition of buds ten days after induction when plants were given one or two inductive cycles. A less sensitive leaf was used and a fairly short night (12 hours), so that the induction for a single dark period is nearer to the threshhold condition. It may be seen that the amount of variation within a treatment decreases as the amount of induction increases. The amount of induction, however, is a factor which is not usually controlled in the normal sense, but is an intricate part of the particular experiment.

Outline of the Procedure for Experimentation with Xanthium

As a summary of this chapter, the complete procedure for experimentation with Xanthium will be presented in outline form:

I. Method of Growing Plants

1. Grown from seed
2. Fertile soil with occasional addition of nitrogen
3. Maintained vegetative with supplementary light

II. Preparation for Experimental Treatment

1. Use as many plants per treatment as possible (20 plants)
2. Plants are sorted according to the size of the #3 leaf
3. Plants are trimmed to a single leaf. The leaf chosen depends upon the sensitivity desired. All extra buds are removed.
4. Plants are chosen from sections of each group (various leaf sizes) to make up each treatment.

III. Experimental Treatments

1. Light intensity before and after induction is controlled or recorded (time of year is recorded)
2. Temperature during the inductive dark period is controlled to within ca. 2^{o} C., but may vary within wide limits both before and after induction.
3. A green safe-light may be used for treatments during the dark period
4. Experiments involving auxin:

    a. Water controls are usually not necessary
    b. Auxin must be carefully prepared:
        pH must be controlled
        Salt must be controlled
        Detergent is added to aid wetting
    c. Method of application depends upon particular experiment

IV. After induction plants are set out on bench so that each treatment is subject to the same variation in environmental conditions.

V. Number of days between induction and dissection of the buds is determined by the rate of development of the fastest growing buds

1. Some extra plants are induced with the most optimal induction of any treatment in the experiment, and a few of these are dissected at intervals after induction to determine the rate of development of the buds.
2. To compare experiments dissected at different times after induction, stages may be adjusted to any given number of days after induction

VI. Plants are dissected and the buds classified into the floral stages described.

From the variables described in the above sections, it is clear that various factors must be recorded along with the experimental results, in order to evaluate these results properly. In this thesis the results are shown graphically or in tables, and the important variables, as well as the experimental procedures, are given either with the results or in the appendix beginning on page 181. The data in the appendix are referred to by numbers in parentheses in the figures and tables.

In addition to the results themselves, the following factors are important:

1. The general set-up of the experiment
   Concentration of chemicals, etc.
   Times of treatment
2. If auxin is used:
   Method of application
   pH of the solution
3. The date, and any unusual weather conditions.
4. The number of plants in each treatment.
5. The number of the leaf or leaves left on the plant during induction
6. Number of days between induction and dissection of apical buds
7. The following factors need not be mentioned unless some deviation is made in the standard procedure:
   Temperature during induction
   Age of the plant
   Number of buds
   Use of green light

Chapter II

THE DUAL ROLE OF AUXIN IN FLOWERING, WITH SPECIAL REFERENCE TO A PROMOTIVE EFFECT

Introduction

As discussed in the thesis introduction, auxin applied to short day plants ordinarily inhibits induction. There are instances, however, in which applied auxin promotes flowering, as with the pineapple, the litchi, some long-day plants, and with various plants to which auxin is applied in low concentrations and at cold temperatures (page 22). In this chapter experiments will be presented which show that auxin applied to Xanthium inhibits the act of induction if applied before induction has been consummated, but that auxin applied after florigen has been formed and translocated out of the leaf, promotes bud development (promotes the induced state).

Experimental

This dual role of auxin is best illustrated by experiments in which auxin is applied at various times during and following an inductive dark period. Groups of plants were treated at various times either by dipping the leaves in auxin solution (10^{-3} M NAA) or by applying 0.5% NAA in lanolin to the stem near the tip. The time of translocation of

the flowering stimulus out of the leaf was measured by defoliating plants at various times after induction, as explained in Chapter I (page 46). The results of such an experiment are shown in Figure 19. It may be seen that auxin applied during or for some time after the inductive dark period inhibits flowering. This is the inhibitory effect discussed above.

Figure 19 also shows that auxin applied after florigen has been translocated out of the leaf promotes the development of the floral bud slightly. Although the differences between controls and auxin treated plants are small, they are statistically significant (page 80), and have been observed in ten separate experiments. It is interesting to note that the inhibition and promotion of flowering were obtained whether the auxin was applied to the leaf or to the tip of the plant. Epinastic bending of the leaf petioles was observed in both instances, indicating that the auxin had been translocated throughout the plant (probably due to the fairly high concentrations). The fact that the effects may be observed regardless of the point of auxin application, indicates that auxin concentration gradients are not responsible for the results.

This promotive effect of auxin may only be demonstrated clearly when light conditions during development of the bud are suboptimal. Thus winter experiments show the effect more strongly than summer ones. That light intensity is the factor involved may be seen by following the floral development of plants treated or not treated with auxin two days after

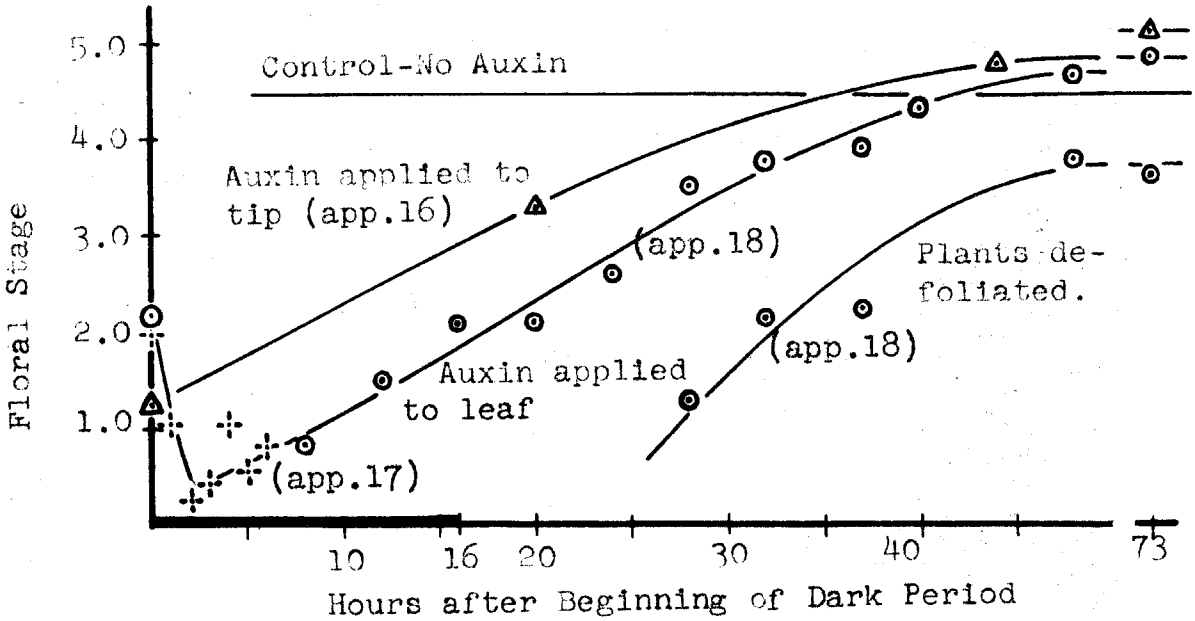

Figure 19. The dual role of auxin in the flowering as demonstrated by time of application. Three experiments are combined. Details of treatments given in the text and appendix.

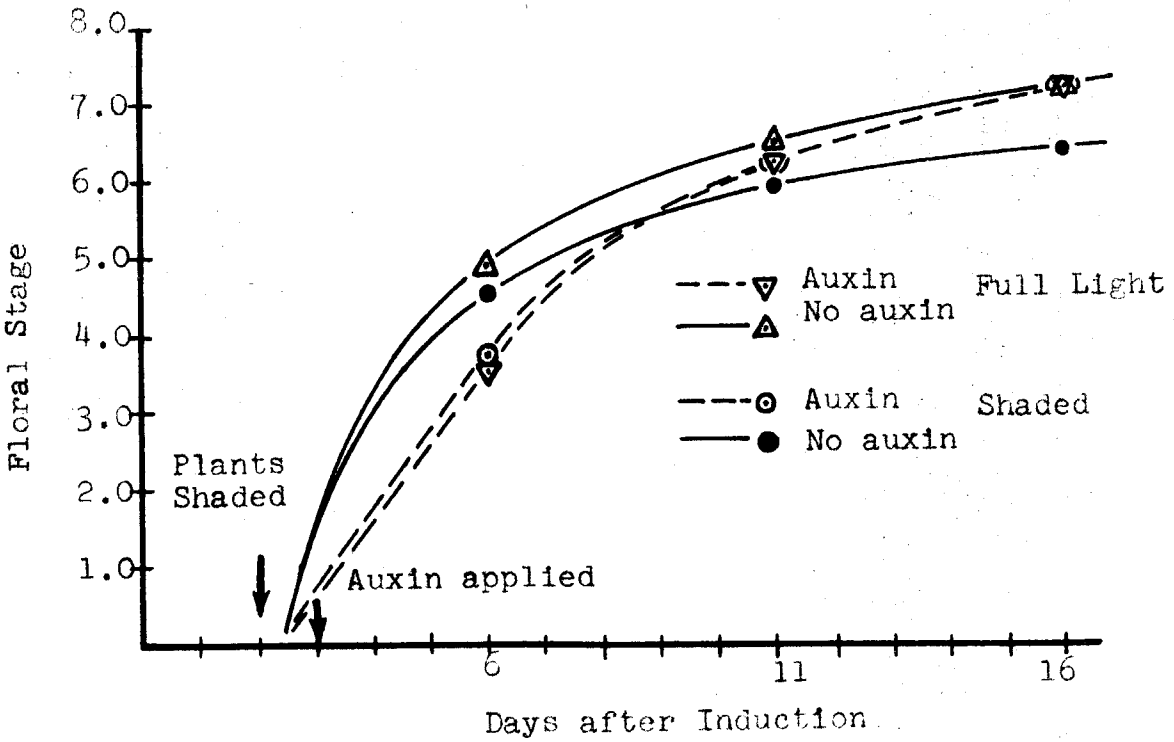

Figure 20. The effect of auxin in overcoming the inhibition in rate of bud development, caused by low intensity light, as discussed in the text (app.19).

induction and grown under high or low light intensity. The results of such an experiment are presented in Figure 20, in which stage is plotted as a function of number of days after induction. The rate of development of non-auxin-treated plants dropped off faster in the plants which were shaded than those which were not (a number of cloudy days resulted in slightly slower development than normal in all plants). The buds of the auxin-treated, shaded plants, however, after a short initial lag, continued to develop at a rapid rate. Similar results were also obtained when auxin was applied to the tip rather than the leaves, and also when two inductive cycles were used instead of one. These results may be interpreted on the assumption that lowered light intensity leads to a lowered auxin concentration within the plant, and that this deficit may be made up by the application of auxin. It has indeed been reported that auxin concentration in the plant is lower under short-day (Cooke) or low intensity light (Resende, 1950, 1952).

It may be demonstrated that the inhibitory effect of auxin on induction occurs only in the leaf, but that the promotion of floral development by applied auxin occurs independently of the leaf. Groups of plants were defoliated at various times after induction. Half the plants of each group were treated by dipping the tips in auxin solution immediately <u>after</u> defoliation. After an appropriate time the tips were examined, and the results are shown in Figure 21 (two experi-

ments). The floral stage of the auxin treated plants was greater than that of the controls, regardless of the time of treatment. Thus the promotive effect of applied auxin is directly upon the bud. Since auxin applied before translocation of florigen from the leaf is complete is inhibitory in the presence of the leaf (Figure 19), and since auxin applied at this time gave no inhibition in the absence of the leaf, it may be concluded that the inhibitory effect of applied auxin takes place only in the leaf.

It has been reported that an inductive dark period is ineffective in the absence of one or more actively growing buds (Carr, 1953; Lincoln, 1953). This has been interpreted by Carr as an indication that the flowering substance (referred to by him, after Gregory (1948), as a hormone precursor) is not stable until it has been "fixed" by the growing point. Carr suggests that if no active bud is present, the hormone becomes dissipated. It has recently been shown that the presence of very young leaves will replace the requirement for an actively growing bud (Lincoln, 1954). It has also been shown that young Xanthium leaves are effective in the inhibition of lateral buds, from which one may infer that these young leaves produce considerable auxin (Hamner and Bonner, 1938). In view of these facts it seemed possible that the lack of an actively growing bud or very young leaves would lead to a low auxin concentration within the plant, and that this might result in a failure of development of floral buds. Thus it appeared possible that the dependence of induction on an actively grow-

ing bud or very young leaves might reflect the dependence of bud development on auxin. This possibility was investigated by the following experiments.

That active buds are essential to induction under the present conditions was first established by experiments in which tips were removed from groups of test plants on successive days before and after an inductive dark period. Either an axillary bud or a cotyledonary bud was left on each plant. The results are shown in Figure 22 in which the percent of the remaining axillary buds which flowered is plotted against time at which the tip was removed. If the tips were cut off two or three days before induction, the axillary bud apparently became active in time to receive the flowering stimulus and differentiate into a flower bud. If the tips were removed just before or for a number of days after induction, the degree of flowering was greatly reduced. Even after the tip has begun to differentiate (two or three days after induction--Figure 4), if it is removed, the remaining dormant bud fails to flower when it becomes active.

To see if auxin can replace the requirement for active buds during induction, tips were removed one day before induction, and auxin in lanolin was applied to the scar on successive days thereafter. Except for one leaf and its axillary bud, all leaves and buds were removed from the plant at the beginning of the experiment. The tip together with part of the one remaining axillary bud was removed as shown in Figure 23. This was to insure that the only meristematic tissue

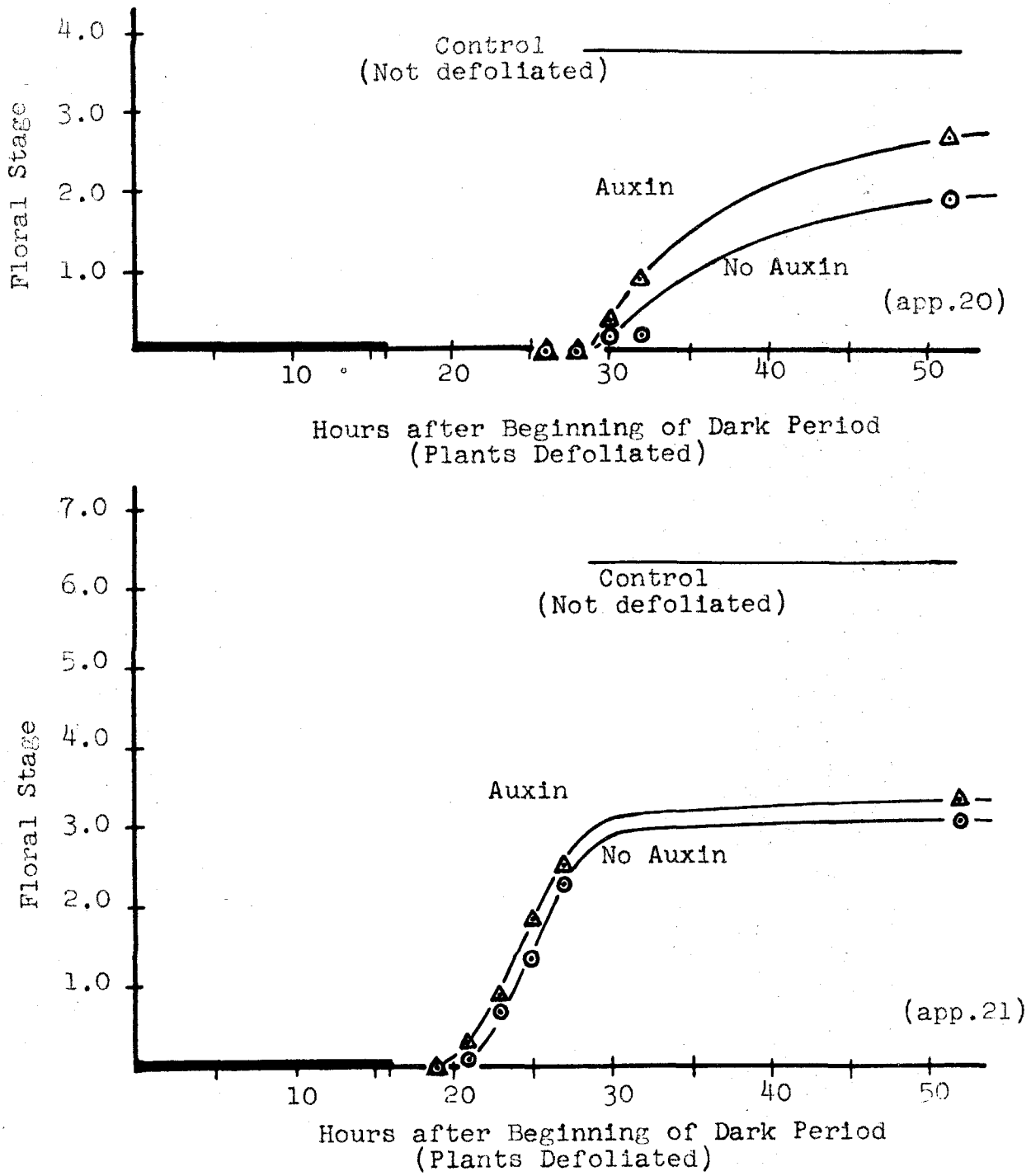

Figure 21. The promotive effect of auxin upon floral bud development, if applied to the stem and tip after removal of the leaf.

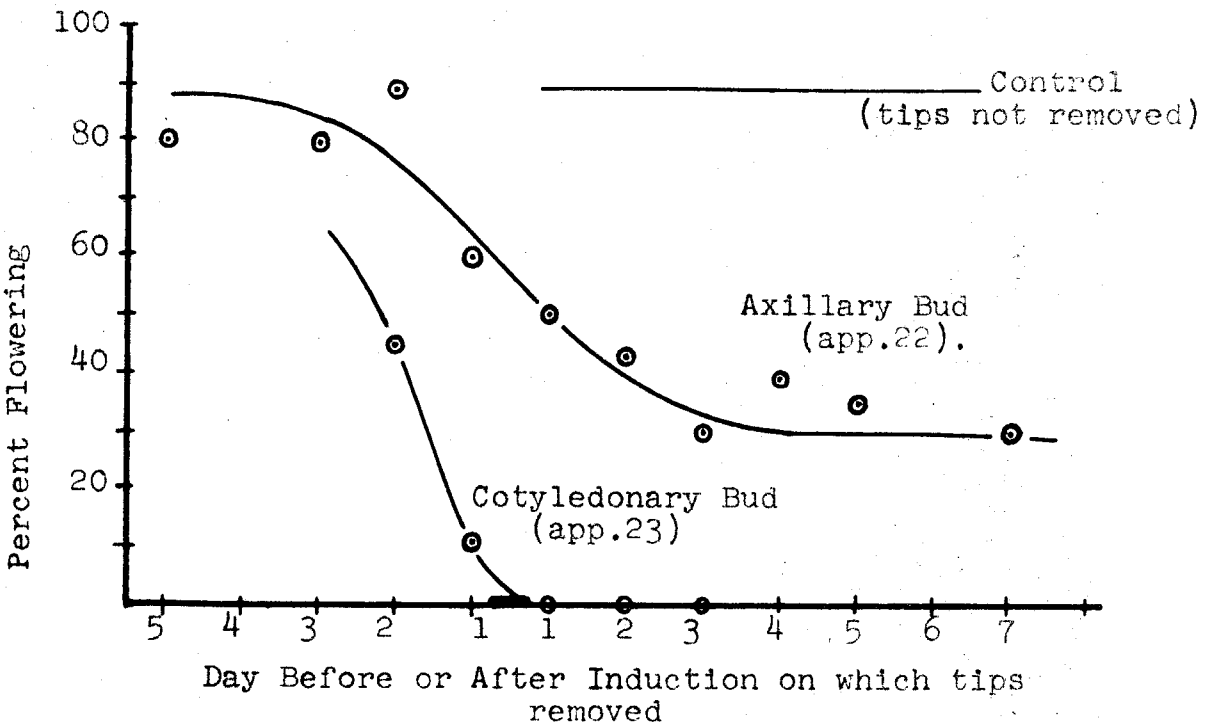

Figure 22. Active buds must be present just before or after inductive treatment in order for flowering to result.

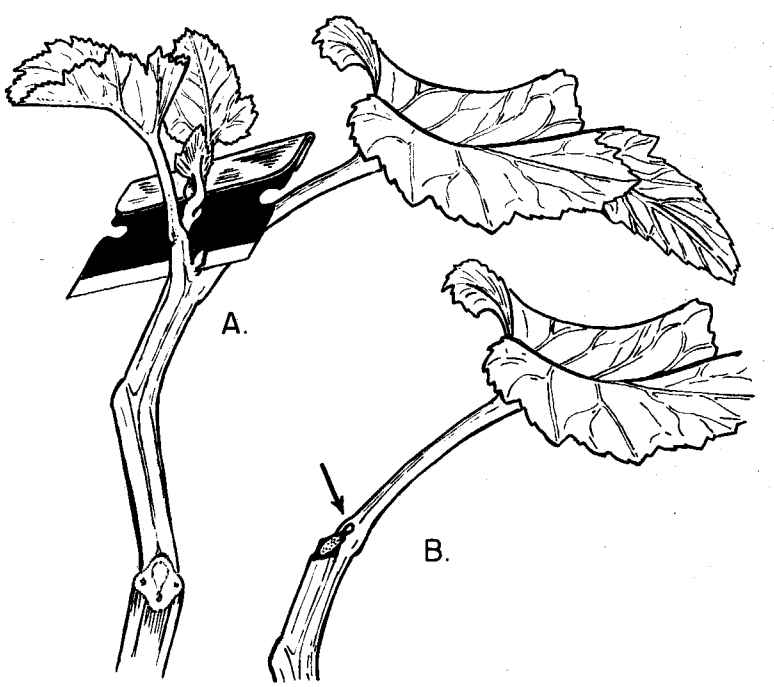

Figure 23. Method of removal of the tip and part of the axillary bud. Arrow indicates remaining meristematic tissue, which will become the growing point.

(later to become the growing point) on the plant was in a completely dormant condition at the time of induction.* The results of this experiment are shown in Figure 24. Forty-five percent of the plants whose tips were removed but which received no auxin flowered. Plants which received auxin just before induction were inhibited in their flowering, as is normally the case. Plants which received auxin two and three days after induction, however, flowered almost as well as controls from which the tips had not been removed. Thus auxin can replace the requirement for active buds in the induction of Xanthium. As shown in Figure 24, there is some indication from the experiments conducted to date, that auxin is not effective more than about six days after induction, indicating, probably, that the flowering hormone has been dissipated in this period of time. It was evident, however, that the meristematic tissue on some of the plants became active within six days, which may account for the flowering which did occur in some of the non-auxin-treated control plants. Experiments demonstrating the replacement of the requirement for active buds with auxin have been repeated three times.

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*Even this method is not entirely satisfactory. In one experiment conducted in the middle of summer, the buds became active within three or four days after the tips were removed, and all plants flowered regardless of the time the tips were removed. In another experiment, the axillary buds had begun to become active at the time the tips were removed, and, in many plants, insufficient meristematic tissue remained to produce new growing points.

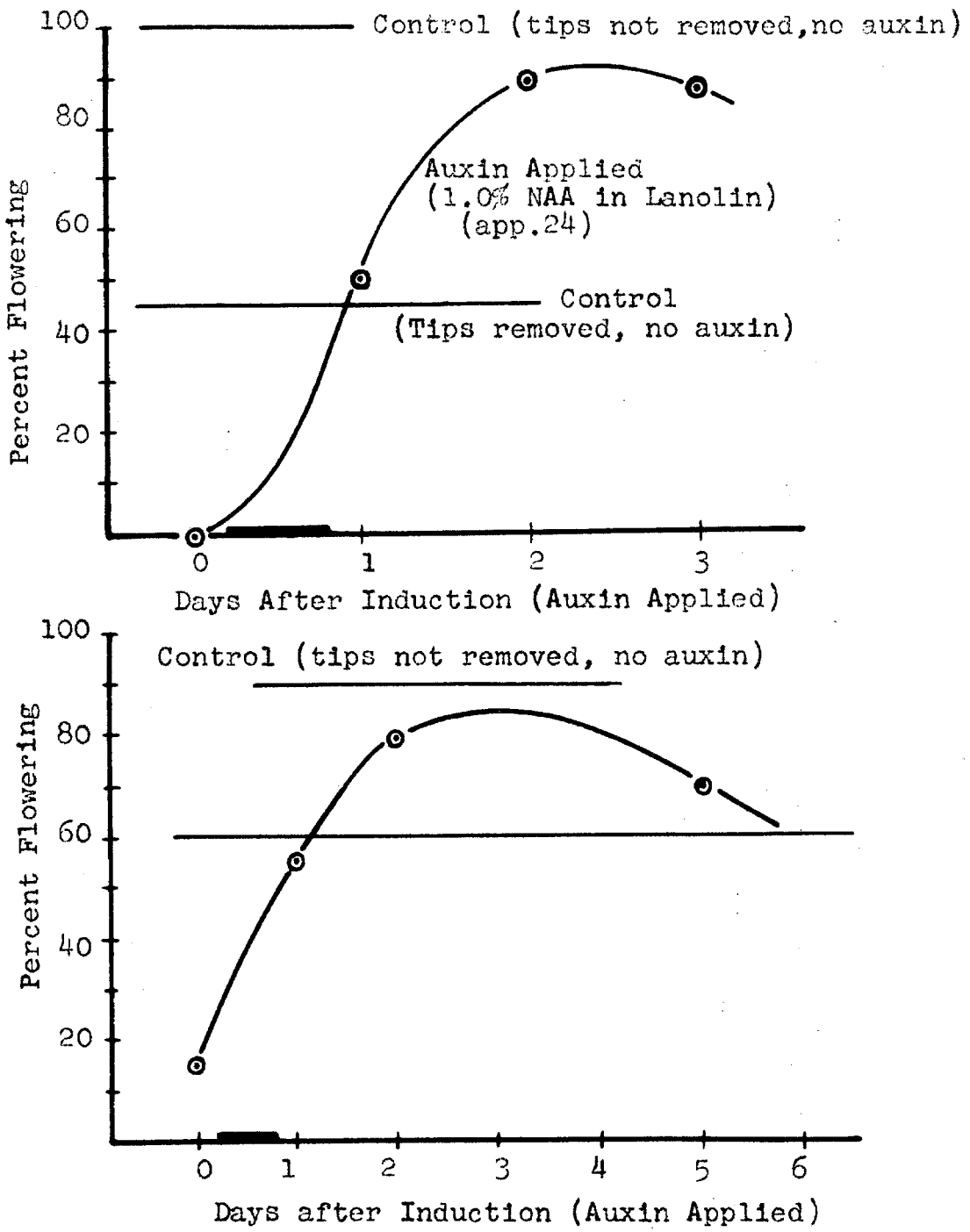

Figure 24. The replacement of the requirement for active buds with applied auxin. Tips (active buds) all removed day before induction, auxin applied on days shown. Auxin applied two days or more after induction to plants having only inactive buds, insures that these buds will flower when they become active. Lower curve suggests that this effect may only be obtained up to five or six days after induction.

Discussion

The promotive effect of auxin on floral development, described in this paper, may aid in the interpretation of some experiments of other investigators which show a promotive effect of auxin upon flowering.

Bonner (1949) and Liverman (1952) were able to obtain formation of flower-like buds in Xanthium by the application of antiauxins, but the buds failed to develop unless they were subsequently supplied with auxin. Since development would only take place after the auxin level had been raised, it would appear to be an example of the phenomenon outlined above.

Leopold and Guernsey (1953 a, b, and c) have reported a promotion of flowering by low concentrations of auxin applied at low temperatures. Auxin concentrations which are too high bring about inhibition even at low temperatures. They were able to obtain this effect by soaking the seeds of long-day, short-day, and day-neutral plants in auxin solutions in the cold, and also by applying low concentrations of auxin to the leaves of soybean (a short-day plant) kept on short-day at low temperature. The explanation for these results may be that low temperature prevents auxin from being effective in inhibiting the act of induction, but that there is an increase in the general level of auxin within the plant so that subsequent bud development is accelerated, as described above.

The experiments showing the requirement for active buds and its replacement by applied auxin are of interest to the understanding of induction, and will be discussed in more detail in this relation in the next Chapter. It may be noted here, however, that the curves showing the effective time of bud removal indicate that active buds are not actually required during the act of induction, but must be present only to receive the flowering stimulus before it becomes dissipated. Thus the requirement for active buds (or auxin) is probably related to the induced state rather than to the act of induction.

Chapter III

THE NATURE OF THE INDUCED STATE

Introduction

Some deductions about the nature of the induced state, or the nature of induction, may be drawn from the experimental results of Chapters I and II coupled with results of earlier workers. The facts pertinent to this discussion are summarized in the following section.

Pertinent Experiments

1. Rate of Bud Development as Measured by the Stage System. Stage of floral bud development is proportional to time beginning about two and one half days after induction. The slope of the stage-time curve gives the rate of bud development in stages per day. (Page 30, Figure 4.)

2. Variation in Degree of Induction. When only one or two inductive cycles are given to the plant the initial rate of bud development is dependent upon the length of the inductive dark period. (Page 35, Figure 5.) It is also evident from the experiments in which plants are defoliated at different times that different degrees of induction are possible. If only a small amount of stimulus has been translocated out of the leaf at the time of defoliation

(soon after induction) the rate of floral development is low. If larger amounts of stimulus have been translocated out of the leaf (long after induction) more rapid floral development takes place. (Page 86, Figure 19.) Many other experiments also illustrate the fact that different degrees of induction are possible (e.g. Figure 35). That the differences in rate of development due to different degrees of induction continue until ripe fruits are produced, is shown by the work of Frances Naylor (1941).

3. Number of Buds on the Plant. The number of buds on the plant influences the rate of development of the terminal bud only slightly and the number of developing buds times the average stage of all buds is not a constant. (Page 51, Table 5.) The implications of this fact are discussed below.

4. Conditions during development of Buds. Exposure of the plant to low intensity light some days after induction slows the rate of development of the buds. (Page 87, Figure 20.) An inductive dark period given some days after initial induction increases the rate of development of the buds (Roberts, 1951). Thus degree of induction may be changed in either direction by proper treatment.

5. Effect of Auxin. Applied auxin inhibits the act of induction, but promotes development in the induced state. Flowering is inhibited if auxin is applied before translocation of the flowering stimulus out of the leaf is complete, but floral development is promoted if auxin is applied after

this time. (Page 87, Figure 19.)

6. Active Buds. If all buds which are actively growing are removed from the plant virtually any time after or just before induction, the dormant buds, when they become active, will be vegetative. This is true even if the active buds are actually flower primordia at the time they are removed. (Page 93, Figure 22.)

7. Auxin and Active Buds. The requirement for active buds can be replaced by applied auxin. It appears that florigen is not only unable to act in the absence of auxin but is also unstable. The length of time that florigen is stable is measured by these experiments. (Page 95, Figure 24.)

8. Translocation out of the Leaf. Defoliation experiments show that the flowering stimulus is translocated out of the leaf some hours after the end of the inductive dark period. (Page 86, Figure 19.)

9. Leaf Grafts. Long (1939) has shown that a leaf from an induced plant, when grafted onto a vegetative plant, will cause it to flower.

10. Grafts through a Number of Generations. It has been shown that florigen may be transmitted through a number of graft generations without any apparent dimunition in activity (Bonner and Liverman, 1953).

Discussion

Induction as defined here (page 12) is the ability of certain plants to continue to develop and produce flowers once they have been exposed to the suitable environmental stimulus, even though the environment under which the development and production of flowers takes place is not of the type which may induce flowering. This definition is the one used by most authors (e.g. Lang, 1952; Bonner and Liverman, 1953), including the earliest known to the present author (Lubimenko and Sceglova, 1931). This definition makes no statement as to the quantitative nature of induction. Yet questions have arisen regarding this point, both from the all or none aspect of flower initiation and from deductions which have been made regarding the induced state.

To some it seems obvious that induction is an all or none process, but this is probably more apparent than real. Certainly the processes which take place during flower initiation are irreversible. The developing flower bud cannot shrink back to a vegetative growing point. Nor is it possible to think of tissue which, at least theoretically, could not be classified either as flowering or vegetative. In this respect, there is no half-way condition. Thus the all or none aspect of flower initiation refers to the differentiation of the bud, rather than to induction itself. A few examples illustrate the fact that the induced state may be reversed completely.

If all the active flowering buds are removed from a plant (Xanthium) a few days after induction, the dormant buds, when they become active, are vegetative (Point 6, above). The author has cut off stems of Xanthium just above the cotyledonary buds, and although the stems possessed macroscopic flowers, the new buds when they became active, were vegetative. Perennials, of course, lose their induction each season.

Cestrum, the night-blooming jasmine, for instance, requires long-day conditions to become induced, but the buds only develop if the plant is exposed to two or more short days. The plant will remain in the induced condition indefinitely until exposure to short day initiates development, but after the fruits mature, the plant must be again induced before flower formation will take place.(Sachs).

Instances are also known where the induced state may change even during flower development. The present author and others (Lincoln, 1954) have observed cases in Xanthium where the average floral stage seems to decrease with time under particularly poor environmental conditions. This is apparently because the individual flower primordia of the inflorescence primordium continue their development as leaves rather than as flowers, under adverse conditions. In extreme instances the tip of the inflorescence primordium may continue its development as a vegetative growing point, without the actual differentiation of flower parts.

It is a striking characteristic of the floral induction

process, that it is in a sense all or none, that the plants either flower or they do not flower. Induction as viewed in this qualitative way might be accounted for by the following general sort of hypothesis. We might imagine that the production of florigen (by the act of induction) trips a mechanism so that florigen is produced autocatalytically within the plant. The autocatalytic process should then continue to build up the florigen concentration to a maximum level, and all buds should develop at the same (maximum) rate. If the mechanisms of induction were simply autocatalytic, plants should either flower maximally, or remain vegetative, depending on whether or not inductive treatment has crossed the necessary threshold.

Obviously, however, induction is not so simple. Different quantitative degrees of induction of Xanthium may be attained (points 1 and 2, above). The induced state is a quantitative one in many other plants as well, and is often measured by number of flowers, days until visible flowers, length of the stalk, nodes to flower, etc. (not all of these criteria, however, give an unambiguous measure of the degree of induction).

Defoliation experiments strongly indicate that different rates of floral development in Xanthium may be caused by different amounts of florigen reaching the growing point. (Points 2 and 8, above.) Hence it is quite logical to assume that the different rates of floral development resulting from different inductive treatments, are also caused by

different amounts of florigen being produced by these treatments. For instance, the increased rate of development caused by longer dark periods may be understood on the basis that longer dark periods result in more hormone formation.

Increasing induction (i.e. longer dark periods or more inductive cycles) apparently results in a build-up in concentration of florigen within the leaf, while defoliation experiments measure the kinetics of the build up in concentration of florigen outside of the leaf. Leaf grafting experiments indicate that the concentration of hormone in the leaf is maintained even after it has been built up in the rest of the plant. (Point 10, above.)

If florigen were consumed by the developing buds, one would expect the rate of development of each bud to decrease as the number of buds on the plant increases. The average stage multiplied by the number of buds, should be at least roughly constant (assuming that the floral stages as defined are directly proportional to amount of florigen--a fairly reasonable assumption on the basis of night length experiments--Figure 5). Since this is not the case--the number of buds affects only slightly the stage of the terminal bud (Point 3, above)--it is suggested that the rate of floral development is determined by the concentration of florigen within the plant, rather than by the total amount. The slight extent to which an increasing number of buds decreases the stage of the terminal bud, may be due to one or both of

the following factors. (1) The competition between the developing buds for nutritional factors may slow the development of each somewhat, as the number of buds increases. (2) Some florigen may be consumed by developing buds.

The constant rate of development under ideal conditions indicates that after the initial induction, the concentration of florigen within the plant is maintained constant. This is also indicated by grafting experiments. Thus it appears that the induced state consists in a maintainance of a given concentration of florigen within the plant, once it has been established by the act of induction. This concentration may decrease under poor environmental conditions, or it may be increased by further inductive treatment. (Point 4, above.)

The induced state is then what might be termed a homeostatic one. The common autocatalytic systems encountered in chemical kinetics do not result in the concentration of a substance being maintained at the original level independent of this level. One might imagine a virus, reproducing itself autocatalytically within an organism, but limited in its maximum concentration by the availability of substrates, the counter reactions of the organism, etc. Under conditions of maximal induction (continuous inductive cycles), factors comparable to those controlling this hypothetical virus may control and limit the rate of floral development, and perhaps the rate of florigen synthesis. But under conditions which result in different degrees of induction or rates of floral development, the concentration at which

florigen is maintained is determined by the original concentration of florigen. It is this characteristic of induction, which is comparable to homeostasis.

One explanation for the self regulation of the inductive system might lie in the possibility that certain cells are capable of producing florigen, and that different numbers of cells capable of florigen production are produced by different inductive treatments. As the concentration of florigen increases within the plant, due to the act of induction, more and more cells become capable of producing florigen. As the plant grows, a certain proportion of the new cells become florigen producers, the number again depending upon the florigen concentration in the plant. This hypothesis has the drawback that a number of unfounded assumptions must be made about the nature of the particular cells involved. Unless this is done, this system also has the kinetics of a typical autocatalytic reaction. At present there is no evidence for any special florigen producing cells. It is highly probable, however, that suitable experiments could be devised to test some of the basic assumptions (e.g. a cytological study of induced and non-induced plants.)

Two homeostatic systems might prove to be closely analogous to the induced state.

(1) Although many molecules within a cell may vary in concentration in response to various stimuli, the genes remain for most of the time at a certain number per cell, and at a nearly constant concentration in relation to the

cell protoplasm. At about the time that the cell divides and begins to double in volume, the genes double in number. Under some conditions (those resulting in polyploidy) the genes increase in number per cell. Perhaps this is analogous to the act of induction which increases the concentration of florigen.

(2) It has been shown by Ephrussi (1953) that the number of mitochondria per cell in yeast (the concentration), may be influenced by various factors, and that after the concentration has been changed by appropriate treatment, it is maintained at the adjusted level throughout subsequent generations of the yeast. This is closely analogous to the system described above for the induced state in Xanthium.

If these analogies are correct, one might ask whether or not florigen resembles the nucleoprotein containing particles of the cell (the genes and the mitochondria). Does its gene-or mitachondrial-like biochemistry account for the difficulty in extraction and for its virus-like properties? Is our lack of knowledge of this biochemistry responsible for our inability to understand quantitative induction? Does florigen affect morphogenesis in a manner similiar to the gene?

At least two things have been learned about the state of induction from the experiments with auxin. First, the condition of induction is different in its chemistry from the act of induction, since applied auxin promotes the induced state but inhibits the act of induction. Thus while both the act

and the condition may consist in a synthesis of florigen, the act requires certain environmental conditions and is inhibited by auxin, while the condition is affected to a lesser degree by the environment and is promoted by applied auxin. (Point 5, above.)

Second, not only is floral development promoted by auxin, but the induced state seems to require auxin. Thus florigen is dissipated in the absence of actively growing buds, but applied auxin will prevent this dissipation. (Points 6 and 7, above.) Perhaps in the induced state florigen is normally in dynamic equilibrium within the plant, being synthesized and destroyed at equal rates. Auxin may be required for the synthetic process. Another possibility is that auxin, applied to induced plants from which the active buds have been removed, causes the dormant buds to differentiate immediately, before the hormone can be dissipated. This, however does not seem likely, as applied auxin tends to delay the bud from becoming active, rather than causing differentiation to occur earlier. Further experimentation is required to learn whether auxin promotes the synthesis of florigen in the induced state, or whether florigen is unable to act upon the bud except in the presence of sufficient auxin.

To summarize: The fact of quantitative induction may be understood on the basis that the act of induction results in the formation of varying concentrations of flowering hormone, and that the concentration thus attained is

automatically maintained within the plant. Florigen is able to act efficiently only in the presence of an adequate supply of auxin, and lack of auxin leads to depletion of florigen already present.

Chapter IV

THE INHIBITORY EFFECT OF AUXIN AND THE LOW INTENSITY LIGHT PROCESS

Introduction

The basic facts regarding the effects of applied auxin on flowering have been summarized in the introduction (page 21). Several kinds of experiments with auxin have been presented in Chapter I (Figures 15, 16, Tables 8, 9). Chapter II deals with the dual role of applied auxin in flowering of Xanthium, and discusses an auxin promotion of floral bud development and the ability of auxin to replace the requirement for active buds in the induction of Xanthium. It was also pointed out that auxin applied before translocation of the stimulus out of the leaf inhibits the inductive process, probably by inhibiting synthesis of the flowering hormone. It was shown that this inhibition occurs exclusively in the leaf. Thus it appears that auxin applied before translocation of florigen out of the leaf is complete directly affects the act of induction. It is of importance to study this inhibitory effect of auxin, since such a study may lead to a better understanding of the dark process. The investigations presented in this thesis are of five kinds:

1. Concentration Relations of Applied Auxin.
2. Relations of Applied Auxin to Length of the Dark Period.
3. The Time of Auxin Application.
4. The Relation of Applied Auxin to Red Light Interruption of the Dark Period.
5. Application of Cobalt.

1. Concentration Relations of Applied Auxin. The earliest work on the effect of auxin on flowering included studies on the concentration relations of applied auxin and inhibition of flowering (e.g. Leopold and Thimann, 1949; Bonner and Thurlow, 1949). Many of the present experiments also included the effect of auxin concentration upon inhibition of induction. The results of a number of these experiments are summarized in Figure 25, in which the data are plotted as floral stage vs. auxin concentration. It may be seen from the figure, that the application of auxin under various conditions of night length, number of inductive cycles, leaf size, method of application, etc., yields concentration curves of the same general shape, but differing somewhat in level of maximum inhibition, and in concentration effective in inhibiting 50% etc.

The auxin concentration curves are all of the saturation (hyperbolic) form. This is best seen by plotting the auxin concentration against the number of stages inhibited by auxin application, as in Figure 26. It is evident that the shape of this curve is similar to that obtained in studying the con-

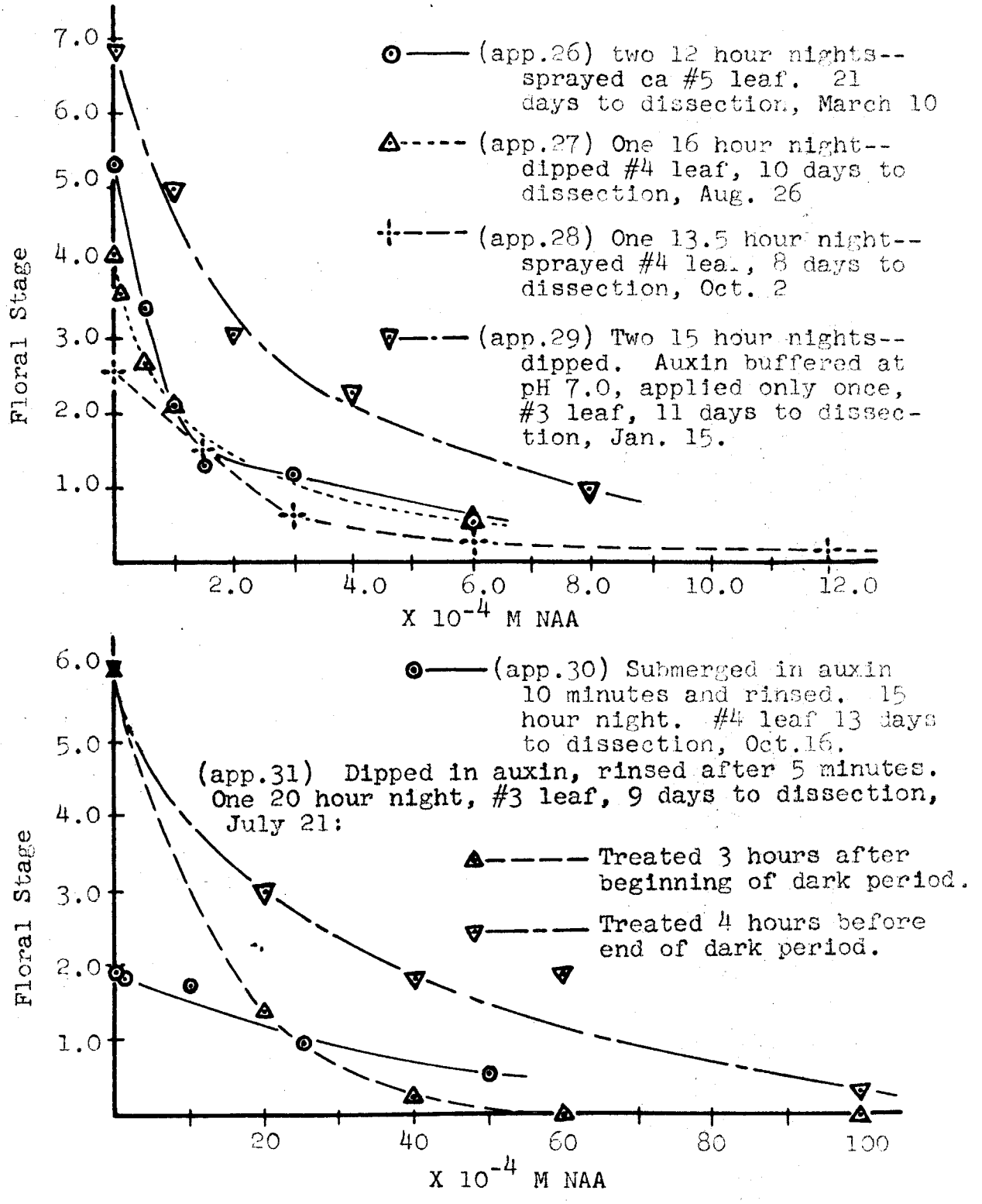

Figure 25. Floral development as a function of different NAA concentrations. Effects of various methods of auxin application, and varied experimental conditions.

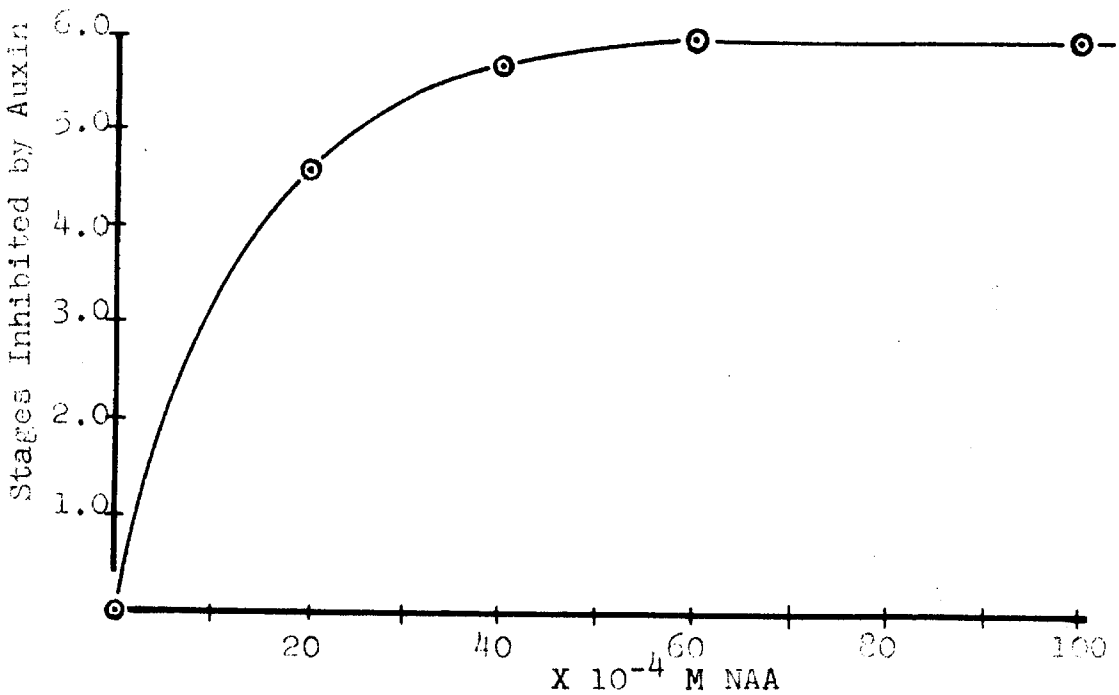

Figure 26. The effect of auxin upon floral stage, plotted as number of stages inhibited by applied auxin against auxin concentration.

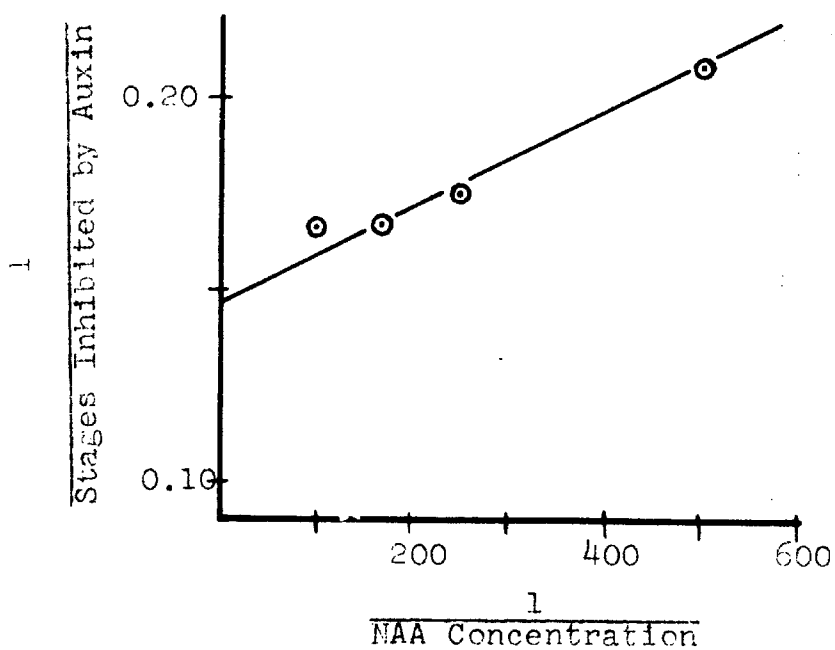

Figure 27. Relation of the reciprocal of the auxin response (floral stages inhibited) to the reciprocal of the auxin concentration.

centration relations of other auxin phenomena. McRae, Foster, and Bonner (1953) have applied Michaelis-Menten enzyme kinetics to the auxin growth responses of Avena coleoptile sections. In this treatment it was shown that the saturation type curve may result from the combination of auxin with a receptor to form an auxin-receptor complex active in the growth reaction.* As is shown in their paper, a test for the saturation type curve consists of a plot of the reciprocal of the auxin concentration against the reciprocal of the response. If the curve is of the hyperbolic type, the double reciprocal plot should be a straight line. In Figure 27 the data of Figure 26 are plotted in this manner, and it may be seen that the result is indeed a straight line.

This result, which applies to most of the curves in Figure 25, together with the fact that antiauxins reverse auxin-induced floral inhibition in the same manner that they reverse other auxin induced responses (page 21), indicates that auxin inhibition of flowering is mediated through the same chemical pathways as other auxin responses. Thus one might speak of an auxin-receptor complex in Xanthium which results in floral inhibition, since the same evidence which stands behind this concept for other plant tissues is also available in the present case.

It is interesting to note, however, that there are a num-

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*Other evidence was also presented to support this conclusion, in particular the interactions with antiauxins.

ber of complications in the concentration relations of auxin and flowering, which must be taken into account if the detailed treatment of McRae et al. is to be applied to this phenomenon. The method of application is perhaps the most serious of these complications. As may be seen by comparing the top of Figure 25 with the bottom of this Figure, ten fold differences in concentration of applied auxin may result in comparable inhibition of flowering depending upon the method of application. Another important factor which must be considered is that of leaf size. In Figure 28 floral stage is plotted against auxin concentration for different leaf sizes, and the floral stage is also plotted against leaf size for different auxin concentrations. Obviously leaf size must be considered. Other factors discussed in Chapter I must also be taken into account, but when this is done, the type of kinetic studies applied to the auxin-induced growth of Avena may also be applied to the auxin inhibition of flowering in Xanthium.

2. Relation of Applied Auxin to Length of the Dark Period. For the determination of the relation of auxin to the synthesis of florigen, it could be of importance to know what relation exists between applied auxin and the length of the inductive cycle. Does auxin applied at the beginning of an inductive cycle increase the critical night length? Or does it only inhibit the degree of response without materially affecting the critical night length? How does the concentration of applied auxin affect the response under different night lengths?

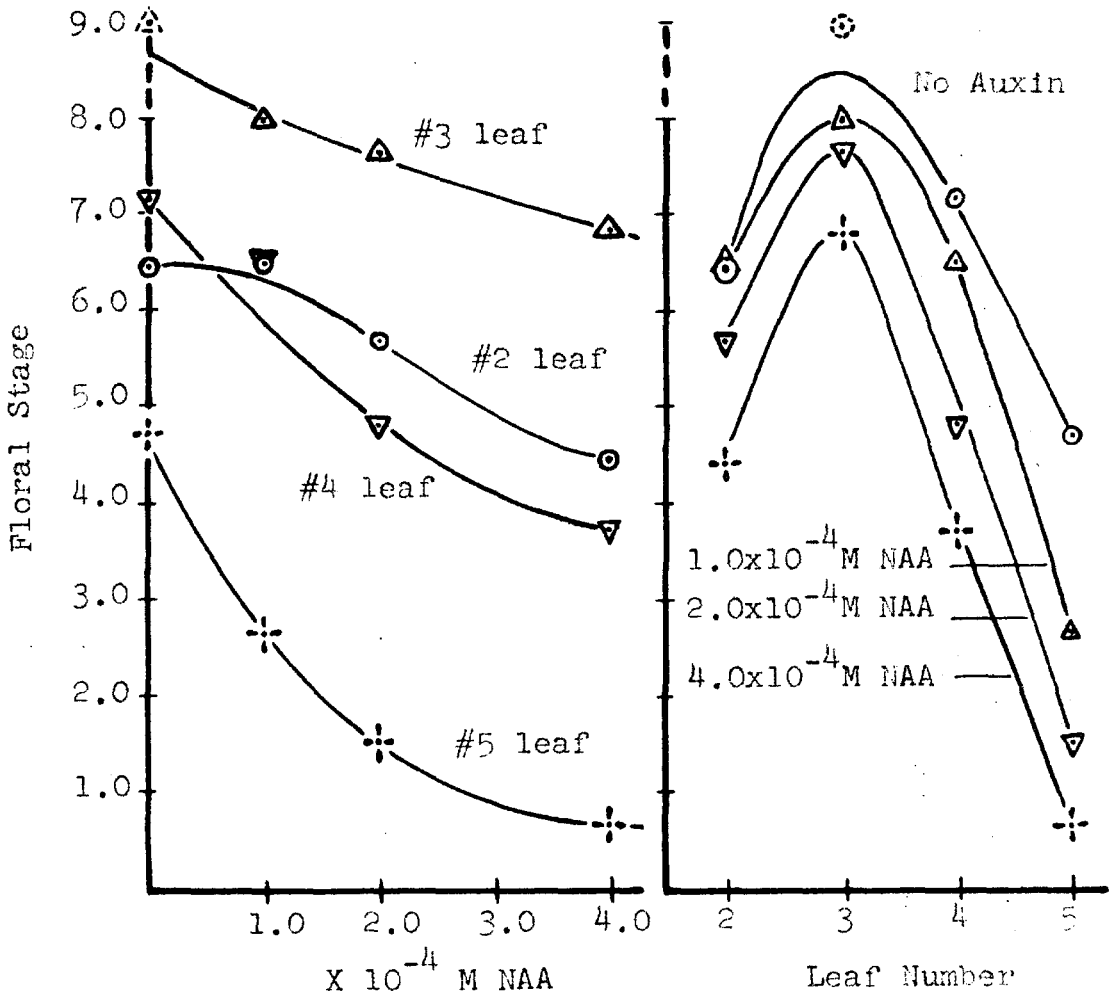

Figure 28. The relation of leaf size to floral inhibition by applied auxin of different concentrations. Inhibition is strongly dependent upon leaf size. Non-auxin-treated controls of plants defoliated to the #3 leaf flowered with buds somewhat larger than the usual stage 8. (app.32)

To investigate these questions, plants were induced with different night lengths and treated with different auxin concentrations. The results of four such experiments are shown in Figures 29 and 30, in which the floral stage (and in Figure 30, percent flowering) is plotted against night length, for different auxin concentrations. From these figures it may be seen that the longer the dark period, the more auxin is required to bring about a given degree of inhibition. The critical night length, however, seems to be affected only slightly if at all by applied auxin. The 6.0×10^{-4} M NAA treatment, shown in the upper half of Figure 29, appears to have increased the critical night length by an hour, but the degree of induction of plants receiving a 16 hour night (almost twice the critical night length) was reduced to less than a third of the non-auxin treated control value. Thus it appears to be primarily the degree of induction which is depressed by auxin.

The variation in flowering response of plants treated with auxin (Figure 16) is particularly evident in these experiments. Some plants are greatly inhibited with low auxin concentrations even at extended night lengths, and some plants flower even when treated with high auxin concentrations and induced with relatively short dark periods.

3. The Time of Auxin Application. Another kinetic study of the auxin inhibition of flowering which might yield insight into the mechanisms of induction involves the effective time of auxin application. Such a study might tell us which of the partial processes of hormone synthesis (see Introduction, page

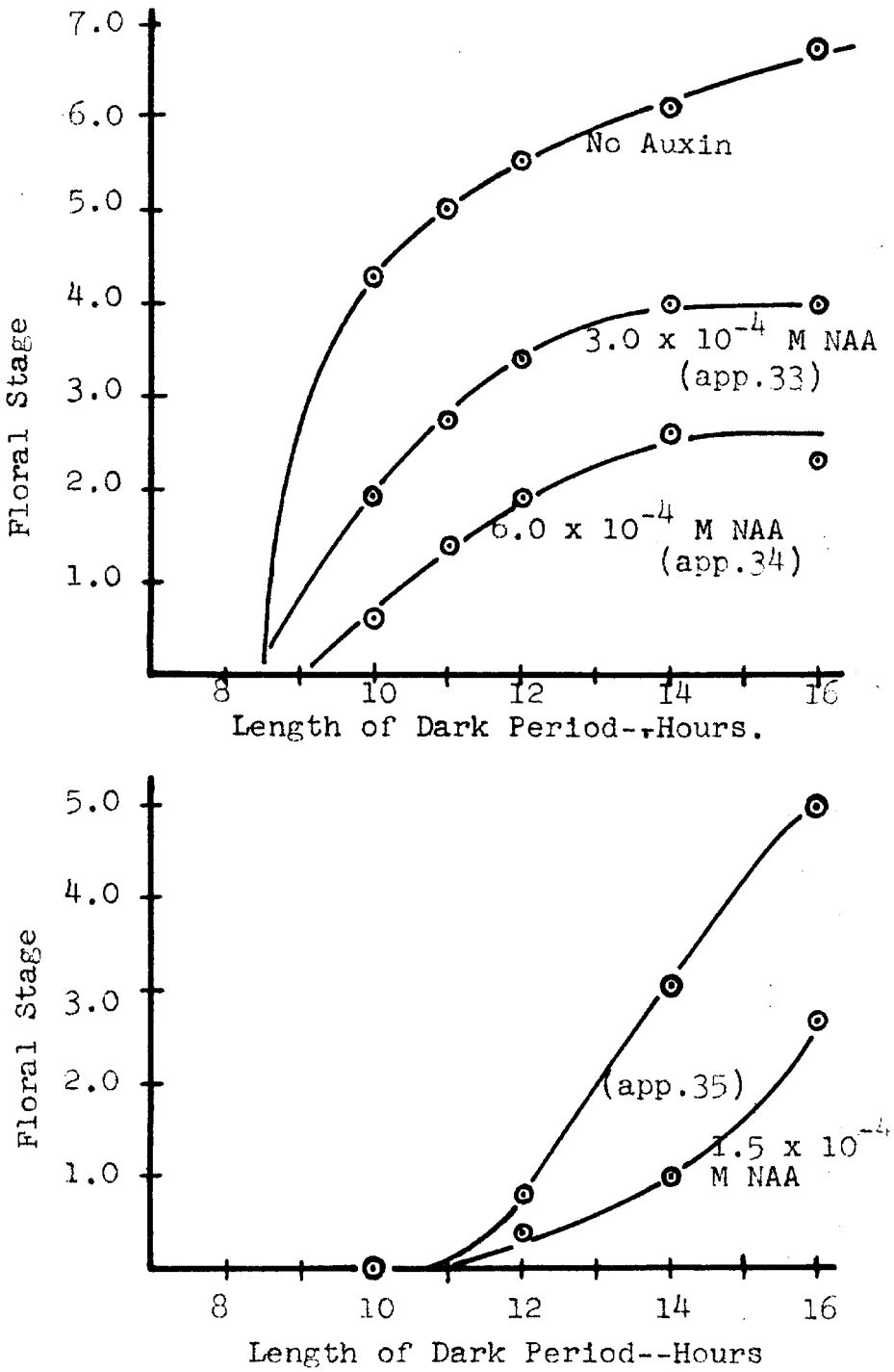

Figure 29. The relation of flowering to length of dark period and to applied auxin. In the top figure, the plants were trimmed to the #3 leaf, and received a single dark period. The stages of curve (app.34) were adjusted so that the controls match those of curve (app.33). The plants in the lower figure received a single dark period, and were trimmed to the #4-5 leaf.

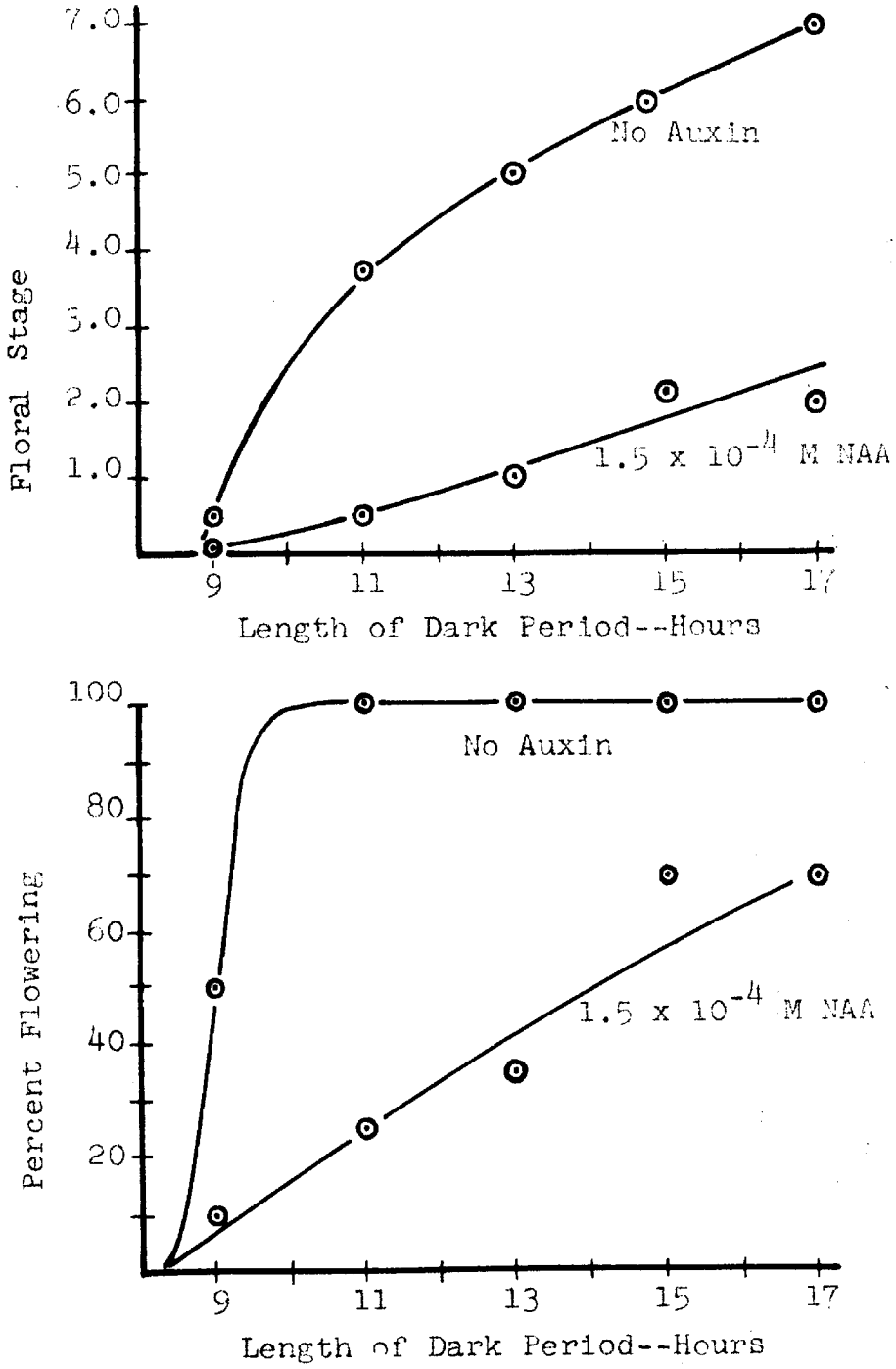

Figure 30. Relation of flowering to length of the dark period and to applied auxin. Data plotted both as floral stage and as percent flowering. Two long dark periods, plants trimmed to #4-5 leaf (app.30).

14) are affected by the application of auxin, or by the auxin already present in the plant. Figure 31 shows the effect upon flowering of auxin applied a number of days before induction. It may be seen that NAA inhibits induction somewhat even when it is applied seven days before the inductive dark period. In another experiment it was found that IAA was ineffective (or slightly promotive) in floral inhibition when applied seven days before induction. This may be due to the in vivo destruction of IAA by the IAA oxidase system. Apparently NAA remains in the plant for a number of days in a condition which inhibits the processes of induction.

Figure 19 in Chapter II shows the results of three experiments in which auxin was applied throughout and after an inductive dark period. Floral stage is plotted against time of auxin application. Two of these experiments, as well as two others, are summarized in Figure 32. In none of these experiments, however, was auxin applied from the beginning of the dark period until it had become ineffective in induction, in a single experiment. Figure 33 shows the results of a single experiment in which auxin was applied from the beginning of induction until it was ineffective in floral inhibition. Two night lengths were used. In all of these experiments the time of translocation of the flowering stimulus out of the leaf was measured by defoliation of groups of plants at different times after the inductive dark period, as explained on page 46 .

The conclusions which may be drawn from these figures

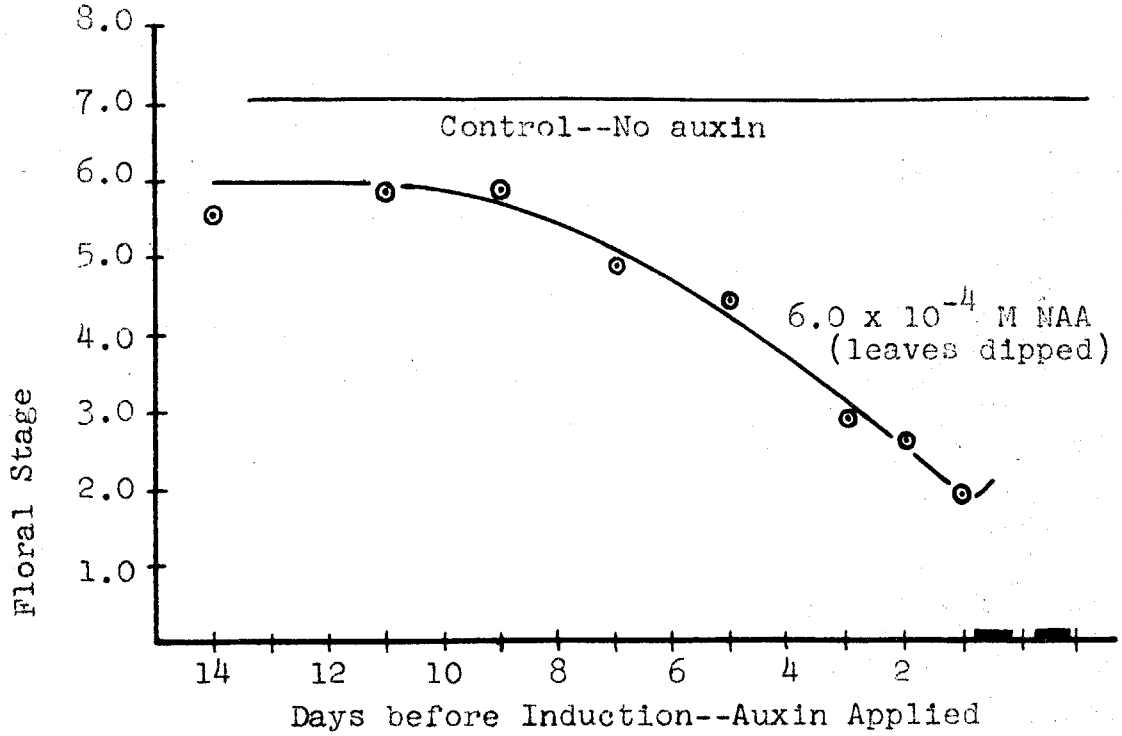

Figure 31. Effect on flowering of NAA applied before induction. In a separate experiment IAA applied seven days before induction was ineffective in floral inhibition.

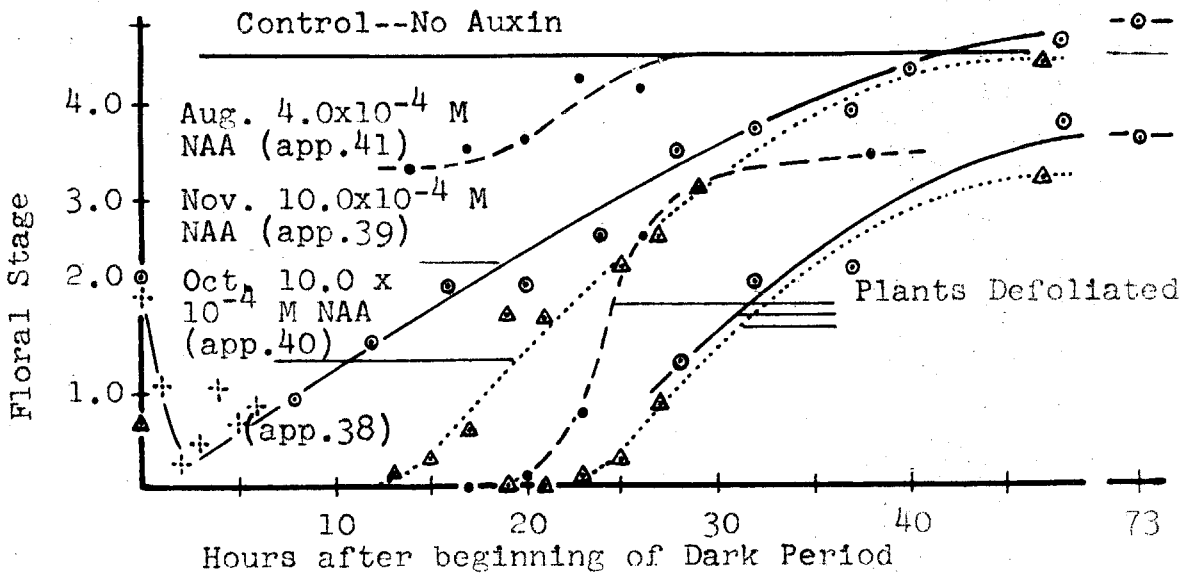

Figure 32. Effect on flowering of auxin applied at different times after beginning of dark period, together with the rates of translocation of stimulus out of leaf. Numbers (40,41) 14 hour night--(38,39) 16 hour night. All stages adjusted to match (39).

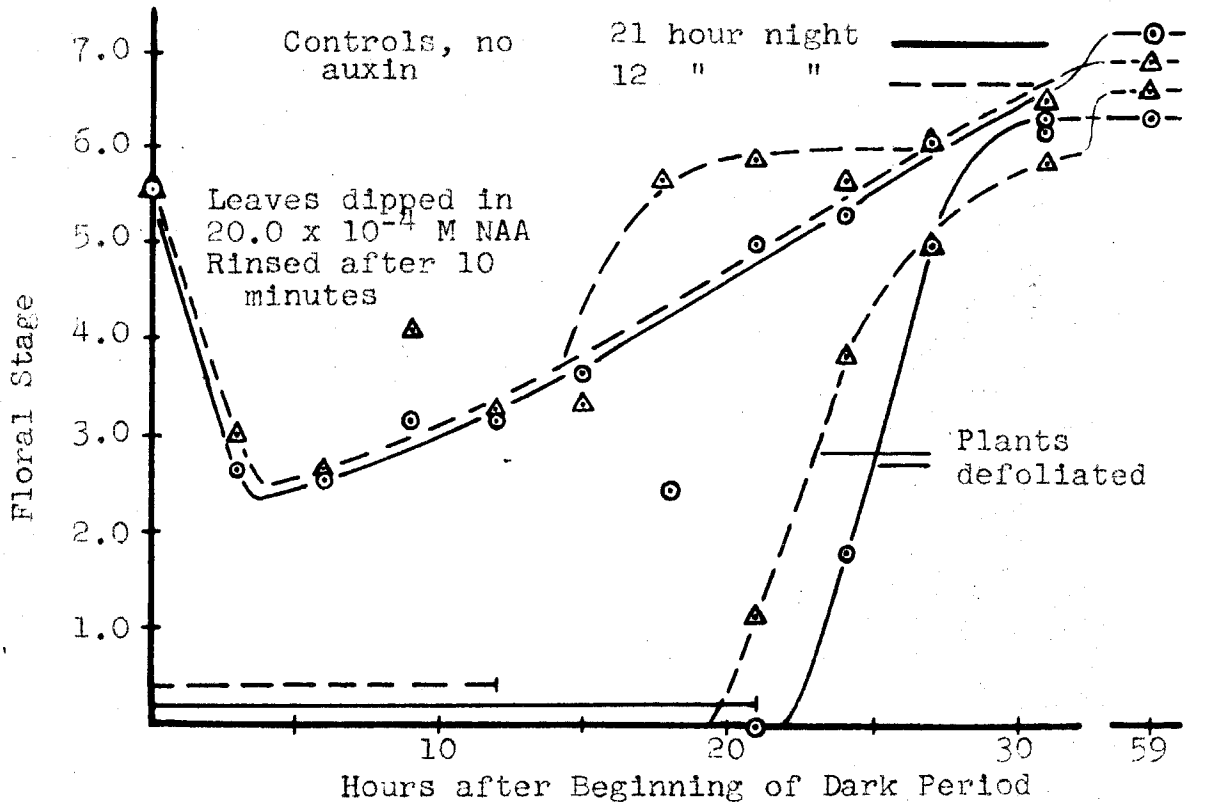

Figure 33. Effect of auxin applied at various times after beginning of induction (app.42) The high point at 9 hours and the low point at 18 hours are quite probably the result of errors in auxin application

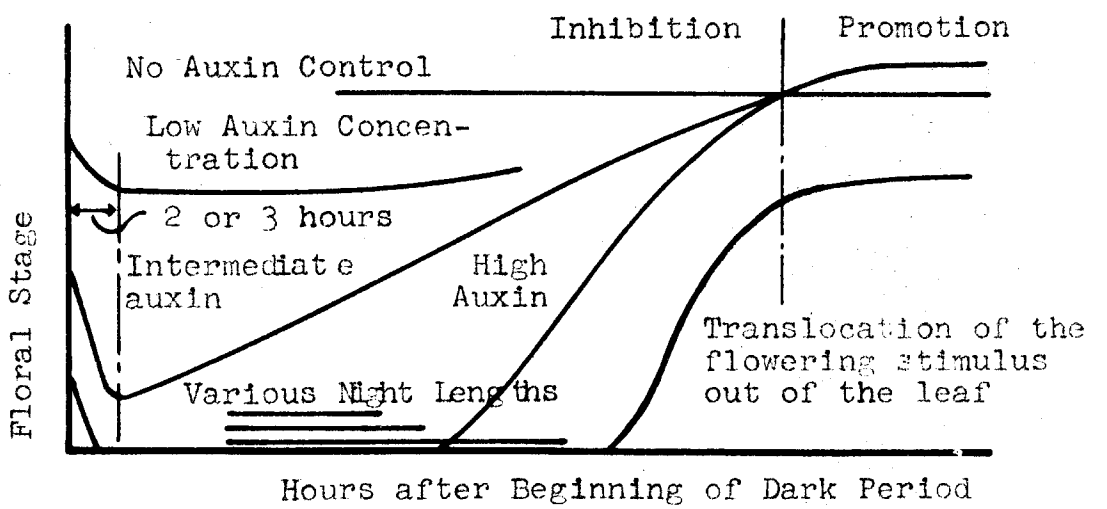

Figure 34. Generalized curves showing the kinetics of floral inhibition due to auxin applied at different times after the beginning of induction.

illustrating the effective time of auxin application are summarized in the generalized curves drawn in Figure 34. Auxin applied at successive times after the beginning of the inductive dark period becomes increasingly effective in floral inhibition through the first two or three hours, and then becomes gradually less and less effective until it is completely ineffective some hours after the end of the dark period. The time at which applied auxin becomes ineffective in floral inhibition seems to be very nearly equal to the time at which translocation of the flowering stimulus out of the leaf is complete. The general shape of the curves does not seem to depend upon the length of the dark period, nor is there an abrupt change in the slope of the curves at the end of the dark period. The slope of the curves tends to increase with increasing auxin concentration. The time at which applied auxin is ineffective in inhibition, related to translocation of florigen, is dependent upon the time of year, as shown in Figure 11, Chapter I.

Thus the conclusions of the kinetic studies on auxin-night length, and time of auxin application must be taken into consideration in any attempt to understand the mechanisms of the dark process, and of hormone synthesis. They will be discussed in this respect in the following chapter.

4. The Relation of Applied Auxin to Red Light Interruption of the Dark Period. As shown above, auxin inhibits the effects of the dark process, and is maximally effective when applied about two hours after the beginning of the dark process.

A red light interruption during the dark period also inhibits the effects of the dark process (page 18). Since both applied auxin and red light interruption of the dark period have the same net result, it is of interest to know what relation there is between the two effects.

The kinetics of applied auxin inhibition of flowering are outlined above, but it was found that in spite of the extensive work on the red light interruption of the dark period (e.g. Borthwick, Hendricks, and Parker, 1952; Liverman, 1952; Liverman and Bonner, 1953 b; Parker, Hendricks, Borthwick, and Scully, 1946; Parker and Borthwick, 1950; Harder and Bode, 1943), kinetic data of the quantitative type described above for auxin were not available for the red light interruption. Most work had been done on the action spectrum for the photoreceptor pigment, and only little had been done on the kinetic aspects of the time and intensity of the light interruption. It had been shown (Parker, et al., 1946) that the reciprocity relations held approximately for the red light interruption of the dark period as for other photo-chemical reactions. A given effect is obtained with a given quantity of light (e.g. ergs/cm^2) whether it is applied at low intensity for an extended period (up to two hours), or at high intensity for a short period. The effect of varying the time of interruption during the night has also been studied, but the results are unsatisfactory for quantitative considerations. Qualitatively it has been found that about the same inhibition is obtained with a given quantity of light from six to eight hours after

the beginning of a twelve hour dark period.

a. Time of Light Interruption during Induction. To obtain more satisfactory data for the time of light interruption, groups of plants were illuminated for brief intervals (from ten seconds to ten minutes, depending on the experiment) by red light of high intensity (approximately 18,000 ergs/cm^2/sec of red light at different times during induction). Figure 35 in which floral stage is plotted against time of red light interruption shows the results of such an experiment. Two night lengths were used (12 and 16 hours), and light interruptions of one minute and ten minutes were used. The two durations of light had almost the same effect (as did 10 seconds in another experiment), and although the data are tabulated in the appendix, only the one minute results are shown in Figure 35.

A number of conclusions may be drawn from Figure 35. The complete floral inhibition brought about by interruption eight hours after the beginning of the dark period (referred to hereafter as the 8 hour interruption) is of interest. In this experiment, interruptions after eight hours seemed simply to stop the processes of induction which had gone on up to the time of interruption (e.g. the 12 hour interruption of the 16 hour night resulted in a floral stage equal to the 12 hour night). Thus from 8 hours (or more probably 8½ to 9--critical night length) to 16 hours, the curves are identical with those obtained by plotting length of the inductive cycle against flowering (Figure 5). The second high intensity light process

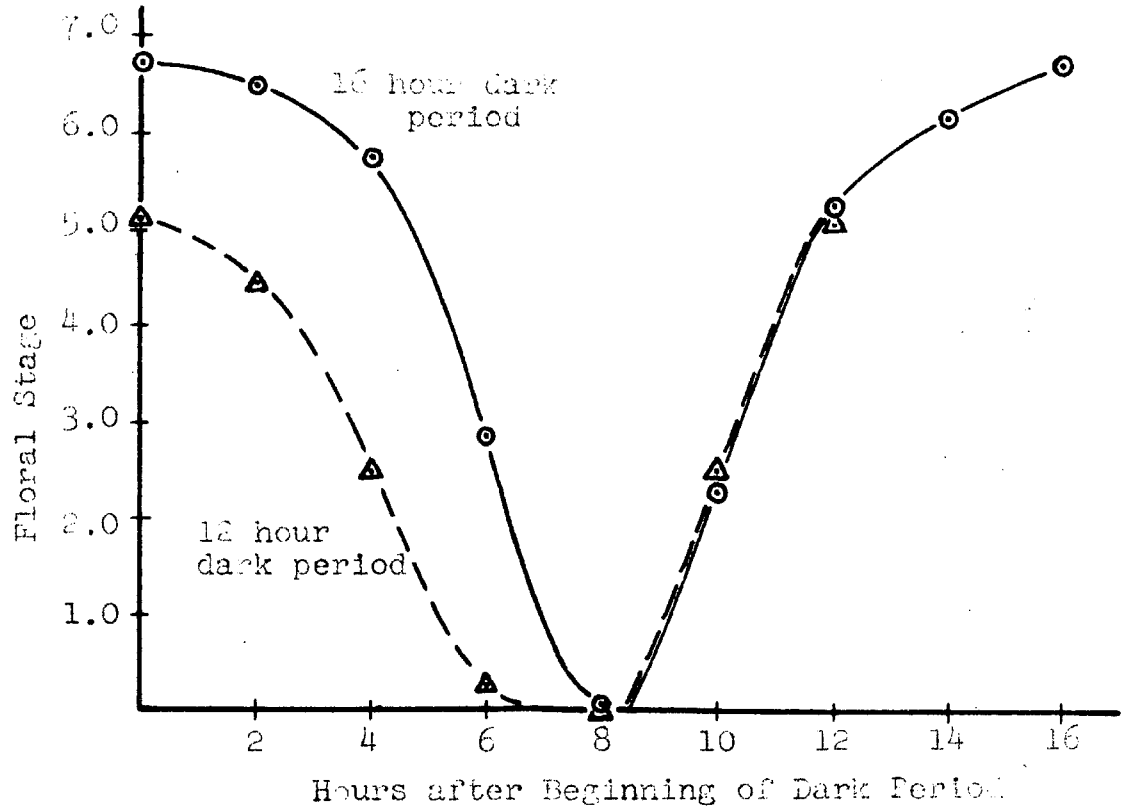

Figure 35. Effect upon flowering of a red light (ca. 18,000 ergs/cm^2/sec) interruption of one minute given at various times during a 12 or a 16 hour dark period (app.43).

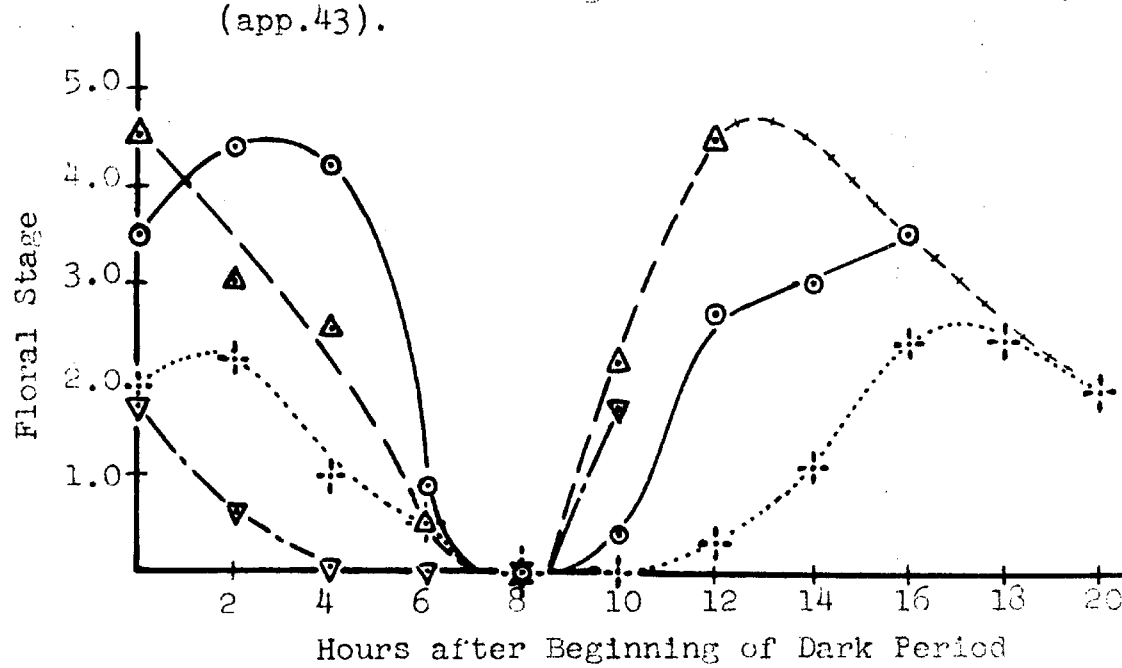

Figure 36. Effect upon flowering of a 12 sec red light interruption given at various times during a 10, 12, 16 or 20 hour dark period (app.44).

(Lockhart and Hamner, 1954) is not evident in these experiments. If it were, one would expect the 12 hour interruption of the 16 hour night to result in a lower floral stage than the 12 hour night (due to the darkness following light interruption).

Response to light interruption during the first eight hours is also of interest. The curves are asymmetrical, with the light interruption being less effective if given in the first half of the dark period. Thus it appears that a given dark period is more effective when it follows a light interruption, than when it is terminated by one. The plants which receive a 2 hour interruption in a 12 hour night (10 hours of darkness to follow), for instance, flower with a much higher stage than plants which receive the 10 hour interruption in either the 12 or the 16 hour night. This effect decreases, however, as the interruption approaches the 8 hour point.

In another experiment, a 12 second light interruption was applied at two hour intervals during 10, 12, 16 or 20 hour nights. The experimental conditions were essentially identical to those in the above experiment (see appendix 43 and 44). In both cases plants were defoliated to the #3 leaf prior to induction, weather conditions were good, temperature during induction was ca. 23° C., and both were conducted in the summer. The plants were younger in the second experiment, but this is probably of no importance (page 51). The length of the red light interruption was very nearly the same as in

another experiment which yields a curve for a 16 hour night much like the one in Figure 35. Yet, as can be seen from Figure 36 in which the results of the second experiment are presented, the results of the two experiments are quite different. The curves for the 10 and the 12 hour nights are very nearly what one would expect from the curves in Figure 35, but the 16 and 20 hour curves deviate greatly both from the curves in Figure 35 and from the curves one might predict on the basis of Figure 35.

The principal difference is the second high intensity light process effect, absent from the first experiment. As can be seen from the line connecting the control values of the 12, 16 and 20 hour nights, darkness after about 14 hours tended to inhibit, rather than to promote flowering. A light interruption at almost any point in the 16 and 20 hour night curves after the critical night length, resulted in decreased flowering. The 2 and 4 hour interruptions in the 16 hour night are also of interest. Here the interruptions promoted flowering above the controls. It would appear that shortening of the dark period, if it would otherwise exceed 14 hours, tends to increase flowering when the interruption occurs within the first few hours after the beginning of the dark period. The same phenomenon is evident in the 16 and 18 hour interruptions in the 20 hour night. It would appear that darkness after about 16 hours is so detrimental to flowering that a light interruption, which apparently stopped the detrimental

processes, was beneficial to flowering. Thus on the basis of the phenomenon described by Lockhart and Hamner (1954), one may account for a portion of the irregular parts of the 16 and 20 hour curves.

That there are differences between the experiments shown in Figure 35 and Figure 36, however, is much more difficult to account for. Lockhart and Hamner (1954) report that the inhibitory effect of darkness following a light interruption some time after the critical night length, could not always be demonstrated. In some instances they were able to obtain the effect in a very striking manner, as in Figure 36, and in other cases the effect was completely absent, as in Figure 35. They attempt to explain these results on the basis of varying auxin levels within the plants, but as will be pointed out below, this explanation is not born out by the present work.

A very important aspect of the curves in Figure 36 is that an 8 hour interruption results in complete inhibition of flowering regardless of the night length. Thus it appears that the processes of induction, if interrupted by a flash of light 8 hours after the beginning of the dark period, cannot be resumed again.

Some experiments of Wareing (1954) are of interest in respect to the two experiments reported above. He found that with a 60 hour dark period, a light interruption just after the beginning and just before the end had an inhibitory effect upon flowering (Xanthium and soybean). Using soybean, he found

that a light interruption near the middle of a sixty hour dark period promoted flowering. These results are in general agreement with the two experiments reported above, although no mention is made in Wareing's paper about different results when experiments are performed at different times. Wareing was unaware of the effect described by Lockhart and Hamner (1954), and his own explanation for his results appears to be needlessly complex.

b. Duration of Light Interruption at Various Times during Induction. In the experiments described above, light interruptions of 10 seconds to 10 minutes duration resulted in response-time of interruption curves of nearly the same shape. To determine what intensities are effective in inhibition or suppression of flowering at different times during induction, plants were illuminated (light source as above) for intervals of 2 to 60 seconds, the illumination again being given at different times during the dark period. The results are presented in Figure 37 as floral stage plotted against duration of red light interruption. It is evident, that although the maximum extent to which flowering is inhibited is greatly dependent upon the time during the dark period when light is applied, the duration of light (amount of light energy) required to bring about this maximum inhibition is the same (or very nearly so) at all times.

According to Borthwick, Hendricks, and Parker (1952) the photo-receptor pigment is capable of existing in two forms: one form capable of absorbing red light, and a second form

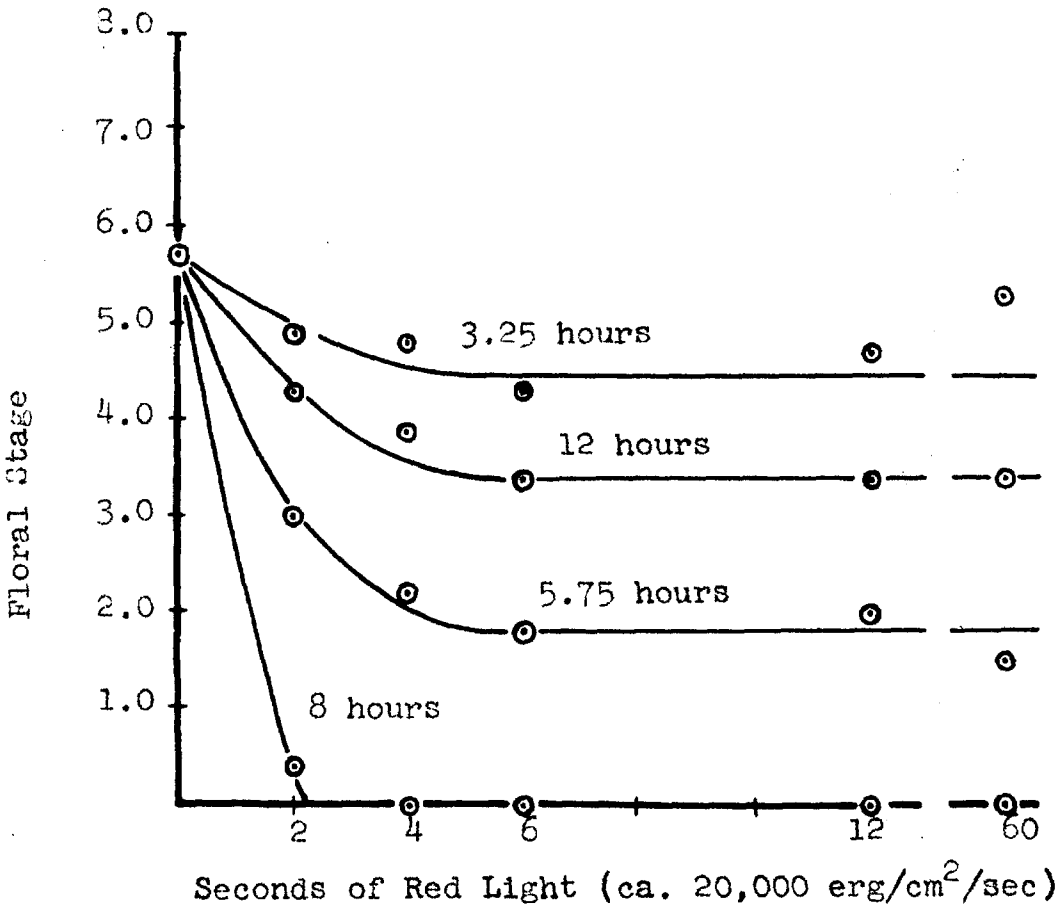

Figure 37. Relation of floral stage to the duration of a red light interruption given at various times during a 16 hour dark period (app.45).

capable of absorbing far red light. As the pigment absorbs one wavelength of light, it becomes converted to the other form, and becomes more receptive to the other wavelength. It is postulated that at the end of the light period preceding induction, all of the pigment is in the far-red-receptive form (since red light predominates in the light which the plants receive during the day), and that during the ensuing dark period, the pigment is converted spontaneously to the red-receptive form. The red-receptive form of the pigment permits the processes of floral induction to take place. A red light interruption converts the pigment to the far red receptive form, which then inhibits induction. If effectiveness of light in pigment conversion depends only upon total light energy, as in the experiments of Parker et al. (1946), then duration of interruption at constant intensity determines the amount of light energy supplied. The experimental results of Figure 37 indicate that the same quantity of light is needed to achieve the maximum inhibition of flowering independent of the time during the dark period at which the light is given. If a given amount of light is required to convert a given amount of the photo-receptor pigment from one form to another, as in other photo-chemical reactions of this nature, then the present results indicate that the same amount of photo-receptor pigment is present in the red receptive form at all the times during the dark period when light was applied. Thus the pigment must have been converted from the far red receptive form to the red receptive form before the first light interruption (3.25 hours).

In the experiments of Borthwick, Hendricks, and Parker (1952) the critical dark period could be shortened by treating the plants prior to induction with far red light. It was assumed that the pigment is converted to the red receptive form by this treatment, and that the time normally required to bring about this conversion spontaneously could be subtracted from the critical night. In their experiments, these workers were only able to shorten the critical night by 2 to 3 hours. These results are in excellent agreement with the deductions made from the curves of Figure 37, and indicate again that the pigment is spontaneously converted from the far-red-receptive form to the red-receptive form within the first three hours of the dark period.

Withrow and Withrow (1944) came to much the same conclusion regarding the time of pigment conversion, from experiments involving a series of light interruptions during an extended dark period.

c. Light Interruption plus Auxin During Induction. Since both applied auxin and a red light interruption inhibit flowering, it has been suggested that the red light interruption is related to the effective auxin level within the plant (Liverman, 1952; Liverman and Bonner, 1953 b). On the basis of work with Avena coleoptile sections it was suggested that red light transforms a precursor into a receptor for auxin. The auxin-receptor complex then is transformed in the dark spontaneously (or rapidly in far red light) to a form inactive in the growth reaction or in the inhibition of flowering.

It was proposed that the transformation into the inactive form regenerated the red light sensitive precursor. Thus it was suggested that the auxin receptor is coupled to or otherwise dependent upon the photoreceptor pigment. The transformations from red-sensitive precursor, to auxin-receptor, to auxin-receptor-complex (sensitive to far-red), to red-sensitive precursor, were envisaged as cyclically interconnected (the morphogenetic photocycle).

Lockhart and Hamner (1954) in their description of the destruction of florigen or florigen precursor in a short dark period following a light interruption at the end of an inductive dark period, also report the results attained by application of auxin near the end of an inductive dark period. They state that the inhibitory effect of auxin (IAA) applied near the end of the inductive dark period could only be observed unequivocally, if auxin were applied after a light interruption. Their results indicate that the combination of applied auxin and a light interruption is more effective in the inhibition of flowering than the sum of each treatment applied separately. The findings of Lockhart and Hamner with Xanthium are thus quite similar to those of Liverman and Bonner with the Avena coleoptile.

The interaction of light and auxin in the flowering of Xanthium has been investigated in detail during the course of the present work.

At two hour intervals during a 16 hour night, groups of plants were either treated with auxin, illuminated with red

light (intensity as above) for 60 seconds, or both illuminated with red light and subsequently treated with auxin. The results are shown in Figure 38 in which floral stage is plotted against the time of treatment during the inductive dark period. It may be seen that the inhibition due to applied auxin was much the same as obtained in previous experiments (Figures 19, 32, 33 and 34). The effects of the red light interruption are also much as those obtained previously (Figure 35). The dotted line of Figure 38 is that obtained by assuming that the inhibitory effects of red light interruption and applied auxin are additive (rather than synergistic). The sum of the inhibitions due to each treatment alone is subtracted from the control level to obtain this curve. It is clear that the inhibitory effects of red light and applied auxin given together is essentially that expected if the two separate inhibitions are additive. Thus it appears that in this experiment, the synergistic effects due to red light and applied auxin, as reported by Lockhart and Hamner (1954), are absent.

Why should there be this difference in result? Lockhart and Hamner used IAA while the present author utilized NAA. To check the possibility that this difference in technique might account for the difference in experimental results, the experiment as described above (12 second light interruptions) was repeated using IAA as the auxin. The results obtained are essentially the same as those of Figure 38.

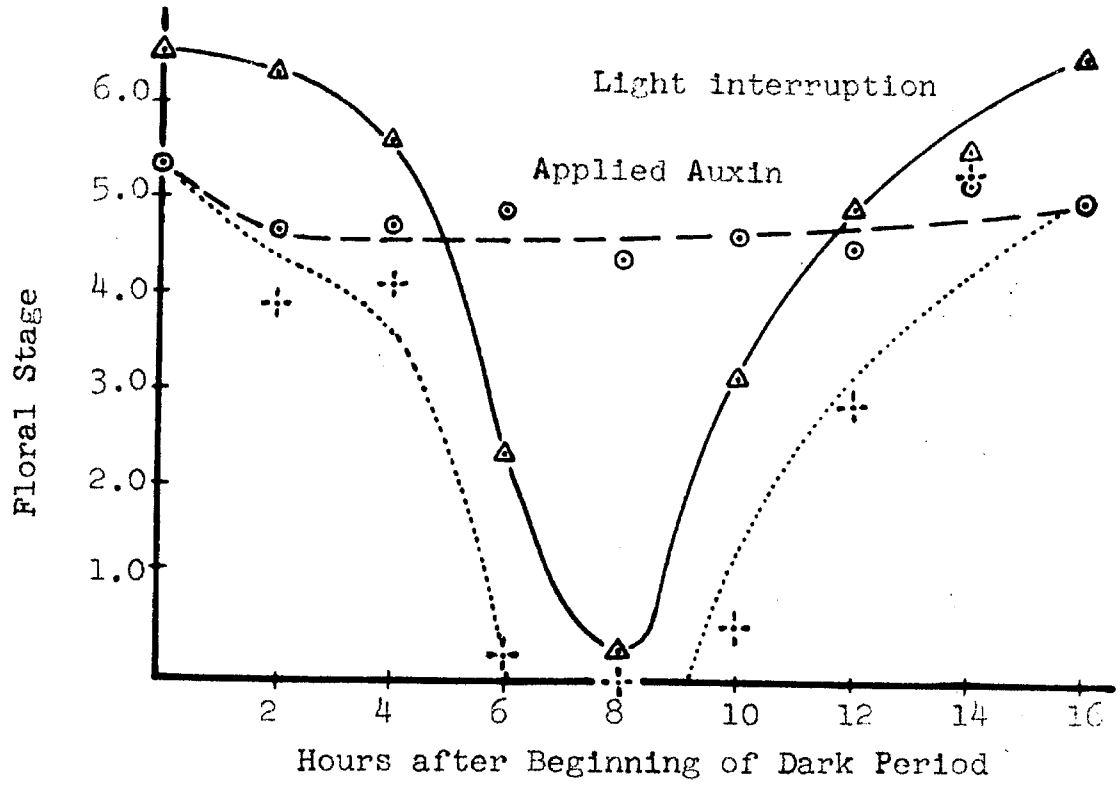

Figure 38. The effect upon flowering of a red light interruption (1.0 min), and auxin, singly or together, given at various times during an inductive dark period (app.46).

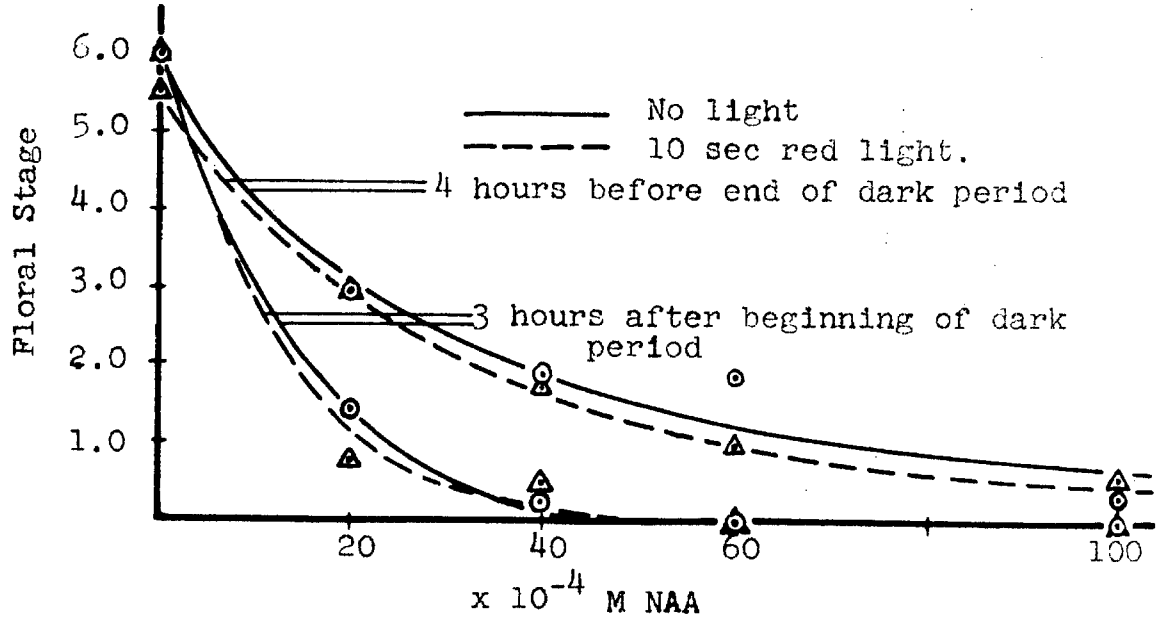

Figure 39. Inhibition of floral induction by various auxin concentrations, applied with or without a red light interruption during a 20 hour dark period (app. 47).

Another difference in technique between the experiments of Lockhart and Hamner and those of the present author involve the method of preparing the auxin solutions. The above workers dissolve the auxin in alcohol, and then add the alcohol to water, while the author dissolves the auxin by neutralization with KOH (page 69). As shown in Chapter I (page 71), the presence of potassium ions in the auxin solution increases its effectiveness in the inhibition of flowering. It is possible that Lockhart and Hamner were unable to obtain auxin inhibition of flowering near the end of the dark period in control plants, because of the lack of potassium ions in their auxin solutions. This possibility has not been tested. It offers no explanation as to why light should increase the effectiveness of auxin in their experiments.

Another possibility is that the proper concentration of auxin was not used in the experiment shown in Figure 38. To test this possibility, plants were treated with light, auxin, or light plus auxin either three hours after the beginning of a 20 hour dark period, or four hours before the end. A series of auxin concentrations was used. The results are shown in Figure 39 as floral stage plotted against auxin concentration for each of the two treatment times. It may be seen that the light interruption by itself (10 seconds) did not reduce flowering below the control level. This is as expected for this light energy applied at these times during a 20 hour night (Figure 36). The energy is sufficient to completely saturate or convert the pigment, as shown in Figure 37.

The combination of light plus auxin was no more effective than auxin alone. Thus in this experiment there is no evidence for a synergistic interaction between a red light interruption and applied auxin.

This same experiment was also performed by using 20 minutes of sunlight for the light interruption. Again no evidence for an interaction between light and auxin could be discerned from the experimental results.

5. Application of Cobalt. In all discussions involving the role of auxin in flowering, the question arises concerning the native auxin concentration within the plant. It has proven very difficult to measure this auxin level directly (Bonner and Liverman, 1953), and so indirect methods have been sought to gain insight into the auxin status of the native plant.

Galston and Siegel have studied the relation of peroxidases in plant tissue to the IAA oxidase system. In one phase of their investigation they have shown that the application of cobaltous ion to plant tissues results in an increase in the auxin concentration within these tissues by preventing peroxidative destruction of IAA by the IAA oxidase system.

If an enzyme system similar to that studied by Galston and Siegel occurs in Xanthium leaf tissue, one might expect application of Co^{++} ion to result in an increase in the auxin concentration within the leaf. Co^{++} ion should therefore inhibit flowering. Figure 40 shows the results of a prelimi-

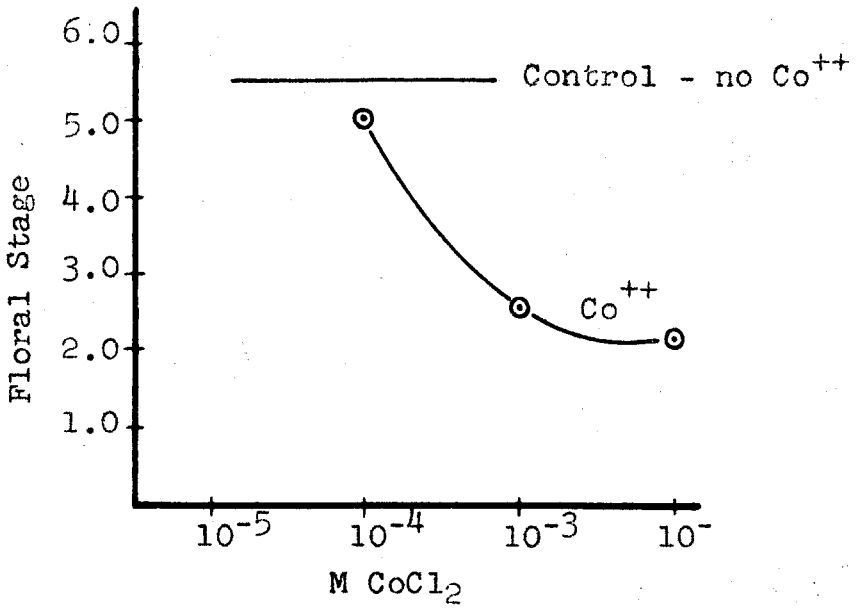

Figure 40. The effect of applied Co^{++} upon flowering. Concentrations shown applied before a single 16 hour dark period (app.48).

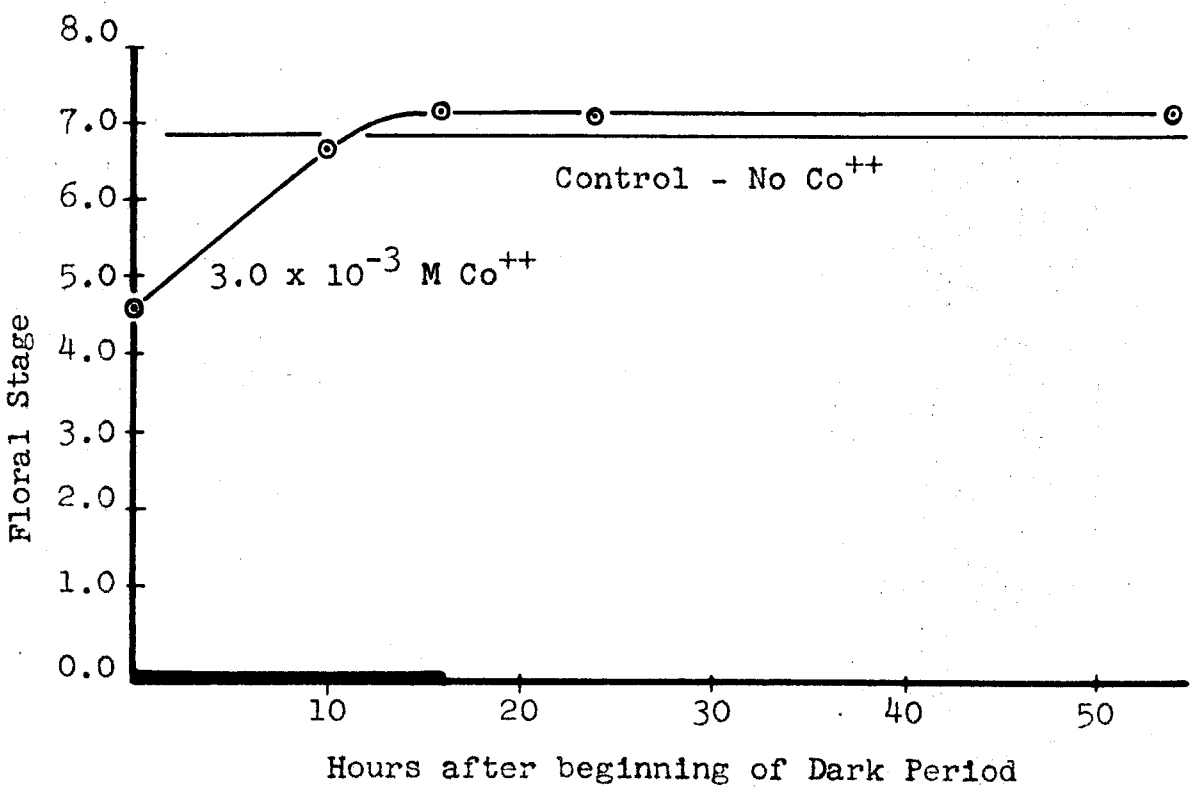

Figure 41. Effect on flowering of Co^{++} applied at different times after the beginning of an inductive dark period (app.49). The curve has the general characteristics of the applied auxin time curves.

nary experiment involving the application of $CoCl_2$ to plants just before a 16 hour inductive dark period. Floral stage is plotted against cobalt concentration. It may be seen that cobaltous ion applied to Xanthium does indeed inhibit the degree of flowering.

To determine whether this inhibition is specifically owing to inhibition of induction, or whether it is owing to an inhibition of growth of the plant (leaves appear yellow near the edges after treatment with cobalt), cobalt solution was applied to plants either at the beginning, during, or for some time after a single inductive dark period. The results are shown in Figure 41 in which floral stage is plotted against time of application of cobalt. The curve has the same form as the curves which relate time of auxin application to floral stage (Figure 34). Applied cobalt becomes ineffective in floral inhibition much sooner after the beginning of induction than does auxin. This may be due to a lag in the build up of auxin within the plant due to applied cobalt. It is clear, however, that the inhibitory effect is specifically upon induction, and not upon growth.

Although the results are only preliminary, they suggest that cobalt may be a promising tool for investigation of the native auxin status of the plant as related to flowering.

Chapter V

THE ACT OF INDUCTION

The Kinetics of the Reactions of the Dark Period

The data of Chapter IV, together with those of other workers, enable us to make a number of deductions concerning the mechanism of the act of induction. The principal results of Chapter IV are summarized in Figure 42. The top part of this figure shows the effects of applied auxin or of red light interruption upon flowering, plotted as a function of the time of treatment after the beginning of the dark period. The relation of flowering to the length of the dark period is also shown. The lower half of Figure 42 shows how the concentrations of the red-receptive form of the photo-receptor pigment and of florigen are believed to change with time after the beginning of the dark period. In Figure 42 the inhibitory effect of extended dark periods or of a short dark period following a light interruption of an inductive dark period, are ignored. Although this effect (as described by Lockhart and Hamner, 1954) may be clearly demonstrated under some circumstances (Figure 36), it is not always present (Figure 35), and therefore may not be an integral and essential part of the inductive process, but rather a side effect.

The major facts summarized in Figure 42 may be listed as

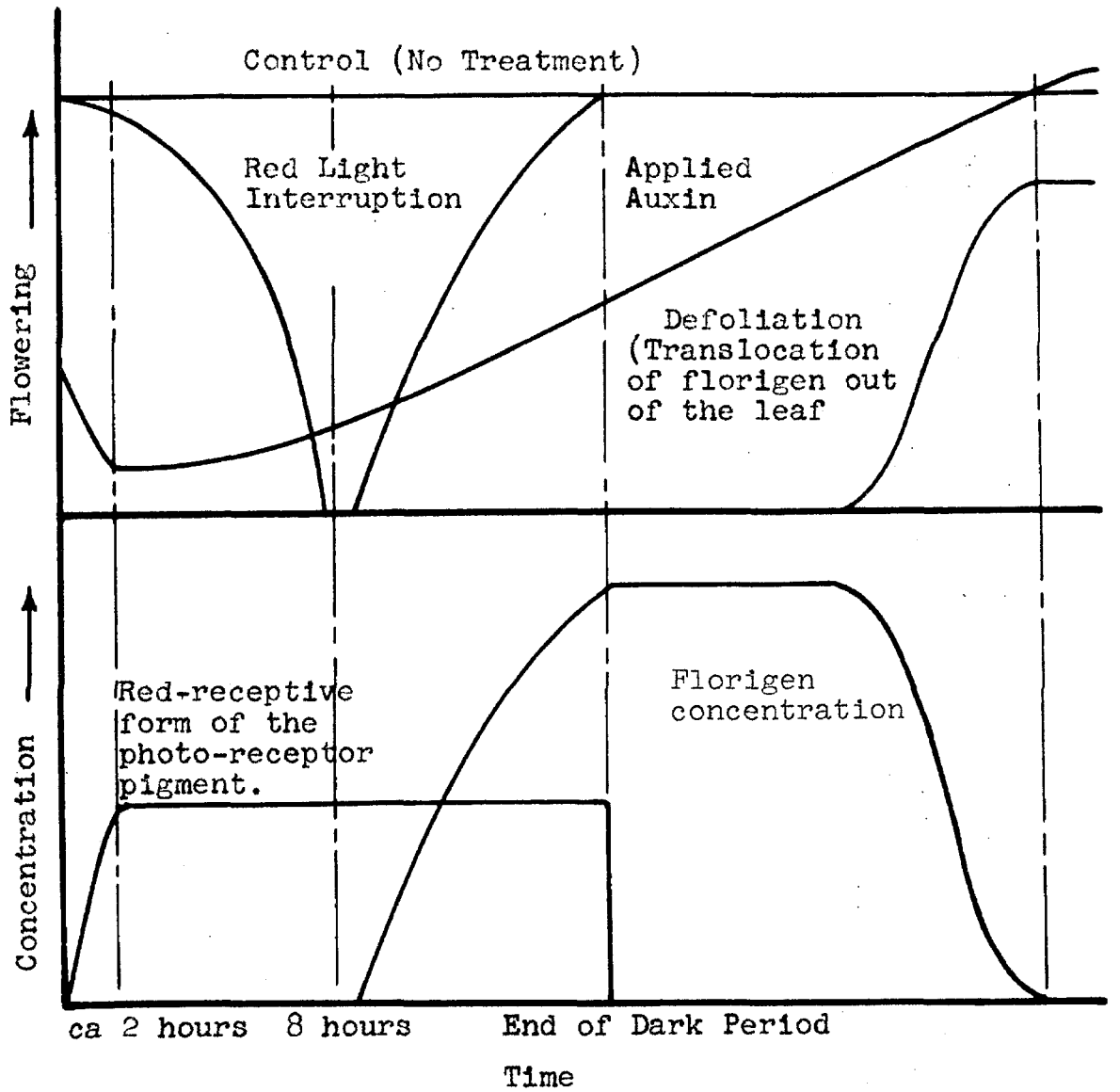

Figure 42. Top half: Generalized curves which summarize many of the effects of auxin application or red light interruption upon flowering as explained in the text. The second half of the red light interruption curve (from $8\frac{1}{2}$ hours until end of dark period) also illustrates the relation between flowering and length of the dark period. Bottom half: Inferred relations of photo-receptor pigment and florigen concentrations to time during induction.

follows:

(1) Auxin is more effective in floral inhibition when applied two or three hours after the beginning of the dark period, than when it is applied at the beginning.

(2) The inhibitory effect of applied auxin decreases with time from two or three hours after the beginning of the dark period to the time when translocation of florigen out of the leaf is complete. This later time is dependent upon light intensity (time of year).

(3) There is no sharp break or inflection in the auxin-time of application curve at the end of the dark period. The length of the dark period seems to have little effect upon the shape of these curves.

(4) Red light is most effective in floral inhibition when applied eight hours after the beginning of the dark period. This effect is independent of the length of the dark period.

(5) The red light inhibition curves are asymmetrical. In the first few hours of the dark period, darkness preceded by a red light interruption is more effective in flower initiation than darkness terminated by a red light interruption and preceded by day light.

(6) In the absence of the Lockhart-Hamner effect, the second half of the red light interruption curve is the same as the curve representing the relation of flowering to length of the dark period. Thus in this case red light simply stops the

inductive processes which have gone on up to the time of light application. This curve, then will be assumed to represent concentration of florigen in the leaf as a function of time, as shown in the lower half of the figure.

(7) The red-receptive form of the photo-receptor pigment must reach its maximum concentration within ca. 2 hours after the beginning of the dark period, as indicated by the experiment shown in Figure 37. This conclusion is based on the fact that the same amount of light is required to saturate the pigment at all times later than 3 hours after the beginning of the dark period. It may be concluded that the same amount of pigment is present at these various times. The work of Borthwick et al. (1952) already cited, supports this conclusion. They were able to shorten the critical night by only ca. two hours with far red radiation given at the beginning of the dark period. On this basis it is concluded that two hours are needed to convert the pigment from far-red-receptive to red-receptive form.

In addition to the facts summarized in Figure 42, the following facts are also pertinent to the discussion which follows:

(8) Applied auxin has little if any effect upon the critical night length.

(9) The application of auxin and red light together, during an inductive dark period, results in floral inhibition equal to the sum of the inhibitions brought about by each of

these agents applied separately.

On the basis of these considerations, the act of induction can be formulated as follows. There appear to be four separate reactions which occur during the dark period:

1. Conversion of the Photo-receptor Pigment. As discussed above, the spontaneous conversion of the photo-receptor pigment from a far-red-receptive to a red-receptive form (Borthwick et al., 1952) is probably complete within approximately two hours after the beginning of the dark period. Since return of the pigment to the far-red-receptive form stops the processes of induction (see below), we may conclude that the conversion to the red-receptive form must take place before any of the ensuing reactions of the dark period may take place. The curves of Figure 37 indicate that only a small quantity of light is required to bring about complete conversion to the far-red-receptive form (ca. 70,000 ergs cm^2).

2. Reaction(s) Preparatory to Hormone Synthesis. From the time of conversion of the photo-receptor pigment to the red-receptive form (ca. 2 hours) until the first appearance of florigen (the critical night length, ca. 8.5 hours), a reaction (or reactions) take place which are preparatory to net accumulation of florigen. Nothing concrete is known about this reaction, but a number of inferences may be made about its characteristics. This reaction may consist of hormone synthesis balanced by destruction. That this is probably not the case will be discussed after some characteristics of

the preparatory reaction as well as hormone synthesis, have been mentioned. It, in combination with the conversion of pigment, determines the critical night length. It proceeds only in the presence of the red-receptive form of the photoreceptor pigment. It can proceed, however, when the pigment is only partially in this form, as shown by the fact that plants can be induced under very low intensity light, as in the threshold experiments of Liverman (1952). If the pigment is completely converted to the far-red-receptive form by red light interruption of the dark period, the reaction is not only stopped, but completely reversed so that the entire sequence of dark reactions must start anew. Thus uninterrupted darkness longer than the critical night length is required after a light interruption to result in hormone synthesis (Figure 35). During the first few hours of the dark period, however, the preparatory reaction (plus pigment conversion) may be resumed after a light interruption in a shorter time than that which is originally required for the reaction to begin after an entire day of high intensity light. This is shown by the asymmetry of the curves in Figures 35, and 42. An uninterrupted dark period of a given length is more effective in producing florigen when it follows a light interruption in the first part of the dark period, than is a dark period of equal length which follows a day of high intensity light.

An important characteristic of the preparatory reaction

is that if it is stopped just before net hormone synthesis begins, that is, very near to the critical night length (8 hours after beginning of dark period), it cannot be resumed. This is shown by the complete inhibition exerted by a red light interruption 8 hours after the beginning of a 20 hour dark period. Although 10 hours of darkness follow this interruption, no net hormone synthesis takes place. Ten hours of darkness following a red light interruption given two hours after the beginning of a 12 hour dark period are ample to initiate flowering. Thus it appears that the conditions which are necessary for resumption of the preparatory reaction do not exist after 8 hours of darkness following high intensity light. This conclusion is in harmony with the results of Carr (1952) and Wareing (1954), who found that light interruption near the beginning of extended dark periods (e.g. 60 hours) resulted in floral inhibition. The effect is probably related to the products of the high intensity light reaction. The application of sucrose, in the experiments of Liverman and Bonner (1953a), reversed the effect significantly.

The preparatory reaction appears to be inhibited only slightly, if at all, by applied auxin. Although the sensitivity of flowering to auxin shows some decrease during the course of the preparatory reaction, the data are not exact enough to draw a firm conclusion. The fact that the critical night length is little effected by auxin indicates, however, that the preparatory reaction is independent of auxin. A slight increase

in critical night length (Figure 29) as a result of auxin application, was found when a single inductive cycle was used. It is possible that the detrimental effect of auxin upon the small amount of florigen produced just after the critical night (see below) should cause an apparent increase in critical night length. When two inductive cycles were used there was no effect of auxin upon critical night length (Figure 30). With two cycles the small amount of florigen produced in the presence of auxin on successive nights may be enough to bring about flowering, even though this amount produced only once is insufficient.

If the decrease in effectiveness of applied auxin between 2 and 8 hours after the beginning of the dark period is real, there appears to be a discrepancy in the conclusion that auxin does not affect the preparatory reaction. Thus auxin seems to be effective during the preparatory reaction, yet the time this reaction reaches completion seems to be independent of applied auxin. Perhaps one must imagine a mechanism whereby the products of the reaction are affected by auxin, while the time for formation of these products is not. Thus the beginning of hormone synthesis (if it differs from preparatory reaction) might depend only upon the presence of some substance rather than upon a given amount of some substance.

3. Hormone Synthesis. Eight and one half hours after the beginning of the dark period (the point at which the preparatory reaction may not be resumed if interrupted, net florigen syn-

thesis begins.* The amount of hormone produced is proportional to the time by which the dark period exceeds the critical night length. This is shown by the relation of night length to flowering response.

The process of hormone synthesis, like the preparatory reaction, requires that the photo-receptor pigment be in the red-receptive form. This requirement is probably indirect. Synthesis continues only in the presence of the products of the preparatory reaction, and is stopped when these products are removed or destroyed by conversion of the pigment back to the far-red-receptive form. After a red light interruption, the process of florigen synthesis is stopped, but the synthetic reaction is not reversed as is the second reaction of the dark period. Thus florigen is synthesized from the critical night length on until either a light interruption is given, or until the plants are returned to sunlight.

The process of florigen synthesis is inhibited by applied auxin. This is clearly shown by the experiments relating applied auxin to night length (Figures 29, 30). It is also apparent from the time of auxin application curves that applied auxin becomes less effective in inhibiting flowering as hormone synthesis proceeds. This will be returned to below.

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* It is possible that a florigen precursor, rather than florigen itself, is produced during this time, and that this precursor is later converted to florigen by high intensity light. This is discussed below.

Since the most rapidly growing leaf (#3) is the one most sensitive to photoperiodic induction, it appears that the process of hormone synthesis takes place most rapidly in this leaf.

One might imagine that the third process, that of hormone synthesis, could be merely a continuation of the second process. According to this hypothesis, florigen synthesis would begin as soon as the pigment has been converted to the red-receptive form, but due to counter reactions (e.g. destruction), net synthesis of florigen would only be apparent after the critical night length. The facts available, however, indicate that this is probably not the case. The second (preparatory) process must start anew after a light flash, while hormone synthesis is only stopped, not reversed, by a red light interruption, as explained above. Further, applied auxin has little or no effect upon the critical night, which is controlled by pigment conversion plus the preparatory reaction, while auxin inhibits the process of hormone formation (or destroys its products).

4. Destruction of Florigen in the Dark. Under some circumstances florigen which has been produced during the third phase of the dark period is destroyed by further darkness. This is clearly shown by the experiments of Figure 36 which include induction by dark periods of 12, 16 and 20 hours. It may be seen that, in this case, the flowering response reaches a maximum after about 14 hours of darkness and decays away with longer dark periods. This appears to be clear evidence of

destruction of flowering hormone by the longer dark periods. Such destruction is even more clearly shown if a light interruption is given after 12 or 14 hours of the 16 hour night. Thus the light interruption stops florigen synthesis, as discussed above, and if conditions are otherwise appropriate, florigen destruction ensues.

Why is the process of destruction of florigen by long dark periods more apparent in some experiments than others? The complete answer to this question is as yet unknown, but some insight into the factors responsible may be gained from the results of an experiment in which the response of leaves of differing physiological age was studied (Figure 43). The experiment was conducted in the winter (November 14) when response of plants to a single inductive cycle is relativity poor (Figure 10). It may be seen that the #3 leaf was most responsive, as usual, and that the maximum response was reached at 13 hours after the beginning of the dark period. Longer darkness resulted in no further net synthesis, but rather a small amount of destruction. The #2 leaf gave no flowering response to dark periods shorter than 12 hours, reached a maximum at 13 hours, and by 14 hours a large amount of destruction had taken place. The #4 leaf showed no response until 13 hours, but between 13 and 14 hours there was considerable net synthesis, and no destruction of florigen as in the #2 leaf. Thus it appears that the ability to destroy florigen after a given period of time in the dark, varies with the physiological

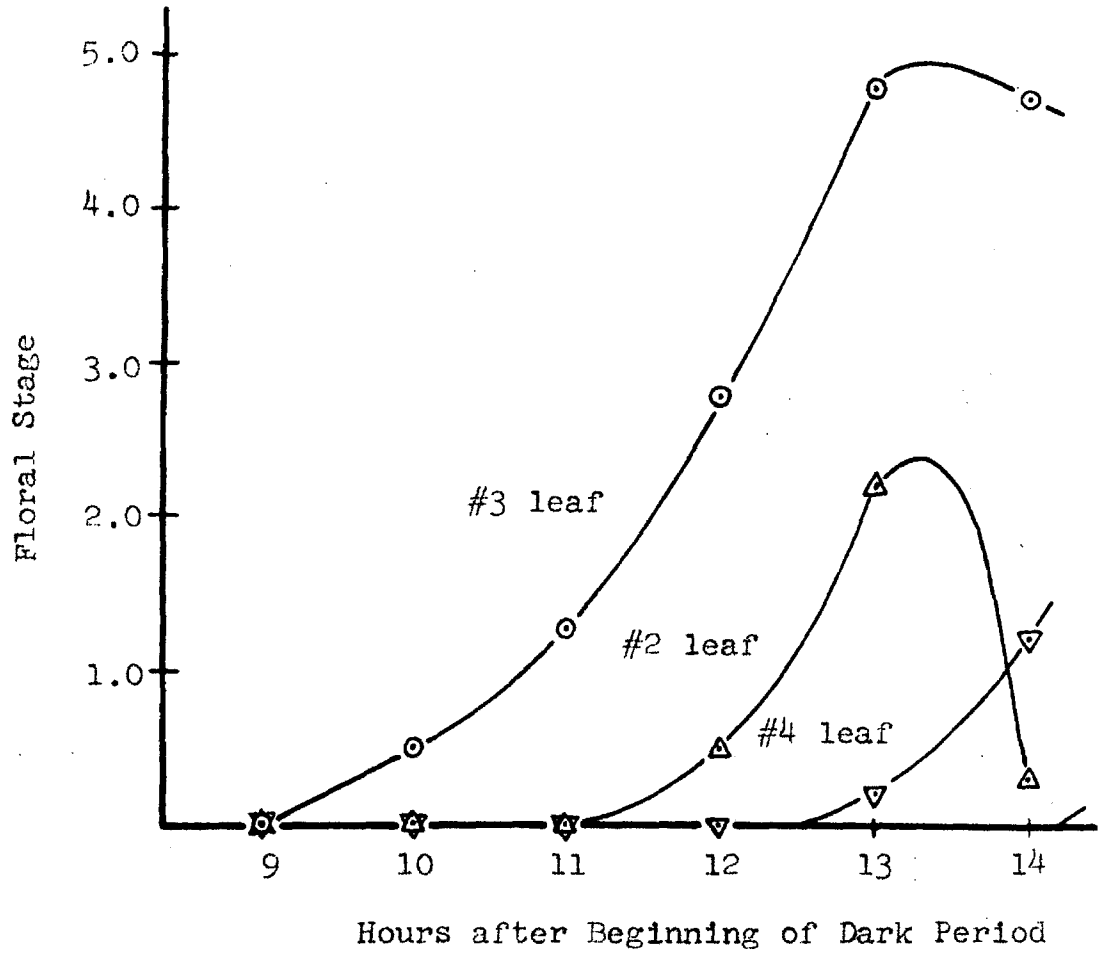

Figure 43. The flowering response of plants defoliated to a single leaf, as shown, to a single inductive cycle of varying length. Plants defoliated to the #5 leaf were vegetative at all night lengths. (Nov. 14, app.58)

age of the leaf, with the younger leaves showing the effect to the greatest extent.

Lockhart and Hamner (1954) have shown that applied auxin (following a light flash) causes inhibition of flowering. They suggest that variations in the auxin level within the plant (resulting from as yet little understood environmental conditions) cause the variability in appearance of spontaneous florigen destruction by excessively long dark periods, or short dark periods following a light interruption.

To the present author this explanation seems unlikely. In the first place, Lockhart and Hamner base their above conclusion partially on the fact that they were unable to consistently demonstrate inhibition of flowering by auxin applied (without a light flash) near the end of the dark period. In the present experiments this has been consistently possible. Even in different experiments, some of which show spontaneous florigen destruction, and some of which do not, consistent inhibitions have been obtained with applied auxin. It has been suggested above that the failure of Lockhart and Hamner to obtain floral inhibition with auxin applied near the end of an inductive dark period may have been due to the lack of potassium ions in their auxin solutions, or perhaps to insufficient control of humidity. In the second place, if the destructive effect of short dark periods following an inductive dark period terminated by light interruption were due to level of native auxin within the plant, then the effect should be nullified by application of an anti-

auxin. (as discussed above, the inhibitory effect of applied auxin must be due to formation of an auxin-receptor-complex, as in other auxin phenomena. Thus application of antiauxin should lower the concentration of this complex.) As shown in Table 4, application of antiauxin does not prevent the destructive effect of short dark periods following light interruption.

Thus the destructive effect of darkness is probably not due to the native auxin within the plant. The possibility that applied auxin may result in destruction of florigen is discussed below.

Lockhart and Hamner (1954 a) suggest that high intensity light following the dark period fixes the florigen produced by this dark period into a form which is stable when the plants are again returned to darkness. They have investigated the duration of high intensity light which is required at the end of the dark period in order to assure that a following exposure to darkness is ineffective in the destruction of florigen. They found that the longer the exposure to high intensity light, up to four to five hours, the less effective is a succeeding dark period in inhibiting flowering. Thus they regard the stabilization of florigen by high intensity light as constituting another step in the complete synthesis of florigen. There is no doubt but what the observations are correct. Their hypothesis concerning stabilization by high intensity light is, however, not the only one consistent with the available facts. In addition their hypothesis does not account for the fact that

plants left in total darkness for a long period of time (e.g. two weeks) produce flowers. Thus there is no absolute requirement that high intensity light follow the dark period.

It appears possible to the present author that the so called stabilization of florigen by high intensity light may have an entirely different basis. The high intensity light given at the end of the dark period may bring about a change within the leaf such that it is no longer capable of destroying florigen in darkness. It is clear that different lots of plants differ in their ability to bring about destruction of florigen in darkness (see above). In some experiments the effect is apparent even without a light interruption, while in others it is completely absent. In Figure 36 it would appear (as discussed above) that the destruction of florigen did not begin to take place until the plants had been in uninterrupted darkness for about 14 hours. Thus it may be that the ability of plants to destroy florigen in the dark increases during the dark period. The rate of increase may depend upon environmental conditions, etc. Exposure to a period of high intensity light may restore the plant to the physiological condition normally present at the beginning of an inductive dark period. A short dark period following this high intensity light would thus be ineffective in florigen destruction, as described by Lockhart and Hamner.

There may, therefore, be no fixation of florigen by high intensity light following the dark period--high intensity light may rather only serve to adjust the metabolism of the plant so

that it is incapable of hormone destruction (as it appears to be under some circumstances anyway). The end result of the two points of view is the same, that is high intensity light causes an apparent stabilization of florigen.

It may be noted that the destruction of florigen proceeds in low intensity light following an inductive dark period (Mann, 1940; Table 4). The process is therefore not dark dependent. According to the above hypothesis, low intensity light serves to stop hormone synthesis, but does not convert the plant to the condition in which destruction of florigen does not occur. Indeed, low intensity light seems to promote the destruction since it is much simpler to demonstrate florigen destruction under these conditions than after a light interruption followed by a short dark period.

Effect of Auxin

Two general effects of auxin have been outlined in Chapter II--an inhibition of the act of induction and a promotion of the development of the floral bud. An understanding of the mechanisms of the inhibitory effect would certainly increase our understanding of the act of induction. Since the general mechanism of auxin action is only poorly understood, our knowledge of the action of auxin upon flowering is also necessarily meager. Some deductions may be made, however, about the role of auxin in floral inhibition.

1. A striking characteristic of the time of auxin appli-

cation curves (Figure 34) is the sharp drop in the curve which occurs during the first two or three hours after the beginning of the dark period. Auxin increases in effectiveness with time, as it is applied at different times during these first two or three hours. The increase in effectiveness between 0 and 2-3 hours means that the auxin applied at the beginning of the dark period has lost some of its effectiveness in floral inhibition during this time. Whether the auxin has been destroyed or simply diverted to some action other than floral inhibition is not clear. Destruction of a small amount of auxin at the beginning of the dark period is not out of line with the data shown in Figure 31. Auxin applied up to 14 days before induction is still inhibitory, but the degree of inhibition decreases gradually with time, which indicates that destruction is taking place at a slow rate. A dilution effect due to growth of the leaf was minimized by using a leaf already mature at the time of auxin application. The significance of this drop is not clear at this time, but it is of interest to note that it coincides in time with the conversion of the photo-receptor pigment from the far-red-receptive form to the red-receptive form. It would be of considerable interest to know whether or not these two processes are related on the reaction level.

2. It has been shown above that the critical night length controlling reactions of the dark period--pigment conversion and preparatory reaction--are not affected by applied auxin.

Thus if pigment conversion is related to the increase in effectiveness of auxin during the first two hours, the end result is not affected by auxin. The same might be said of the second reaction.

3. It has been shown above that applied auxin inhibits florigen synthesis. The mechanism of the effect will be discussed below.

4. Perhaps the most significant, yet unexpected aspect of the time of auxin application curves (Figures 34, and 42) is that floral inhibition is caused by auxin applied after the inductive dark period. The rate at which floral inhibition by auxin decreases with time is fairly constant from about two hours after the beginning of the dark period up until the time when it becomes completely ineffective (when the flowering stimulus has been translocated out of the leaf). Although the rate does change somewhat over this entire range, there is no abrupt change at the end of the dark period.

The coincidence between the time at which auxin becomes ineffective and the time at which translocation of florigen out of the leaf is complete, is particularly striking. This would first suggest the possibility that the effect of auxin is upon translocation of the flowering stimulus out of the leaf. Upon closer examination, however, it is apparent that this is probably not the sole action of applied auxin. Hamner (1954) and Khudairi and Hamner (1954) have shown that translocation out of the leaf does not take place during the inductive dark

period (except in extreme cases such as continuous darkness for two weeks, etc.). Yet inhibition by auxin of a given concentration decreases as auxin is applied later in the dark period. The data of this thesis certainly show that the major effect of applied auxin cannot be upon translocation of florigen. They do not show that there is no effect of this kind. This must be decided by further experimentation.

It is clear then that florigen synthesis is inhibited by applied auxin. Can applied auxin also inhibit the stabilization reaction postulated by Lockhart and Hamner (1954)? Aside from the fact that such stabilization by light has not been conclusively demonstrated, it appears unlikely that auxin could inhibit it if it did exist. Lockhart and Hamner (1954 a) report that the so called stabilization takes place within 4 or 5 hours after the end of the dark period. Their test for stabilization consisted of returning the plants to dark and noting whether inhibition of flowering results. If plants are left in high intensity light, application of auxin continues to result in inhibition of flowering up to 20 hours after the end of the dark period. Indeed, the time at which auxin becomes ineffective in inhibition is not dependent upon the end of the dark period, but upon conditions at the time of the experiment (light intensity) (Figures 11 and 34). One might also expect that if auxin affected both a dark phase and a high intensity light phase of hormone synthesis, an inflection might be evident in the time of auxin application curves at the end of the dark

period. This is not so. The effect of auxin is then, not easily interpreted as an effect only upon translocation of florigen, nor as a double effect upon synthesis and the post-inductive high intensity light process.

Thus a single paradox makes both of these explanations unlikely: At least one phase of hormone synthesis is completely terminated by the end of the dark period or by a light interruption given more than 8 hours after the beginning of the dark period, and yet there is no apparent change in the rate at which auxin becomes ineffective in floral inhibition at the time synthesis is stopped. Let us consider a further possible mode of auxin inhibition of inductions. Let us suppose that applied auxin results in a destruction of florigen at any time after the first perceptible florigen has been synthesized up until florigen has been translocated out of the leaf (it has been shown that applied auxin does not cause a destruction of florigen once it has been translocated out of the leaf--Figure 21). The assumption must further be made that for a given auxin concentration, the rate of destruction is proportional to the amount of florigen present.

The concept of florigen destruction is in agreement with the facts at hand. As shown above, darkness may result in florigen destruction, and as discussed in Chapter II, florigen appears to be destroyed when the induced state is terminated, as when active buds are removed, etc. Hence it is not unreasonable to consider the idea that applied auxin may lead to

florigen destruction.

The idea that a constant fraction of the available florigen is destroyed by a given concentration of auxin is in at least qualitative agreement with the kinetic studies on auxin application with different night lengths (Figures 29 and 30). The control (no applied auxin) curve for flowering against night length is a measure of the amount of florigen produced at the various night lengths. The curves for the several auxin treatments show that auxin application inhibits flowering by an amount which is very nearly a constant fraction of the control.

Figure 44 illustrates the application of these assumptions concerning florigen destruction by auxin to the picture of florigen synthesis presented in Figure 42. In Figure 42 the curve representing florigen synthesis during the dark period might apply equally well to a precursor which is subsequently fixed by high intensity light. In the following discussion, however, it is assumed that it is the final florigen molecule which is synthesized in darkness. For the purpose of this discussion we will consider a concentration of auxin which inhibits flowering response by half if applied at the end of the dark period (when florigen synthesis is complete). This auxin concentration will then destroy half of the florigen produced during the dark period before translocation out of the leaf is complete. It will be assumed for purposes of the discussion that translocation is instantaneous. The slope of the broken line, A, indicates the rate of florigen destruction

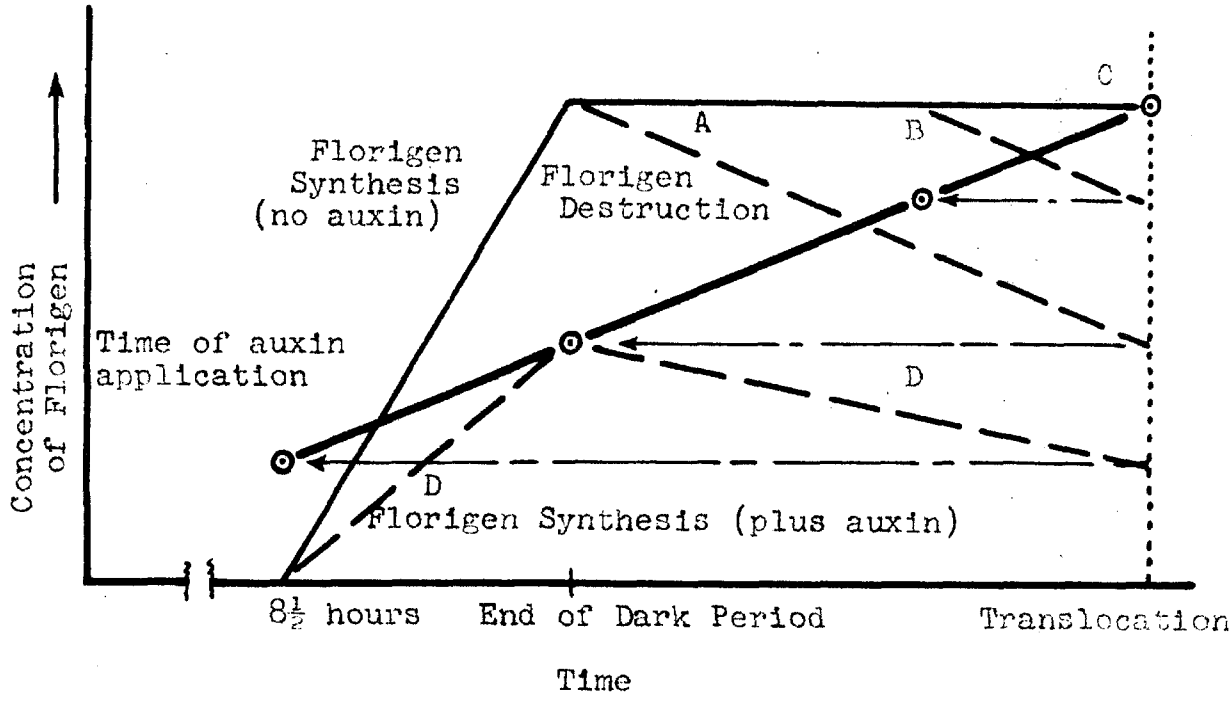

Figure 44. The kinetics of auxin inhibition of flowering, based on the hypothesis of auxin caused destruction of florigen, as explained in the text.

after the end of the dark period for this particular auxin and florigen concentration. Auxin applied at any time after the end of the dark period will result in a rate of destruction identical with that shown by A (same initial florigen concentration). If auxin is applied after some interval following the end of the dark period, the remaining time to translocation for destruction to be carried on is shorter, and hence the net destruction is decreased as indicated by line B. Auxin applied at the time translocation out of the leaf is complete will be ineffective in florigen destruction (Point C). Auxin applied before florigen synthesis begins will destroy a fraction of that which is subsequently produced. This is apparent as a decreased net rate of synthesis, indicated by the first part of the broken line, D. After the end of the dark period a further proportion of the florigen produced (and not already destroyed by auxin), will be destroyed before translocation out of the leaf is complete, as indicated by the second part of curve D. The final concentrations of florigen which might be expected to result from auxin application at the four times discussed above are plotted for each time of auxin application. The heavy line connecting these points represents the auxin-time of application curve as shown in Figures 34, 42, etc. It is evident that the kinetic treatment outlined above produces a qualitatively satisfactory result.

The above treatment is, however, greatly oversimplified. Even with this system a specific relation between time of

florigen synthesis and time from end of the dark period to translocation, is required to yield an exactly straight line for the time of auxin application curve. (With 50% inhibition, as above, time to translocation must be twice time of synthesis to yield a straight line.) But the time of auxin application curves are not exactly straight lines. They are curves changing gradually in slope over the entire range. A number of factors might account for this, but would complicate the above treatment considerably. Translocation is not instantaneous, as assumed above, and its rate also changes with time. Rate of florigen synthesis can seldom be represented by a straight line, as in Figure 44, but usually has the form of a saturation curve. The assumption was made above that, at a given auxin concentration, the rate of destruction of florigen is proportional to the amount of florigen present. In the treatment shown in Figure 44 this is only followed to a first approximation. The destruction curves are shown as straight lines. Actually the destruction curves should probably be hyperbolic, with a decreasing rate of destruction as more and more florigen has been destroyed. Additional experiments involving application of a range of auxin concentrations at different times during and after induction would help confirm or deny the assumptions made above. With these additional data these assumptions could be expressed as reaction rate equations, and a more detailed treatment of the type above could be applied to the data. The treatment shown in Figure 44 is

sufficient to indicate that the approach has promise.

It may be concluded that of the three suggested mechanisms of auxin inhibition of flowering (effect upon translocation, effect upon both synthesis and stabilization, and destruction of florigen at a rate essentially proportional to florigen concentration), the last best fits the experimental data. The proposed mechanism would seem to warrant critical experiments in the future. One aspect of the problems yet to be solved concerns the fact that auxin appears to decrease in effectiveness before the critical night length, as discussed above.

An actual mechanism for auxin-induced destruction of florigen is of course unknown, but one may visualize either a direct effect of the auxin-receptor-complex upon the hormone, or an indirect effect caused by rechanneling of energy from florigen maintainance to other processes. No precedents are known for auxin-induced destruction, and it is difficult to draw any conclusions when virtually nothing is known of the florigen molecule, or of the usual mechanisms of auxin action. The idea of energy redirection by auxin is simpler to apply in the present case. For instance, florigen may be kept from spontaneous decay in the leaf by the utilization of ATP as an energy sourse. Auxin might redirect the available ATP towards the process of water uptake, etc. This would result in a net decay of florigen in the absence of sufficient energy supply. The idea is pure conjecture, but some action of auxin which

would result in an indirect destruction of florigen is not out of the question.

The Role of Native Auxin in Flowering

These investigations have concerned the effects of applied auxin upon flowering. Can we draw from these results conclusions about the relation of the auxin normally present within the plant to flowering? It is reasonable to assume that auxin present within the plant may effect floral initiation qualitatively in the same manner as auxin applied artifically. The question is then, primarily one of the concentrations involved. Are the auxin concentrations within the plant high enough to ellicit responses similar to those obtained by auxin application?

That the native auxin may have an effect upon flowering is indicated by experiments with the application of antiauxin. Liverman (1952) and Bonner (1949) were able to obtain initiation of flowers by applying antiauxins to plants grown under light of an intensity just high enough to normally inhibit flowering. It would appear that the auxin normally present within the plant suppresses the net formation of florigen under these light conditions, but that application of antiauxin prevents such suppression, so that a net synthesis of flowering hormone may take place. Liverman (1952) also obtained flowering by application of antiauxin to plants otherwise inhibited in their flowering by a light interruption in the middle of a 10 hour dark period. Either some synthesis must have gone on before

the light interruption, or the light flash was not sufficient to completely saturate the photo-receptor pigment and reverse the reactions preparatory to synthesis, again allowing some synthesis to take place. In either case the applied antiauxin must have prevented destruction of florigen by the native auxin. The data of this thesis suggest that no synthesis takes place before the critical night length, and hence the first explanation seems unlikely. In Liverman's experiments the light flash was just sufficient to suppress flowering in controls. The plants had up to three mature leaves per plant, and probably these leaves caused some shading. Thus the lower leaves must have received an insufficient amount of light to completely saturate the photo-receptor pigment. None the less, at this night length the controls remained vegetative without antiauxin application. In any instance, the experiments with antiauxin indicate that the native auxin is of importance to flowering.

The experiments with application of cobalt (Figures 40 and 41), if the interpretation is correct (that applied cobalt results in an increase in the native auxin concentration), also indicate that the native auxin may effect flowering.

It has been suggested that the critical night length is controlled by the time required for the decay of the effective native auxin level within the plant to a level where florigen synthesis may proceed (Liverman, 1952). The data of this thesis indicate that the critical night length is controlled by two

reactions (pigment conversion, preparatory reaction) which are virtually independent of auxin. Thus it appears that the native auxin does not control the critical night length. A corollary to the idea that auxin controls the critical night length, is that plants maintained on long-day fail to flower because the native auxin level is too high for florigen synthesis to proceed. Although there is experimental evidence that auxin levels within the plant are higher on long-day than on short-day (Bonner and Liverman, 1953; Cooke), the conclusions of this thesis again must be that plants maintained on long-day fail to flower because pigment conversion and hence preparatory reaction cannot take place, and that this is a direct response to illumination, independent of auxin.

An argument against the idea that plants on long-day have a high enough auxin level to inhibit flowering is that applied auxin, if its concentration is high enough to inhibit flowering, brings about severe epinasty of the leaf petioles, yet plants on long day never show this epinasty. Auxin is usually applied, however by high concentration and low volume treatment. There are also complications due to penetration and localization of the applied or native auxin. Hence this argument in itself is not sufficient to permit conclusions to be drawn.

It would appear to the present author, that the native auxin concentration in the leaf may be much lower at all times than the auxin concentration in the leaf after artificial application of concentrations which inhibit flowering. Native

auxin must suppress net florigen synthesis under normal inductive conditions, but the suppression appears to be slight, and can be best demonstrated only under threshold conditions. The inhibition brought about by auxin application, therefore, may be an artifact. Although auxin within the plant is connected with specific phases of the act of induction, its influence may normally be only a minor complication, rather than a controlling factor.

Other Hypotheses on the Act of Induction

It is interesting to note that the extensive mass of data (see e.g. Lang, 1952) on the flowering reponses of plants to photoperiod has given rise to many theories and hypothesis which attempt to explain or at least coordinate the facts of photoperiodism. Naylor (1953) reviews the proposals of Hamner (1940), Harder and Bode (1943), Gregory (1948), Chouard (1943), the Withrows (1944), and Borthwick, Parker and Hendricks (1948). Lang (1952) also reviews some of the proposed mechanisms of photoperiodism. Bakhuyzen, in a series of papers (1947, 1951, 1953), proposes a mechanism to account for the photoperiodic responses of both long-day and short-day plants. Bünning (e.g. 1952) has shown a relationship between photoperiodism and an endogenous daily rhythm exhibited by many plants (e.g. soybean).

A number of authors, as well as some other than those mentioned above, have investigated or considered the question

of auxin in flowering, and some have suggested mechanisms for the noted effects. Liverman and Bonner (1953 b, and Bonner and Liverman, 1953) propose an interaction between light and auxin, as explained briefly above. Laibach and Kribben (1951, 1953) deduced the auxin status of some photoperiodically sensitive plants by observing epinastic movements of the petioles when the leaves are illuminated from one side or the other, and integrated their findings into a concept of flowering, which involves the native auxin levels within the plant. Resende (1950), and Resende and Viann (1952) have also made deductions about the auxin status of the plant from physiological responses under different light conditions. They conclude that flowering results from the balance within the plant between auxin and antiauxin. They reject the concept of a flowering hormone in favor of the auxin/antiauxin ratio. Still other authors have made proposals concerning the photoperiodic mechanisms, as discussed in some of the reviews referred to in the introduction (page 7).

A number of these hypothesis are simply descriptions or condensations of the facts of photoperiodism, as shown in Figure 1 (The Partial Processes). The sections in this chapter are largely an extension or finer definition of these descriptive proposals. Thus while the requirement for an uninterrupted dark period was represented by a single condition in the scheme of Hamner (1940), it has here been divided into three separate parts or reactions.

Some of the proposals include suggestions on kinetic changes in concentration of substances, precursors, etc. within the plant during induction. These suggestions have been alluded to above where pertinent. Again the conclusions of this thesis are largely an extension and finer definition of the concepts held by others (as in the discussion of the conversion of the photo-receptor pigment from one form to another). It has been made clear above when new proposals were put forth, or when disagreements with the concepts of other workers have arisen. Thus it would appear superfluous to further compare the work of the above investigators with the data presented in this thesis.

Even though we now have a massive amount of data in photoperiod, and even though there have been many proposals as to the photoperiodic mechanisms, we appear to be still a long way from complete solution of the problem. Since Garner and Allard's discovery in 1920, most of this massive amount of data has been gathered by the application of purely physiological techniques. Only whole plants have been used (very few exceptions), and the methods of experimentation could seldom at best be called biochemical. Yet this work has pointed out above all else that the real problems are of a biochemical nature. Although this is apparent to all, up to date no really suitable method of approach has been found to investigate the biochemical nature of photoperiodism. The deductions made, for instance, concerning the mechanism of the photo-receptor pigment

are striking, yet to date we have only pure conjecture as to how a pigment can halt a given reaction or allow it to proceed. The nature of the reaction which is controlled is also a complete mystery. The problem of hormone synthesis may have to await advances in understanding of protein synthesis for its solution. The work with auxin has led to some further understanding, yet real advances in this field must await a better understanding of the mechanism of auxin action in general. Thus although the only means of approach available at present are purely physiological, the real problems all appear to be biochemical.

Yet it is the author's belief that more is to be learned about photoperiodism by the physiological approach. Further refinement of techniques and wider application of quantitative kinetic studies will yield increasingly better founded conclusions about the intricate reactions taking place during photoperiodic induction. In spite of the many years of study on the effects of light, much is yet to be learned about these processes (as indicated by the recent work of Borthwick et al., 1952, on the effects of far-red radiation). Further work with application of auxin (perhaps with strains of plants homozygous in their response to auxin) may well provide clues for solution of the problem introduced by the inhibition of applied auxin after the end of the dark period.

Even in future physiological and biochemical research solves the problem of florigen synthesis as controlled by

photoperiod, the most intriguing problem of all may still remain: what are the physio-chemical mechanisms involved in the re-direction of growth by a chemical entity--a hormone or morphogenetic agent--from vegetative growth and the production of leaves and stem to reproductive growth and the production of flowers and ultimately the embryos which may become new organisms?

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APPENDIX

The data presented below are of importance in the careful evaluation of the material presented in the Tables and Figures, but are of secondary importance to the conclusions drawn within the text. In the Tables and Figures, the data below are referred to by number (e.g. app. 20). In general, data or experimental procedures given in the Tables, Figures, or text, are not repeated in the appendix below.

From January, 1954, until the present, the pH of all auxin solutions was adjusted between pH 7.0 and pH 8.0, unless otherwise noted. Before January, 1954, the pH of auxin solutions was adjusted to approximately pH 5.5.

Abbreviations are used in the following manner:

Aug. 3, 1954.....The first inductive dark period began on August 3, 1954.

Diss. 9 days.....Dissected 9 days after the beginning of induction (e.g. beg. of ind. August 3, Diss. August 12).

20 P/T.....20 Plants per Treatment.

#3 leaf.....Plants defoliated so that only the #3 leaf remained on each plant, as explained on pages 39, 40, 78, and 84.

Roman Numerals following appendix number refer to the number of the experiment in the authors original notebooks.

1. (XXXV) Development with continuous cycles.

July 3, 1953	10 P/T	
11 hour nights	all leaves	

2. (XXVI) Development with two 12 hour nights.

June 6, 1953	25 P/T ca. #5 leaf	Earhart Laboratories

3. (A-XXIX) Development with one 12 hour night.

July 12, 1954 — 12 P/T, #4 leaf

4. See (app. 35)

5. See (app. 36)

6. (A-XXXIII) One night -- various lengths.

July 7, 1954 — 12 P/T, #3 leaf
Diss. 9 days

7. (XXVIII) Continuous Inductive cycles.

July 9, 1953 — 20 P/T, #2,3,4 leaves.
Diss. 6 days

8. (A-VI) Leaf size and sensitivity.

Dec. 11, 1953 — 10 P/T
Two 16 hr. nts.
Diss. 13 days

9. (A-XIX) Age of plant -- from planting of seed.

March 17, 1954 — 20 P/T — weather cloudy
Three 16 hr. nts.
Diss. 9 days

10. (A-XLV) Temp. and light intensity --Night temp. 6°C below day temp.

July 26, 1954 — Earhart Laboratories
One 16 hr. night — #3 leaf
Diss. 11 days

(A-IX) Temp. and photoperiodic sensitivity --Night temp. 3°C below day temp.

Jan. 27, 1954 — Earhart Laboratories
One 16 hr. night — #3 leaf
Diss. 10 days

11. (XXI) Rate of development with IAA and NAA.

April 30, 1953 — 20 P/T auxin treated
Two 12 hr. nts. — 10 P/T controls.
4-5 leaf.

12. (A-V) pH of auxin solution -- no buffer. Green light, KCl.

Dec. 12, 1953	16 P/T	
Two 16 hr. nts.	#1,2,3, leaf--auxin treatments, KCl	
Diss. 14 days.	#3 leaf--green light.	

13. (A-X) pH of auxin solution -- citrate buffer.

Jan. 15, 1954	16 P/T	
Two 15 hr. nts.	#3 leaf	.001 citrate buffer.
Diss. 11 days		(leaves dipped)

14. (LXIX) Variability and applied auxin.

Nov. 7, 1953	#1,2,3 leaves	
Two 16 hr. nts.		plus $5.0X10^{-4}$ M NAA (leaves dipped)
One 15 hr. night		no auxin.
Diss. auxin treated, 13 days, no auxin, 9 days.		

16. (AXII) Time of auxin application --applied to stem near tip in lanolin paste (0.5% NAA).

Feb. 6, 1954	20 P/T	
One 16 hr. night	#3 leaf.	
Diss. 17 days		

17. See (app. 38)

18. See (app. 39)

19. (AXXVIII) Development with and without auxin; with and without shade.

May 18, 1954	16 P/T	
One 10 hr. nt.	#4 leaf	$5.0X10^{-4}$ M NAA (leaves dipped)

20. (LXVI) Auxin applied immediately after defoliation following induction.

Oct. 30, 1953	10 P/T	tips of defoliated plants
One 16 hr. night	#3 leaf	dipped in $10.0X10^{-4}$ M NAA.
Diss. 15 days		

21. (A-XXXII)

June 6, 1954	13 P/T	Plants shaded
One 15 hr. night	old #3 leaf	Tips of defoliated plants
Diss. 11 days	young #4 leaf	dipped in $6.0X10^{-4}$ M NAA.

22. (A-VIII) Removal of tips before and after induction.

Jan. 6, 1954	20 P/T	(0.5% NAA in lanolin
One 16 hr. night	Trimmed Jan 2 to old	added after induction,
Diss. 14 days.	#2 or young #3 leaf.	see bottom Figure 24)

23. See (app. 16)

24. (A-XXVI) Auxin applied to plants with tips removed just before induction.

April 24, 1954 14 P/T
One 16 hr. night #4 leaf
Diss. 23 days

Additional data, not shown in Figure 24:

Auxin Applied in Lanolin

Days after induction, auxin applied	0.25% NAA		0.50% NAA		1.00% NAA	
	% Fl	*	% Fl	*	% Fl	*
0	0	1	0	7	0	12
1	45%	3	62%	6	50%	10
2	83%	2	71%	0	89%	5
3	92%	1	73%	3	88%	6

Tips not removed -- flowered 100%
Tips removed, no auxin -- flowered 45%, 3 inactive tips*.
Stages of almost all buds were higher than eight.

* Number of plants out of 20 whose buds did not become active

25. See (app. 22)

26. (VI) Auxin concentration curves (up to app. 31).

March 10, 1953 10 P/T Further data in Figure 25.

27. (LVIII)

Aug. 26, 1953 14 P/T 10° C. during induction
Further data given in Fig. 25.

28. (LXII)

Oct. 2, 1953 16 P/T Further data in Figure 25.

29. (A-X)

Jan. 15, 1954 16 P/T Further data in Figure 25.

30. (LXIII)

Oct. 16, 1953	10 P/T	Further data given in Fig. 25.

31. (A-XLIII)

July 21, 1954	20 P/T	Further data given in Fig. 25.

32. (XLIV) Auxin -- Leaf size.

July 30, 1953	16 P/T	
Two 12 hr. nts.		
Diss. 12 days.		

33. (A-XVIII) Auxin -- night length.

March 11, 1954	20 P/T	
One inductive cycle	#3 leaf	$3.0X10^{-4}$M NAA--leaves dipped.
Diss. 13 days		

34. (A-XVI) Auxin -- night length.

March 1, 1954	20 P/T	poor weather
One inductive cycle	#3 leaf	
Diss. 10 days.		$6.0X10^{-4}$M NAA--leaves dipped.

35. (XXV) Auxin -- night length

May 26, 1953	20 P/T	
One inductive cycle	#4 or 5 leaf.	
Diss. 17 days		$1.5X10^{-4}$M NAA sprayed on leaves.

36. (VII) Auxin -- night length.

March 5, 1953	20 P/T	
Two inductive cycles	#4 or 5 leaf	
Diss. 21 days		$1.5X10^{-4}$M NAA sprayed on leaves (twice)

37. (AXI) Time of auxin application --days before induction.

Jan. 29, 1954	23 P/T	All leaves dipped in $6.0X10^{-4}$M NAA
Two 16 hr. nts.	Trimmed to #6 leaf	
Diss. 9 days	just before induction	

38. (A-XXIV) Time of auxin application--during induction.

May 1, 1954	20 P/T	
One 16 hr. nt.	#3 leaf.	dipped in $25.0X10^{-4}$M NAA,
Diss. 9 days		Rinsed, after 10 minutes.

39. (LXVIII) Time of auxin application.

Nov. 6, 1953	20 P/T	
One 17 hr. night	#3 leaf	dipped in $10.0X10^{-4}$M NAA,
Diss. 14 days		

40. (LIX) Time of auxin application.

Sept. 24, 1953	20 P/T	
One 13 hr. night	Old #4 leaf	dipped in $10.0X10^{-4}$M NAA.
Diss. 14 days		

41. (LI) Time of auxin application.

Aug. 3, 1953	16 P/T	
One 14 hr. night	#3 leaf	Sprayed with $4.0X10^{-4}$M NAA.
Diss. 13 days.		

42. (A-XXII) Time of auxin application.

April 10, 1954	16 P/T	weather poor
	#3 leaf	
Diss. 13 days		

43. (A-XXV) Red light interruption.

May 4, 1954	14 P/T
One inductive cycle	#3 leaf
Diss. 10 days	

44. (AXLVII) Red Light interruption.

July 28, 1954	10 P/T
One inductive cycle	#3 leaf
Diss. 9 days	

45. (A-XLVI) Intensity at various times during induction.

July 26, 1954	10 P/T
One 16 hr. nt.	#3 leaf
Diss. 9 days	

46. (A-XXIX) Auxin and/or light interruption during induction.

May 19, 1954	15/P/T	
One 16 hr. nt.	#3 leaf	Dipped in $20.0X10^{-4}$M NAA,
Diss. 9 days		rinsed after 5 minutes

(A-XXXVII)

July 6, 1954	16 P/T	
One 16 hr. nt.	#3 leaf	Dipped in $30.0X10^{-4}$M IAA,
Diss. 10 days		rinsed after 5 minutes.

47. (A-XLII) Auxin, various concentrations, and/or light interruption.

July 21, 1954	20 P/T	
One 20 hr. nt.	#3 leaf	Leaves dipped in auxin, rinsed after 5 minutes. Auxin follows red light interruption.
Diss. 9 days		

48. (A-XXXVI) Cobaltous ion -- concentration.

June 24, 1954	16 P/T	
One 16 hr. nt.	#4 leaf	Leaves dipped in solution.
Diss. 9 days		

49. (A-XXXVIII) Time of application -- Co Cl_2.

July 6, 1954	16 P/T	
One 16 hr. nt.	#3 leaf.	Leaves dipped in solution.
Diss. 11 days		

50. (XXXVI) Application of sucrose --florigen destruction.

June 30, 1953	27 P/T	Earhart Laboratories
One 16 hr. night	ca. #4 leaf	Sucrose sprayed on leaves
Diss. 13 days		

51. (XL) Application of antiauxin--florigen destruction.

June 17, 1953	16 P/T	
One 14 hr. nt.	#3 leaf	Antiauxin sprayed on leaves
Diss. 9 days		

52. (LII) Application of antiauxin--florigen destruction.

Aug. 4, 1953	15 P/T	
One 13 hr. nt.	#4 leaf	Antiauxin sprayed on leaves
Diss. 12 days		

53. (LV) Florigen destruction occurs in the leaf.

Aug. 13, 1953	8 P/T
One 16 hr. nt.	ca. #1,2,3 leaves
Diss. 9 days	

54. (A-XXXIV) Number of buds.

June 24, 1954	16 P/T
One 16 hr. nt.	#3 leaf
Diss. 9 days	

55. (A-XXIII) Temp. during induction.

April 16, 1954	20 P/T
One 16 hr. nt.	#3 leaf
Diss. 13 days	

56. (A-XLIV) Green light.

July 11, 1954 24 P/T
One 16 hr. nt. #3 leaf.
Diss. 10 days

57. (A-XLII) Potassium effects.

July 17, 1954 15 P/T
One 19 hr. nt. #3 leaf
Diss. 9 days

58. (LXV) Leaf size--night length.

Oct. 26, 1953 6 P/T
One inductive cycle
Diss. 21 days