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THE RELATION OF PLANT GROWTH
HORMONES TO PHOTOPERIODISM

by
Anson R. Cooke

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THE RELATION OF PLANT GROWTH HORMONES TO PHOTOPERIODISM

by Anson R. Cooke

The purpose of this study has been to determine if a change in auxin concentration is associated with flower initiation in plants responding to photoperiodic treatment and if this is so, is this change in auxin the cause of flower initiation.

In the first part of the study, the auxin content of several species of plants, as measured by the standard Avena method, was followed during the photoinduction period. In these experiments it was discovered that there is a decrease in auxin content in both long-day and short-day plants associated with the appearance of flower primordia. This decrease occurs regardless of the method of induction. If *Xanthium* plants are induced for only 3 days or if only a single leaf is used in induction, there is still a decrease in auxin accompanying the appearance of flower primordia. However, the auxin change for the first few days following the start of induction is quite variable. In a series of experiments it was shown that if a plant was suddenly changed from long days to short days there is an increase in auxin content for the first few days. Likewise, a plant suddenly changed from short days to long days generally has a decrease in auxin content for the next few days. However, these changes in auxin content are associated with the sudden change in photoperiod and not with any photoinduction process. After the

plants have come into flower, their auxin content depends upon the photoperiod under which they are growing. It was shown that plants growing under long days, whether they are flowering or vegetative, contain more auxin than plants growing under short days.

In the second part of this study an attempt was made to induce flowering by lowering the auxin content of the plant. It was shown that decapitation lowers the auxin content but this has no effect on the initiation of flower primordia. Likewise, continuous gamma irradiation lowers the auxin content of plants but in general, has no effect on flower initiation. High dosages of gamma irradiation that stunt the plant also suppress flower initiation. The plants receiving these high dosages of irradiation contained more auxin than the plants receiving lower dosages. However, high dosages of gamma irradiation hasten the flowering of Nicotiana rustica, var. Olsen 68. Treatment with antiauxins hastens the flowering response in Biloxi soybeans but will not bring about flower initiation under unfavorable photoperiods. Treatment of long-day plants with antiauxins may decrease the flowering response of these plants. In general, lowering the auxin content will not bring about flower initiation under unfavorable photoperiods.

It may be concluded from these experiments that flower initiation in both long-day and short-day plants is associated with a decrease in auxin content. However, this decrease in

auxin is not in itself the cause of flower initiation, as lowering the auxin content of the plant by various methods has not brought about flower initiation under unfavorable photoperiods.

This study was carried out under the direction of Dr. Felix G. Gustafson to whom the writer is indebted for much helpful criticism and suggestions. A part of the study was made possible through a fellowship at the Brookhaven National Laboratory, Upton, L.I., N.Y.

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INTRODUCTION

One of the least understood processes in the field of plant physiology today, is that of flower initiation. The cause of the rapid and dramatic change from a vegetative state to a reproductive state has been the object of much thought and experimentation. However, the study of such a problem is difficult due to the fact that in most cases the initiation of flowers cannot be predicted and hence controlled experiments cannot be readily devised. The great majority of plants grow vegetatively for a period of time, then suddenly initiate flowers. The factors bringing about this initiation are either not known or else very little understood at the present time.

In some instances, however, flower initiation can be controlled by changes in external factors. A number of plants, especially biennials, will initiate flower primordia subsequent to a low temperature treatment. This phenomenon is usually referred to as vernalization or yarovization (47). In this treatment the germinating seeds are exposed to a temperature of one to ten degrees centigrade for a varying period of time. The longer the cold treatment the sooner the plants will come into flower after being returned to normal growing temperatures. During the cold treatment some chemical reactions occur which will subsequently influ-

ence flower initiation. A definite chemical substance is postulated because when a stem of a vernalized plant is grafted onto the stem of a non-vernalized plant, it will cause flower initiation in the receptor plant.

Another external factor that causes flower initiation in some plants is the relative length of daylight to which the plants are exposed. This response to day length, discovered by Garner and Allard (22) in 1920, is termed photoperiodism. The response of different plants to photoperiods can be divided into four groups: short-day plants, long-day plants, day-neutral or indeterminate plants and intermediate plants. Short-day plants are those that flower when placed under conditions of short day length, generally less than 12 to 15 hours of light in a 24 hour cycle. In most short-day plants the day length that separates vegetative growth from reproductive growth is quite sharp and is referred to as the critical day length. Therefore, short-day plants in a stricter sense are those plants that flower when placed on a photoperiod less than the critical day length and remain vegetative when grown on a photoperiod longer than the critical day length. Long-day plants flower in photoperiods greater than the critical day length. This is generally a photoperiod longer than 12 hours. Day-neutral plants flower independently of the photoperiod and it is in this group of plants that flower initiation is so difficult to control. A small number of plants are placed in an intermediate group (1) in which flowering

occurs only in photoperiods of 12 to 14 hours. Photoperiods longer or shorter than this inhibit flower initiation.

In any study of flowering the initiation of flower primordia must be separated from the development of the flower primordia into macroscopic flower buds. This is especially true when working with plants sensitive to photoperiod as in many cases a specific treatment will produce flower primordia but will not necessarily result in flowers. It is usually necessary that a plant receive a definite number of cycles of the proper photoperiod to cause flower initiation. If after this minimum number of cycles the plants are returned to unfavorable photoperiods, the plants will still initiate flower primordia. However, in many cases these induced flower primordia will not develop into flower buds when the plant is returned to unfavorable photoperiods. Additional correct photoperiodic cycles are necessary for flower development and in general, an increased number of cycles results in a greater flowering response. When a plant comes into flower as a result of photoperiodic treatment, the process is known as photoperiodic induction.

The site of perception of the photoperiodic stimulus appears to be the leaves (8,65), although there is some evidence that stems and even roots are capable of perceiving this stimulus (19,34,40). This stimulation results in the formation of an unknown substance of a hormonal nature which moves out of the leaves to the meristems of the plant

and there brings about the initiation of flower primordia. The postulation of a chemical substance is based on grafting experiments (45), as was the case in vernalization studies, and also on experiments with branched plants. A branch from an induced plant grafted onto a plant grown under photoperiods producing vegetative growth will bring about flowering in this receptor plant. Many attempts have been made to isolate a flower forming substance from blossoming plants but so far the results of such experiments have not been very successful (6,53).

Vernalization and photoperiodism can both be used in controlled experiments to study the process of flower initiation. However, in vernalization the treatment must be applied several months before the flowers are initiated and hence in studying the chemical control of flowering, analyses must be made over an extended period of time. In photoperiodism, on the other hand, flower initiation usually occurs within two weeks after the beginning of the treatment. It is for this reason that considerably more attention has been paid to photoperiodism as a factor in flower initiation. The results of such experiments do not necessarily give us a true picture of the flower initiation process in all plants but a better understanding of the process in plants responding to photoperiod will undoubtedly aid in an understanding of flowering in those plants that do not respond to day length treatment.

HISTORICAL REVIEW

The discovery of photoperiodism in 1920 by Garner and Allard (22) was followed by many investigations to determine the nature of the photoperiodic stimulus. It was soon discovered that the site of perception of the stimulus was in the leaves (8,65). Lona (44) was able to show that a leaf could be removed from a plant and photoinduced while growing as a cutting. When the leaf was grafted on the plant it caused flower initiation in the receptor plant. As the morphological changes accompanying flower initiation take place at the meristems of the plant, it is necessary for this stimulus to flower to move from the leaves to the growing meristems. If a single branch, (29) or in some instances a single leaf (29), is subjected to a minimum number of photoperiodic cycles, a flowering stimulus results which travels to the noninduced parts and brings about flower initiation. Grafting an induced plant to a noninduced plant likewise results in the transmission of the floral stimulus and subsequent flowering of the noninduced receptor (31). Lang and Melchers (37) showed by means of reciprocal grafts that the flowering stimuli of long-day plants and short-day plants are interchangeable and it is quite possible

that the stimulus to flower is identical in these two groups of plants. It thus appears that the flowering stimulus formed in the leaves is of a hormone like nature. Cajlachjan (10) has tentatively named this flower hormone "florigen".

The movement of the flower hormone out of the leaves has received considerable attention. Moskov (46) showed that the stimulus travels only in living tissues such as the phloem, parenchyma and leaf mesophyll. The stimulus does not move in xylem cells or through sections of tissues that have been killed by means of steam. The movement appears to be a diffusion process involving living cells. Withrow and Withrow (64) showed that the diffusion of the stimulus across surfaces of grafted plants closely pressed together did not take place unless a living connection was made. Cajlachjan (10) showed that the flower hormone both ascends and descends the stem in the bark. This non-polar transport of the flower hormone is in direct contrast to that of the plant growth hormones which are translocated basipetally in the plant.

Several attempts have been made to extract an active material from flowering plants and to introduce this material into vegetative plants in an effort to induce flowering. The results of such experiments have been inconclusive or irreproducible. Bonner and Bonner (6) were able to induce floral primordia in *Xanthium* with an aqueous extract

of floral primordia of a palm. However, further extracts made from other specimens of the same species or from other species of palms were ineffective. Roberts (53) reports that he obtained an active extract with an organic solvent but other attempts to extract such materials with the same solvent have met with no success. Failure to obtain an active extract does not necessarily negate the idea that a chemical substance is responsible for flower initiation. It may only be that an effective method of extraction has not yet been devised or that once it is extracted it has been impossible to get it back into a vegetative plant in an active form.

In spite of the large amount of indirect evidence that tends to show the existence of a definite flower hormone, there is still the possibility that the native growth hormones may account for the change from vegetative to reproductive growth.

After careful examination of the problem Cholodny (12) came to the conclusion that auxins were involved in flower initiation. The term auxin is used in this thesis to designate any substance capable of causing cell elongation while the term plant growth hormone is used for any naturally occurring substance that brings about cell elongation. The term antiauxin is used for any compound that brings about competitive inhibition of growth; the inhibition in this case can be reversed by the addition of an auxin.

The evidence that auxin is involved in flower initiation is based on a number of investigations. The first direct control of flowering by auxin treatment was demonstrated by Clark and Kerns (13). They found that low concentrations of the auxin naphthaleneacetic acid induced formation of an inflorescence in the pineapple plant when applied as a foliage spray. However, high concentrations, especially when applied in solution at the apex of the plant, delayed flowering. Van Overbeek (66) demonstrated that as little as 50 micrograms of naphthaleneacetic acid per plant were sufficient to induce flowering in the pineapple. Cooper (14) found that pineapples, as grown under Florida conditions, responded somewhat differently to auxin treatment. October treatments over a wide range of concentrations induced premature flowering, while July treatments in the same range of concentrations did not induce flowering. It has been suggested (67) that since flowering can be artificially induced in the pineapple by the application of auxin, then flowering under natural conditions may be the result of an increased level of free auxin which results from an increased rate of conversion of bound auxin into its free form.

The effects of auxin treatment in pineapple appear to have a direct relationship to flower initiation. This is not the case, however, in the litchi plant (54). Here, auxin is effective only in hastening the maturation of the vegetative terminals and preventing their further growth.

If a 100 p.p.m. solution of sodium naphthalene acetate is sprayed on a litchi plant, vegetative growth immediately stops and in due time the hormone sprayed terminals mature and flower. If high concentrations of the auxin are used the succulent terminals are killed. As a result the previous growth, which has been mature for at least 3 to 4 months, becomes the actual terminal and in due time this mature portion will develop an inflorescence. Another case of auxin-induced flowering was reported by Laibach and Kribben (35). When the undersides of the primary leaves of a cucumber plant were smeared with a naphthaleneacetic acid paste, normal flowers developed in the axils of the second leaf. These flowers were induced much earlier than the corresponding flowers on the control plants. This treatment was most effective when the primary leaves were only half grown and the treatment usually resulted in the formation of female flowers instead of the normal male flowers. In this instance, as in the previous two examples, naphthaleneacetic acid is more effective than the naturally occurring indoleacetic acid growth hormone. Other attempts to induce flowering, especially in photoperiodically controlled plants, have met with little success.

Auxin treatment of plants responding to photoperiod will not in itself bring about flower initiation. A few instances have been reported, however, where auxin treatment has hastened and increased flowering of long day plants. Leopold and

Thimann (41,42) found that in barley, treatment with auxins at the concentration of 10 mg./l. had an inhibitory effect on the vegetative buds and that the low concentrations which produced vegetative inhibition definitely increased the number of flower primordia. The application of auxins, however, did not induce flower primordia in plants exposed to day lengths unfavorable to flowering. At high concentrations of auxin the number of flower primordia was decreased, the decrease being approximately proportional to the logarithm of the auxin concentration. Hyoscyamus niger and Silene armeria have been induced to flower by treatment with auxin (43) when grown just below the threshold photoperiod. With certain intensities of supplementary light most of the treated plants formed flower primordia, whereas the controls remained vegetative. With higher light intensities, floral initiation in the treated plants was significantly accelerated, but at high light intensities that normally permit rapid flower formation, auxin treatment inhibited flowering. Inhibition of flowering in Calendula officinalis by spraying the leaves with growth substances has also been reported (15).

Auxin treatments of short-day plants usually result in an inhibition of flowering. Thurlow and Bonner (7,61) inhibited flower initiation in Xanthium by spraying the leaves with solutions of indoleacetic acid and naphthalene-acetic acid. When the plants whose response to photoperiodic induction had been inhibited by auxin were subsequently

grafted on vegetative receptor plants, they did not bring about any flowering response in the receptor plants. This would seem to indicate that the effect of applied auxin is to inhibit the production of the stimulus to flower in the induced leaves or its transport. Green and Fuller (27) immersed the roots of petunia plants in solutions of auxin and showed that such a treatment greatly delayed flowering. The effect of auxin, therefore, is probably not an indirect effect of leaf injury but is directly related to the formation of the flowering stimulus. Auxin treatment has also been shown to inhibit flower initiation in the short-day plant Kalanchoe blossfeldiana (30). Naylor (48) attempted to prevent flowering in Xanthium by introducing large quantities of growth substances including indoleacetic acid, beta-naphthoxyacetic acid, 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid into the plant. None of these completely prevented floral initiation but in all cases the treated plants were weeks later than the controls in developing flowers.

Considerable work has been done to induce flowering with compounds that possess antiauxin activity. Bonner and Thurlow (7) were able to suppress the effect of auxin in inhibition of floral initiation with 2,4-dichloroanisole. When this antiauxin compound was used alone it hastened floral development. In a later experiment, Bonner (5) found that the application of 2,4-dichloroanisole and triiodobenzoic

acid to vegetative *Xanthium* plants resulted in the initiation of "flower-like" buds, although true flowers were not obtained. This effect was obtained under conditions of long days in which the untreated plants remained in the vegetative condition. The effect of triiodobenzoic acid was first noted by Zimmerman and Hitchcock (69) in 1942. When tomato, a day-neutral plant, was treated with this compound it was noted that axillary buds which normally produce leafy shoots were induced to grow flower clusters. The main shoot of the plant also lost the shoot producing bud and terminated in flower clusters. Galston (20,21) sprayed vegetative and photoinduced Peking soybean plants with .0004 M triiodobenzoic acid. No vegetative plants were induced to flower but photoinduced plants showed a tenfold increase in the number of flower buds formed. Another compound that appears to possess some type of antiauxin activity is maleic hydrazide. Naylor (49) found that this compound is a very good inhibitor of flower buds as well as of vegetative buds. However, suppression of flower primordia in this case might very well be due to a general suppression of growth. Flower initiation generally takes place only when the plant is in an actively growing state.

Summarizing the preceeding experiments we can draw several conclusions as to the role of auxin in flower initiation. In short-day plants flower initiation can be inhibited by high levels of auxin. Treatment with antiauxin compounds increases

the flowering response but does not initiate it. It thus appears that flower initiation in short-day plants is associated with a decrease in auxin content in the plant. The role of auxin in flower initiation in long-day plants is not so clear. In some long-day plants auxin treatment increases the flowering response while in other species auxin treatment seems to suppress flowering. No results have been obtained with antiauxin treatment of long-day plants. Whether flowering is naturally accompanied by a change in auxin level has not been studied in any detail. Cajlachjan (11) found that flowering long-day plants contained more free growth hormones than vegetative plants while the reverse was true for short-day plants. However, the plants used in his experiments were in flower and any differences in auxin content might be attributed to the difference in day lengths to which the plants had been subjected and are not necessarily related to flowering.

The purpose of the experiments to be reported in this thesis will be to determine if a change in auxin content does accompany flower initiation in photoperiodically induced plants and, if this is so, whether these changes in auxin content are responsible for flower initiation.

METHODS OF INDUCTION, AUXIN ANALYSIS AND PLANTS USED.

The plants used in this investigation have been used frequently before and are well known as to their reaction to photoperiod. These included the short-day plants, soybean (Glycine Soja (L) Sieb. and Zucc., var. Biloxi), tobacco (Nicotiana tabacum L., var. Maryland Mammoth) and cocklebur (Xanthium italicum Moretti). The long-day plants included stock (Matthiola incana (L) R. BR.) and snapdragon (Antirrhinum majus L., var. Afterglow). The seeds were germinated in flats and when the seedlings had attained the desired size they were transferred to 2 inch and later to 4 inch pots. During their vegetative growth the plants were kept under photoperiods that inhibited flowering. The short-day plants were given supplementary light to provide day lengths of 16 hours. The long-day plants were kept vegetative by removing them from the greenhouse to a dark room each afternoon and returning them to the greenhouse the next morning. They received an 8 hour photoperiod.

As it has been shown that the temperature often affects the critical day length (36,50,51) in photoinduction, it was necessary to keep the experimental plants at some day length far removed from the critical day length, as the temperature of the greenhouse and darkroom could not be closely controlled.

However, an attempt was made to maintain the temperature of the darkroom the same as that of the greenhouse. This was accomplished by a blower which drew air into the darkroom from the greenhouse.

As soon as the plants had reached the proper size they were photoinduced. This was accomplished in short-day plants by putting them under an 8 hour photoperiod. At the same time a similar group of plants were left under a 16 hour photoperiod as controls. Long-day plants were induced by just the reverse treatment, that is, the experimental plants were put under a 16 hour photoperiod. The use of control plants was necessary due to the variation in auxin content of the plants and the variation in sensitivity of the *Avena* test plants from day to day. Gustafson (28) found that, in general, plants contained more free auxin in the morning than in the afternoon if the light intensity was great as on a sunny day; however, the reverse was true if the day was cloudy. He also noted that, in general, more hormone was present when plants were grown at high temperatures than when grown at low temperatures. Because these external factors of light and temperature could not be controlled to any great degree, the results of growth hormone analysis were expressed as percentage increase or decrease over the controls. In this way, the effect of external factors was more or less cancelled. As a further measure of control, the material for analysis was always collected in the evening between 5

and 6 o'clock.

Wildman and Muir (63) in their study of free auxin in plant tissues proposed the following method of extraction as giving the closest approximation of free auxin in the tissues. The material was immediately frozen and then lyophilized. This was followed by an ether extraction at 0° C. for a period of 16 hours. Preliminary experiments in this investigation show that rapid drying of leaf tissue in a "speed" oven gave comparable results to lyophilizing the tissue. The speed oven utilizes an infra-red lamp and forced draft. The results of two typical experiments are shown in Table I.

TABLE I

A comparison of the extractable free auxin from lyophilized and oven-dried plant tissues in degrees curvature of Avena coleoptiles.

	<u>Lyophilized</u>	<u>Oven-dried</u>
Tomato leaves (2.0 g. fresh wt.)	14.8	13.2
Soybean leaves (3.5 g. fresh wt.)	13.8	14.3

The following was the procedure used for free auxin extraction in the present investigation. The material, after being weighed, was placed in wire baskets in the speed oven and dried for 20 minutes at a temperature of 75° C. This resulted in complete drying. The dried material was then transferred to 125 ml. flasks and covered with 75 ml. of peroxide free ether. The material was allowed to extract overnight (about 12 hours) at 5° C. The ether was decanted

from the material and the sample washed with a small portion of fresh ether. The combined extracts were then reduced in volume to a few milliliters which were quantitatively transferred to melted agar and poured into a mold. The solidified agar block was cut into 12 small blocks which were used for the Avena assay. The standard Avena assay (62) was used for all hormone tests.

Briefly, the Avena assay is as follows. The Avena seeds are husked and germinated in red light at a temperature of 26° C. At the end of 30 hours the seedlings are placed in glass holders and allowed to grow in total darkness for another 40 hours. At this time one or two millimeters are removed from the apex of the coleoptile. This decapitation greatly increases the sensitivity of the coleoptiles to applied auxin. Three hours later another millimeter of the coleoptile is removed with decapitating scissors in such a way as to leave the primary leaf intact and then the primary leaf is pulled loose in the coleoptile. The agar block, containing the test material, is then placed on one side of the cut surface of the coleoptile, resting against the primary leaf. A rack of 12 seedlings is used for each test. The coleoptiles are allowed to grow for 90 min. as this length of time has been shown to result in maximum curvature. A permanent record of the resulting curvature is obtained by making a shadowgraph of the rack of coleoptiles. The curvature is measured on the shadowgraph by means of a transparent celluloid protractor and the average curvature for

the rack is determined. The curvature of the coleoptiles is proportional to the concentration of the applied auxin up to a maximum of 25° curvature. Therefore, the material was always diluted so that the resulting curvature was between 5° and 25° . The standard deviation for a rack of 12 coleoptiles was generally between 1.0° and 2.0° or approximately 10 % of the average curvature. All experiments in which the auxin content of plant material was assayed were repeated at least once although in most cases the results of a typical experiment are reported in this thesis.

RESULTS

Inhibitor in Xanthium Leaves.

Much experimental work has already been done on Xanthium and it was therefore only natural to use it in this investigation as one of the short-day plants. However, preliminary extractions made to determine the amount of leaf material needed for an auxin analysis failed to show any activity. Either the leaves contained no free auxin or else they contained an ether soluble material that masked any auxin activity in the Avena test. To test this latter suggestion, known concentrations of indoleacetic acid (IAA) were added to an extract of Xanthium leaves. One tenth of a gram of dry leaves was extracted with ether and the extract evaporated down to dryness. IAA was added to this extract and the mixture tested on Avena coleoptiles. The results are shown in Table II.

TABLE II

Inhibition of IAA induced growth in Avena coleoptiles
by extract from Xanthium leaves.

<u>Concentration of IAA</u>	<u>Curvature in degrees</u>
Extract	0.0
30 µg/l. IAA	21.0
30 µg/l. IAA + extract	0.0
60 µg/l. IAA + extract	6.0
90 µg/l. IAA + extract	11.3

These results show that there is an inhibitor in Xanthium leaves and that the inhibition can be overcome by the addition of IAA. As the Avena test actually assays total growth activity, that is, the sum of the activity of all extractable auxins and inhibitors, the inhibitor in Xanthium leaves was further studied.

Stewart (56) found an ether extractable substance in the cotyledons and leaves of radish plants that was capable of causing growth inhibition in the Avena test. This inhibitor, which has approximately the same molecular weight as IAA, can readily be hydrolyzed to form a growth promoting substance. As the inhibitor in Xanthium leaves might be of this type, acid and alkaline hydrolysis of the tissue was tried. Three tenths of a gram of dried leaves was hydrolyzed in 1 N NaOH or in .1 N HCl for 20 minutes at 15 lbs. pressure. The mixture in each case was then adjusted to a pH of 4.5 and extracted with ether over night. The ether extract was separated from the mixture, reduced in volume and the auxin activity tested in the standard manner. No growth activity was noted in either the acid or alkaline treated material. This indicates that the inhibitor is not a bound form of auxin or an auxin complex.

Attempts were made to separate the inhibitor in the leaf extract from the auxin that might be present by standard diffusion methods. An agar block containing the leaf extract was placed on top of 3 plain agar blocks and diffusion was

allowed to take place at 25 °C. for three hours. No activity was noted in any of the blocks. Even when 60 µg IAA was added to the extract of 3 grams fresh weight of leaves, no growth activity was obtained. This indicates that the molecular weight of the inhibitor is approximately the same as that of indoleacetic acid. Attempts were made to oxidize the inhibitor with 3 % H_2O_2 to an inactive form. The extract was treated with H_2O_2 , the peroxide removed with catalase and the resulting extract added to a known amount of IAA so that the final IAA concentration was 30 µg/l. Here again, no curvature resulted. The activity of the inhibitor is not destroyed by oxidation with H_2O_2 .

Goodwin (23,24) has isolated a number of natural inhibitors and has tested these growth inhibitors and their derivatives on the growth of Avena roots. These inhibitors are derivatives of coumarin and are especially active in inhibiting root growth. Thimann and W. Bonner (60) showed that coumarin also inhibits cell enlargement in Avena coleoptiles and in etiolated pea stems. The molecular weight of these coumarin compounds is in the range of that of the inhibitor in Xanthium leaves; therefore, an attempt was made to isolate such a compound from Xanthium. Most of the coumarin derivatives are fluorescent and can be identified by the relation between the intensity of fluorescence and the hydrogen ion concentration of the solution. The method of extraction followed was that of Goodwin (23). Two hundred grams

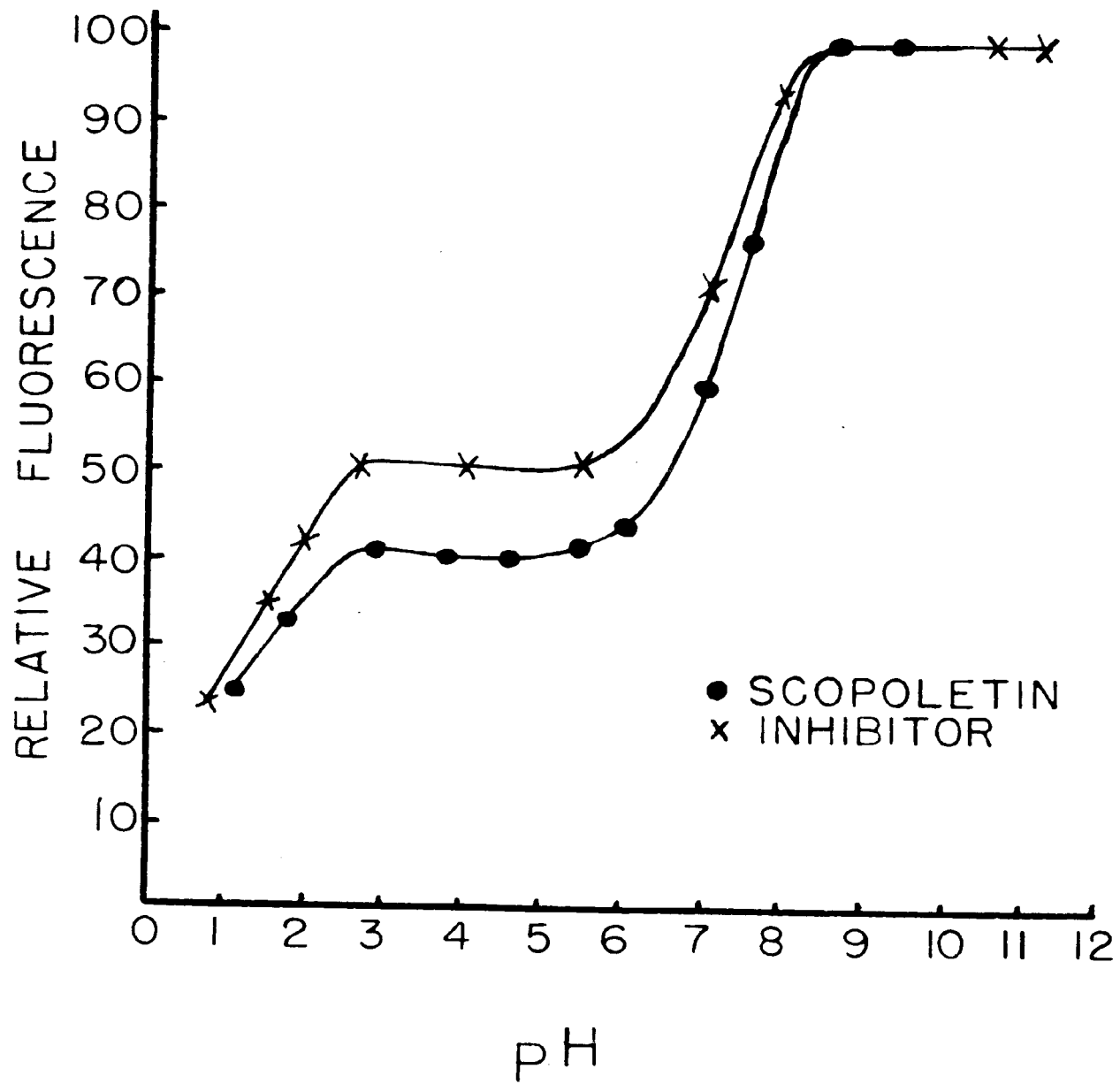
of leaves were covered with 300 ml. of .05 N H_2SO_4 and 100 ml. of chloroform and placed on a mechanical shaker overnight. The chloroform was then decanted and another 100 ml. were added. After another 12 hours the second lot of chloroform was removed. The two chloroform fractions were combined and shaken repeatedly with 25 ml. portions of .005 M ammonium hydroxide which removes the inhibitor from the chloroform. These portions were then combined, acidified to a pH of 2.5 and extracted with 5 portions of 50 ml. of chloroform. The chloroform extract was evaporated to dryness under vacuum and the residue taken up in a small quantity of .005 M ammonium hydroxide for fluorometric study. The fluorescence was measured at various hydrogen-ion concentrations on a Lumetron fluorimeter-colorimeter employing filters normally used for thiamine determinations. The fluorimeter was set to 100 with a 1 mg./l. quinine sulfate standard. The pH was varied by adding a drop or two of concentrated ammonium hydroxide or HCl to the solution. Upon irradiation of the solution with ultra-violet light a blue fluorescent color was noted. The intensity of the fluorescence varied with pH as is shown in Figure 1.

A comparison of the pH-fluorescence curve with published data for coumarin derivatives (24,25) indicated that the inhibitor might possibly be scopoletin or a closely related compound. Through the kindness of Dr. R. H. Goodwin, a sample of scopoletin was obtained. This scopoletin had been ex-

tracted from *Artemisia* in the Wellcome Research Laboratories, London, England. The pH-fluorescence curve of this compound turned out to be slightly different from that of the inhibitor as is shown in Figure 1. The scopoletin, when run in buffered solutions, gave an identical curve to that obtained by Goodwin for the same compound.

The fluorescing extract obtained from *Xanthium* leaves was tested for its IAA inhibiting activity on *Avena* coleoptiles. The same extract as was used in the fluorescence studies was adjusted to a pH of 4.5 and added to IAA so that the final IAA concentration was 60 µg/l. No curvature resulted when this mixture was tested on *Avena*, indicating that the inhibitor is present in the fluorescing extract. However, when pure scopoletin was tested in this manner for growth inhibiting activity, it showed very little inhibition of growth. A 10^{-4} M solution of scopoletin decreased the activity of 30 µg IAA by only 20 %. This concentration of scopoletin is considerably greater than would be obtained from the extract of *Xanthium* leaves. Because of the difference in pH-fluorescence curves and activity in the *Avena* test, it must be concluded that the inhibitor in *Xanthium* leaves is not scopoletin. The inhibitor may, however, be closely related to this type of coumarin derivative. A number of coumarin derivatives have been isolated from plants. Bennett and J. Bonner (3) isolated a group of furocoumarins from *Thamnosma montana* that were highly toxic to the growth

Fig. 1. The relationship between fluorescence and pH for scopoletin and the inhibitor extracted from Xanthium leaves. The pH was varied by the addition of HCl and NH_4OH . Fluorescence was measured on a Lumetron fluorimeter using the standard thiamine filters.



of tomato seedlings. As the inhibitor of Xanthium leaves could not be separated from the auxin in the leaves, it was impossible at the time to follow the changes in inhibitor concentration during the induction of flowering.

Changes in Auxin Activity During the Induction of Short-Day Plants.

Since it has been shown that the leaves of Xanthium contain a very strong inhibitor and cannot, therefore, be used, some other part of the plant would have to be employed in investigating the auxin changes occurring during photoinduction. In a preliminary experiment, the distribution of auxin in various parts of the plant was investigated. The results are shown in Table III.

TABLE III

Distribution of auxin in Xanthium as measured by the Avena test.

Apex (including immature leaves)	0.22	°/g. fresh wt.
Mature leaves	0.00	°/g. fresh wt.
Stem and petioles	3.17	°/g. fresh wt.

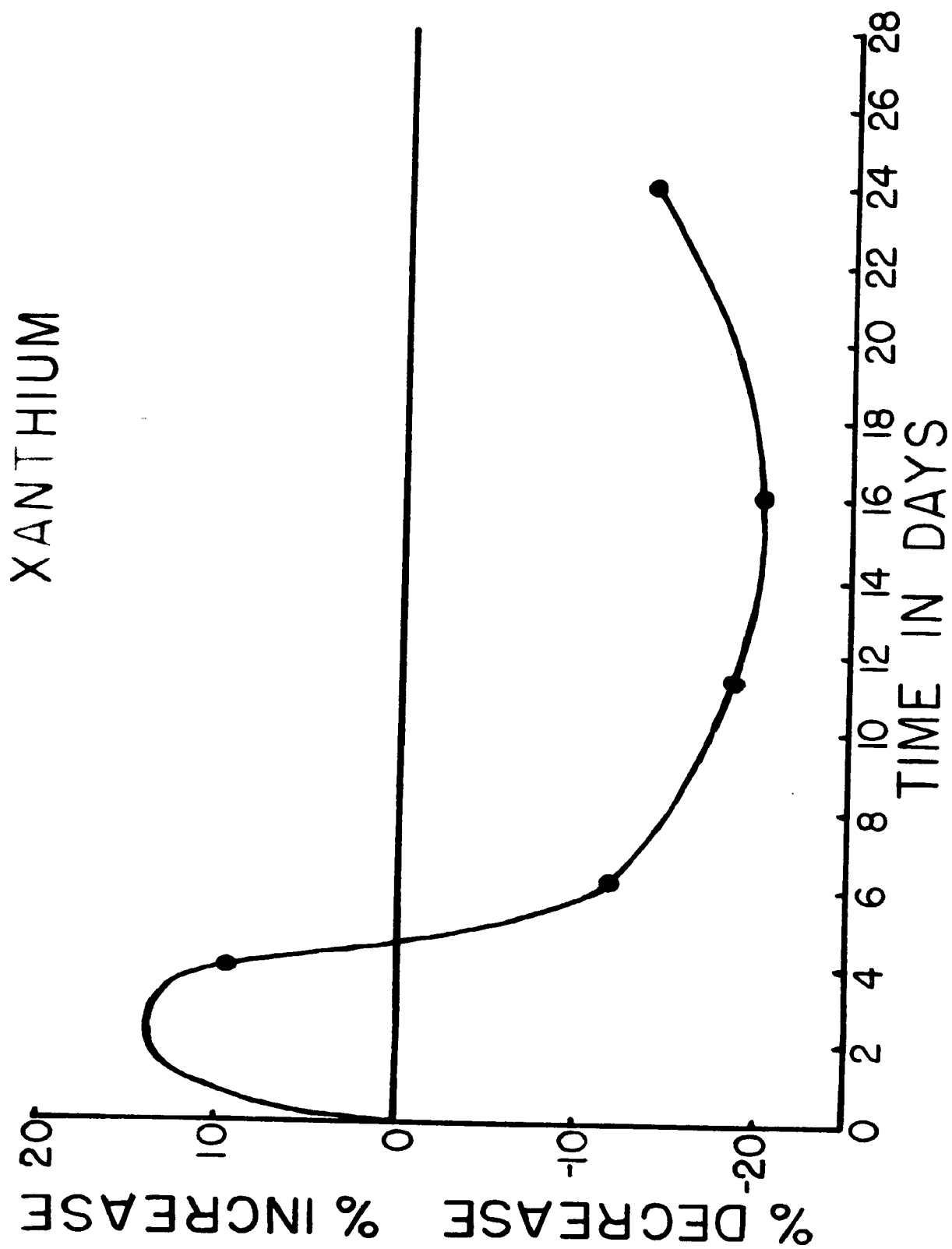
The results show that even the immature leaves contain a considerable amount of inhibitor. The stem, however, contains a large amount of extractable auxin and probably very little inhibitor. The auxin content of the stems was therefore followed during the induction of these plants. As a

standard procedure the top centimeter of the stem as well as the leaves were discarded and the next 15 centimeters of the stem used for auxin analysis.

On April 10, a group of vegetative Xanthium plants were divided into two groups. One group was left under continuous long days (16 hrs.) while the other group was placed under continuous short days (8 hrs.). Samples from each group were taken at intervals for a period of 24 days and analyzed for their auxin content. The data for this experiment are expressed as percentage increase or decrease over the vegetative controls. A graph of the data is show in Figure 2.

For the first two days there was an increase in auxin content in the induced plants. This was followed by a decrease in auxin; the induced plants actually had less auxin than the controls after the fifth day of treatment. At the end of 17 days the auxin content began to increase once again in the experimental plants, but at the end of 24 days it was still considerably below the control. During the induction period, the sampled plants were examined under a binocular dissecting scope for evidence of flower primordia. The terminal bud of the Xanthium plant is the first to differentiate inflorescence primordia. In the above experiment the inflorescence primordia could be detected after 6 days of treatment. The morphological change from vegetative to reproductive growth took place during the decrease in auxin content but before the auxin content reached its minimum value. The

Fig. 2. Changes in free auxin content during the photoinduction of Xanthium plants. The experimental plants were kept under continuous short days. The auxin content is expressed as percentage increase or decrease over the vegetative controls.

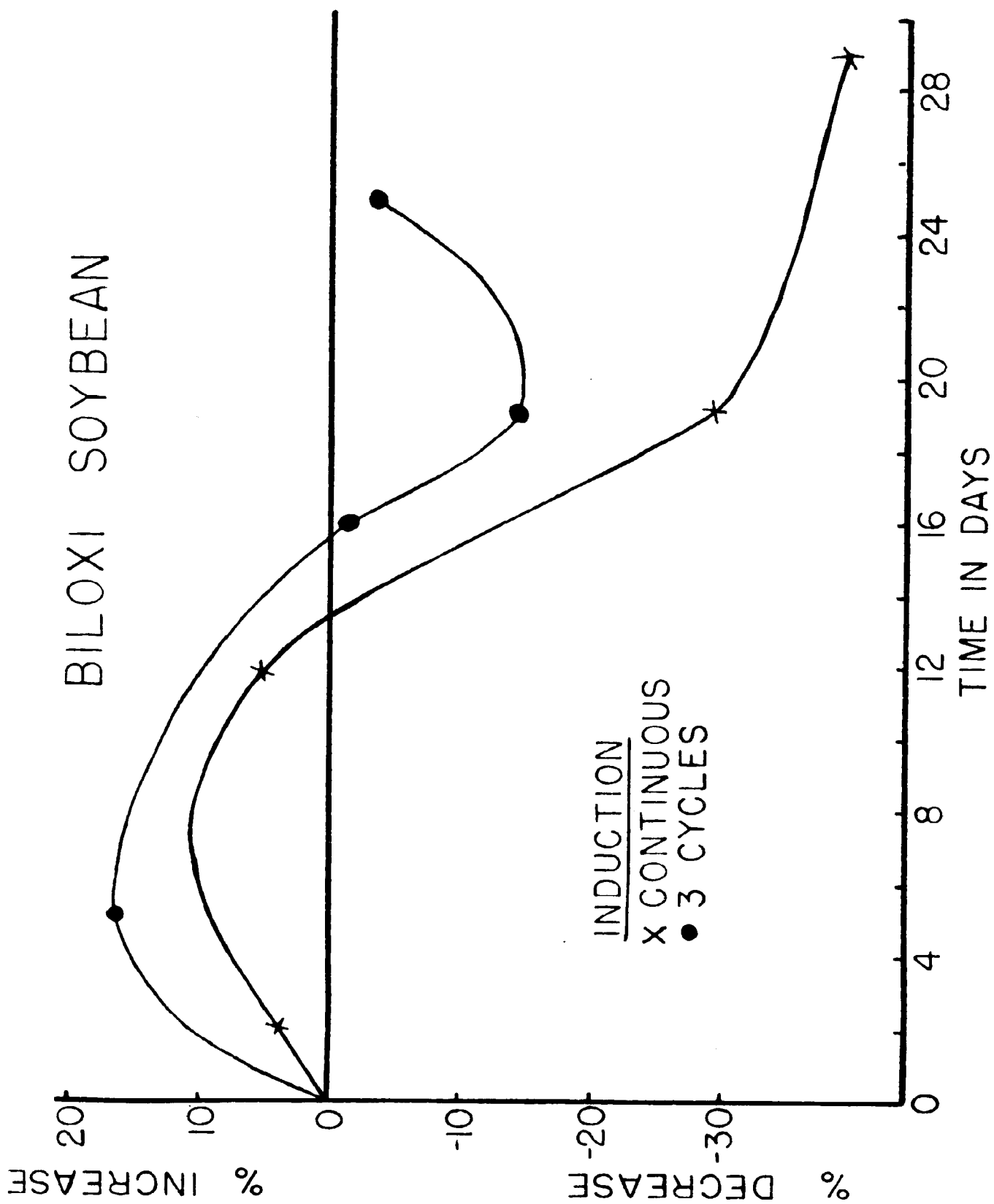


internal events preceding the actual appearance of inflorescence primordia must have been in operation for several days before the primordia were detected. This would have been at a period before there was any significant decrease in auxin content.

In order to compare the changes in auxin content which take place in the leaves during induction with these results for *Xanthium* stems, an experiment was set up with Biloxi soybean. The leaves of this plant contain no detectable inhibitor and so any change in auxin content during photoinduction could be followed at the site of the reception of the stimulus. On March 21, a group of soybean plants was placed under an 8 hour photoperiod while the control group was left under a 16 hour photoperiod. The auxin content of these plants was determined periodically for 29 days. The results of this experiment are graphically represented in Figure 3.

The results of this experiment are, in general, similar to those obtained with *Xanthium*. Biloxi soybean plants, however, do not respond as rapidly to photoinduction as do *Xanthium* plants and hence the induction period is considerably longer. Initially, there is an increase in auxin content which continues in this case for approximately two weeks, the maximum content being reached, however, at the end of one week. Following this, there is a decrease in auxin content. Dissection of the plants periodically showed the beginnings of flower primordia after 14 days of treatment.

Fig. 3. Changes in free auxin content during the photoinduction of Biloxi soybean plants. The lower curve is for the group of plants left under continuous short days. The upper curve is for the group of plants induced for 3 days and then returned to long-day conditions. The auxin content is expressed as percentage increase or decrease over the vegetative controls.



This corresponds with the period when the auxin content in the experimental plants drops below that of the vegetative controls. However, the actual induction must have occurred several days earlier.

It is possible to initiate flower primordia in Biloxi soybean by treating the plants to 3 short days and then returning the photoinduced plants to long days. This procedure was followed in a second experiment which began on April 30. The experimental group of plants was exposed to 3 short days and then returned to long days. The auxin content was then followed for a period of 25 days. The results of this experiment are also shown in Figure 3.

The results obtained in this experiment during the first 19 days after the start of induction parallel those in which the plants were kept under continuous short days. Flower primordia were noted 16 days after the start of the experiment. This is two days later than in the previous experiment. However, 19 days after the beginning of the experiment the auxin content of the experimental plants suddenly showed a rapid increase. This increase can probably be correlated with the fact that after 19 days the plants reverted back to the vegetative state. The flower primordia degenerated and the plants began growing vegetatively once again. In no case were flower buds formed. It has been shown (59) that Biloxi soybean needs 17 short days to form mature flowers, although 3 days will bring about the

initiation of flower primordia.

Auxin Changes During the Induction of Long-Day Plants.

The role of auxin in the induction of flowering might be related to the auxin level in the plant. Thus, flowering might occur in short-day plants when the auxin level had dropped to a certain level, while in long-day plants, flowering might occur when the auxin level had been raised to a certain level. The auxin changes during photoinduction would thus be different for these two groups of plants. To test this hypothesis an experiment was set up with long-day plants to follow any auxin changes that might take place in them during flower initiation. Snapdragon plants were used in this set of experiments. Although many varieties of snapdragon plants are day neutral with respect to flowering, the variety Afterglow used in this experiment responded as a true long day plant. Preliminary experiments with this plant during the summer of 1952 showed that it flowered readily under long days and remained strictly vegetative under short days. One group of plants with flower buds reverted back to the vegetative state when placed under short days. A preliminary auxin analysis showed that the leaves contained a readily extractable auxin with no evidence of an interfering inhibitor.

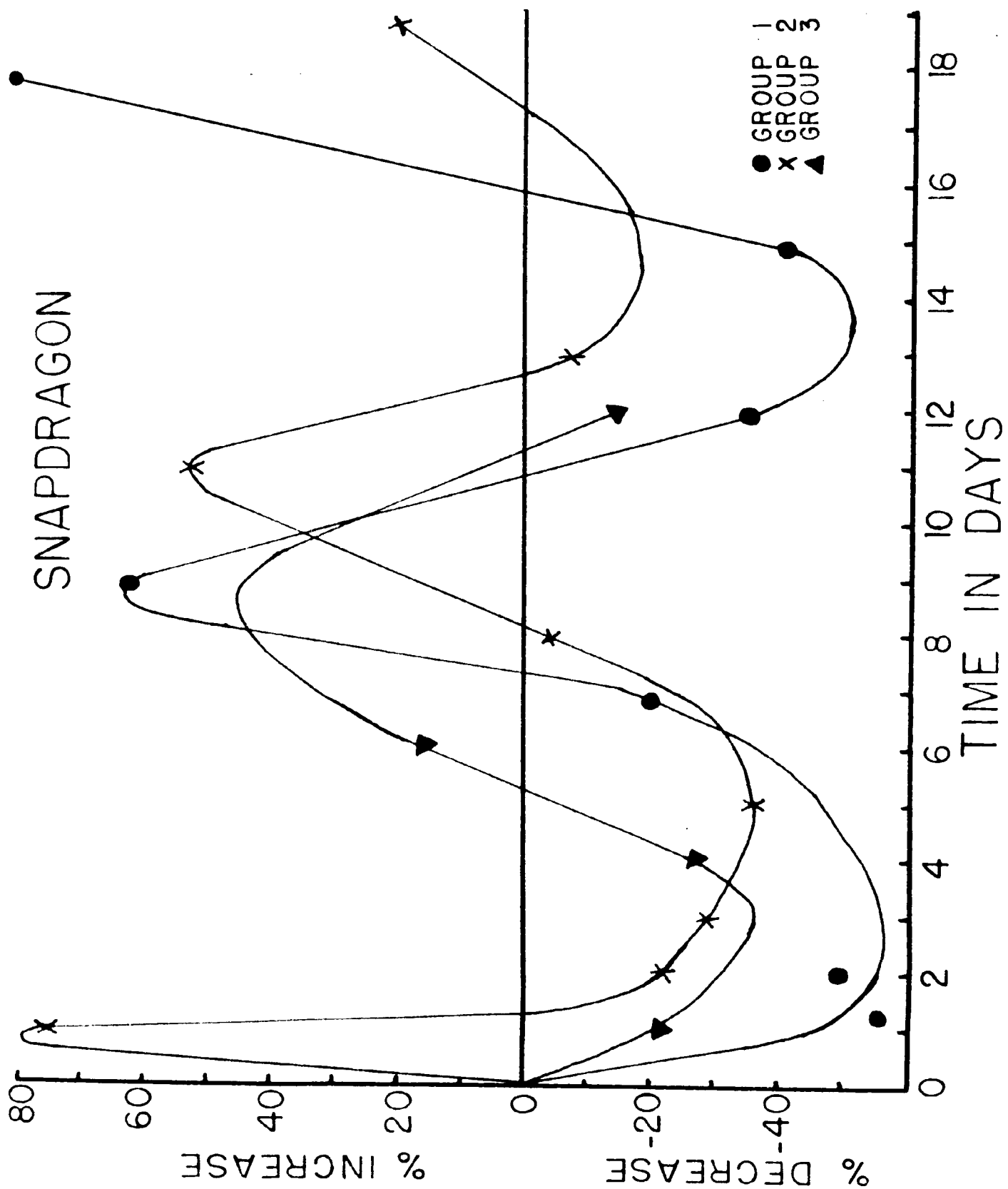
Three groups of Afterglow snapdragon plants were used in the experiments on long-day plants. Group one was in-

duced on Nov. 16, group two on Dec. 1 and group three on Dec. 8. The plants in group 3 were slightly larger and older than the other two groups at the time they were induced. The experimental plants were kept under day lengths of 16 hours while the control plants were maintained under a day length of 8 hours. The plants were analyzed periodically for their auxin content. Throughout the whole experiment the weather was very cloudy. The results of these 3 separate experiments are presented in Figure 4.

The change in auxin content after one day of induction was not the same in the three groups of plants. In groups I and III there was a drop in auxin while in group II there was an increase in auxin content. After two days of induction, however, there was a drop in auxin content in all three groups. This was followed a few days later by a rise in auxin content. After 9 days of treatment there was a second decrease in auxin activity. This second decrease in auxin was never noted in short-day plants, and may occur in all long-day plants or it may only hold in the case of snapdragon plants. The auxin changes during the induction of other long-day plants have not yet been followed.

In groups I and II there was a slight development of the lateral buds on the upper part of the stem after 7 days of treatment. This development of the laterals corresponds to a period when the auxin content had dropped to its lowest value and the apex of the plant was initiating an inflores-

Fig. 4. Changes in free auxin content during the photoinduction of Afterglow snapdragon plants. Group I was induced on Nov. 16, group II on Dec. 1 and group III on Dec. 8. The auxin content is expressed as percentage increase or decrease over the vegetative controls.



cence primordium. At this period the control of the lateral buds by the apical bud is very weak and with the decrease in auxin content the lateral buds start to develop. The two strap-like leaves at the base of these buds grew to a length of 1 to 2 mm. As the plants in group III were slightly larger and older at the start of induction, they responded to the long-day treatment much quicker. Development of laterals was noted 5 days after the start of induction. These lateral buds did not continue to develop in any case during the further growth of the flower buds.

Flower primordia could be detected rather early in the snapdragon plants. In groups I and II flower primordia were noted 8 days after the start of induction while in group III they were detected 5 days after the start of induction. The appearance of flower primordia occurs just after the period of minimum auxin content. In both long-day and short-day plants the appearance of flower primordia occurs after the auxin content has started to drop. After 12 to 15 days of treatment the flower primordia are very large and are starting to differentiate floral parts. This further development of the flower primordia may be associated with the second decrease in auxin content although this is not clearly shown by the data obtained in these experiments.

Effect of Day Length on Free Auxin Content in Vegetative Plants.

Any change in free auxin content, accompanying photoinduction, might be due directly to the change in day length on auxin synthesis rather than to any internal changes brought about by flower initiation. This would be a very real possibility if the auxin content of a vegetative plant was proportional to the photoperiod in which the plant was grown. Some evidence in support of this hypothesis has been reported in the literature. Leopold (39) found that Wintex barley showed a seasonal variation in tillering. During the short days of fall and winter tillering was much greater than at other times of the year. Tillering, or the growth of lateral shoots, is generally attributed to a decrease in auxin content. Leopold also found that short days decreased the amount of diffusible auxin in red-leaved *Coleus*. Von Witsch (68), working with *Kalanchoe blossfeldiana*, found that the auxin content was lower in the plants grown at a short photoperiod than in those grown under long days. He measured the auxin content by applying an acid alcohol extract in lanolin to the side of an *Avena* coleoptile and measuring the curvature. The difference in auxin content was noted in both the leaves and the shoot. Cajlachjan (11) compared the auxin content of flowering and vegetative plants of the same age. The plants growing under long days contained more diffusible auxin than those under short days, regardless of whether they were flowering or vegetative. This difference in

auxin content might, therefore, be due strictly to day length rather than to any vegetative or reproductive state of the plants.

To further test the effect of day length on the auxin content of vegetative plants, a number of plants were grown under both long and short days. After germination the seedlings were placed under 8 hour photoperiods and 16 hour photoperiods. Two to three weeks later the plants in each group were analyzed for auxin content. These plants were still vegetative. The results of this experiment are shown in Table 4.

TABLE 4

Effect of day length on free auxin content of vegetative plants.

<u>Species</u>	<u>Short days</u>		<u>Long days</u>	
<u>Nicotiana tabaccum</u>	6.3°	curvature	8.2°	curvature
var. Maryland Mammoth	4.4°	"	6.4°	"
Biloxi soybean	6.0°	"	9.7°	"
	11.0°	"	9.0°	"
Stock	4.8°	"	6.7°	"
Barley	10.3°	"	13.5°	"
	6.4°	"	7.9°	"

Maryland Mammoth tobacco and Biloxi soybean are short-day plants while stock and barley are long-day plants. In both cases, however, the auxin content of the vegetative plant depends upon the photoperiod in which it is grown. The

plants grown under long days generally contain more free auxin than those plants grown under short days. This difference in auxin content is also correlated with the size of the plants. The plants grown under long days were considerably larger than the plants grown under short days.

The effect of day length on free auxin content was also investigated in older plants. A number of plants of the same age were grown under different photoperiods until one group of plants under the proper photoperiod came into flower. The parallel group under the opposite photoperiod was still vegetative at the time of sampling. Samples of comparable leaves were taken from each group and analyzed. The results are shown in Table 5 along with the vegetative or reproductive state of the plant under the different photoperiods.

TABLE 5

Effect of day length on free auxin content of flowering and vegetative plants of the same age.

<u>Species</u>	<u>Short days</u>		<u>Long days</u>	
Stock	7.3°	veg.	9.7°	flo.
Nasturtium	4.1°	veg.	6.5°	flo.
Calendula	6.4°	veg.	5.8°	flo.
	8.2°	veg.	10.5°	flo.
	7.1°	veg.	8.5°	flo.
Biloxi soybean	12.0°	flo.	19.7°	veg.
Zinnia	2.0°	flo.	3.8°	veg.
Xanthium	12.2°	flo.	17.4°	veg.

The results of this experiment agree with those of Cajlachjan. The plants under long days, whether they were vegetative or flowering contained more auxin than the plants under short days. It then becomes evident that any change in auxin content must occur during the initiation period in order to be associated with flowering and not just with day length.

Different Methods of Induction and the Resulting Auxin Changes.

In the early experiments of this investigation it was shown that the appearance of flower primordia was accompanied by a decrease in auxin. This was especially true for short-day plants. The experiments discussed in the previous section, however, showed that plants in either the vegetative or reproductive state usually contained less auxin when grown under short-day conditions than when grown under long days. It might therefore be argued that the drop in auxin during photoinduction of short-day plants is caused by the change in day length and is not necessarily associated with flowering.

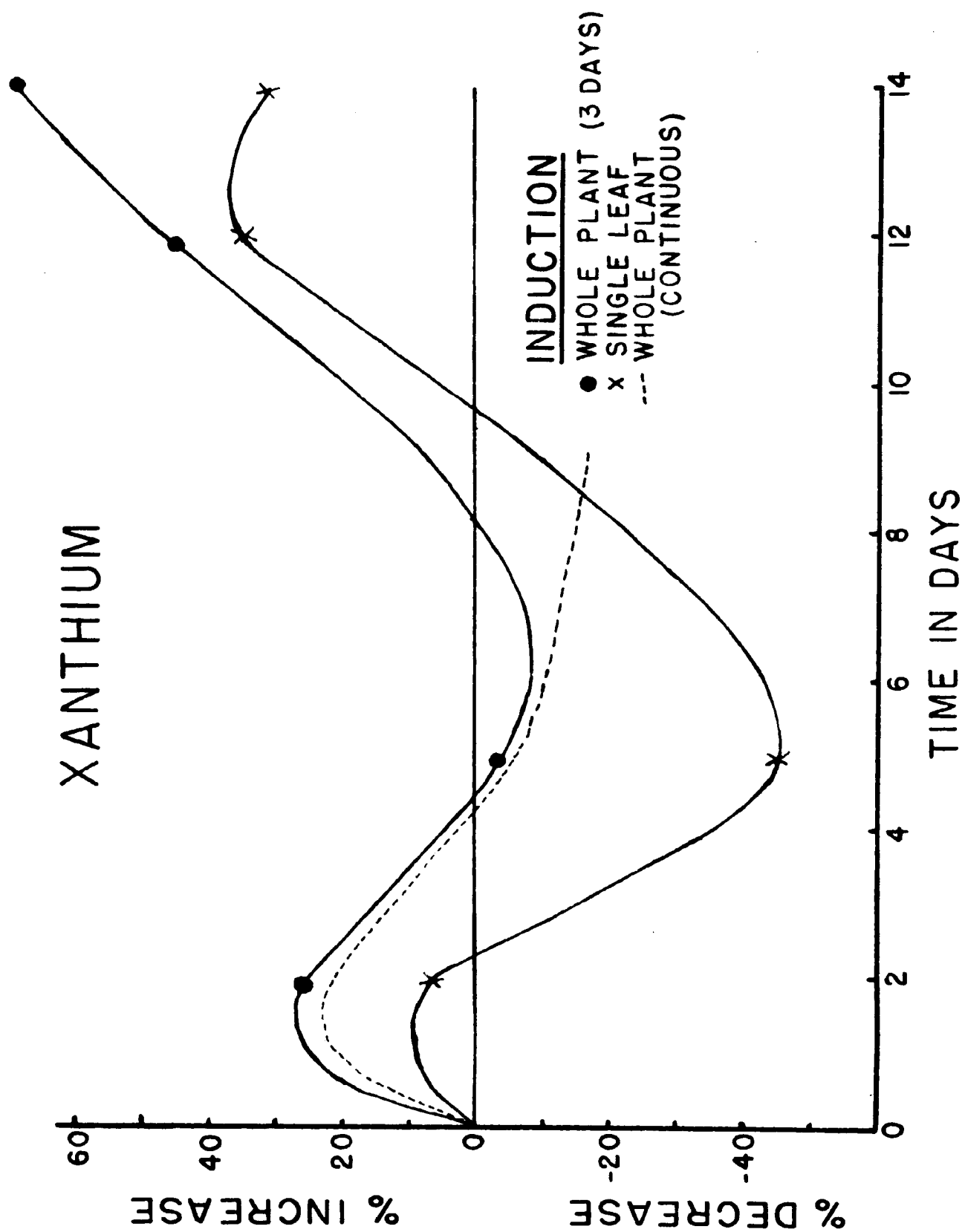
To determine whether a decrease in auxin in plants exposed to short photoperiods is actually associated with flower initiation, several different methods of flower induction were employed. In one experiment *Xanthium* plants were placed under short photoperiods for only three days and then

returned to long-day conditions. These plants came into flower, and the only differences in the amount of illumination between these plants and the vegetative plants was that they received 3 short photoperiods whereas the vegetative plants were continuously under long days. This method of induction cuts down considerably any influence of day length on auxin content. The results of this experiment are shown in Figure 5.

In another experiment a group of *Xanthium* plants were induced by exposing a single leaf on the plant to short photoperiods. This was accomplished by enclosing a single leaf on the plant in a black envelope from 4:00 P.M. to 8:00 A.M. each day. This single leaf, therefore, was under an 8 hour photoperiod while the rest of the plant was maintained under a 16 hour photoperiod. The controls were also kept under a 16 hour photoperiod and hence, any differences in auxin content between the control and induced plants would be due to flower initiation and not to differences in day length. The youngest fully mature leaf was used for short-day treatment throughout this experiment. The results of the auxin analysis made on these plants are shown in Figure 5.

The two experiments just described were started on March 8th. Both groups of plants, those induced for only 3 days and those induced by continuously treating a single leaf with short photoperiods, reached the flowering stage at the same time. Flower primordia were noted in both groups 6 days after the start of treatment. The general shape of

Fig. 5. Changes in free auxin content during the photo-induction of Xanthium plants. The upper curve shows the results of a 3 day induction period of the whole plant, while the lower curve shows the results obtained when a single leaf was continuously induced. The dotted curve shows the results when the plant is continuously induced, the data being taken from Fig. 2. Flower primordia were observed at the end of 6 days.



the auxin curves is also the same in both experiments and compares favorably with the results obtained when the whole plant was continuously exposed to short days. The appearance of flower primordia also occurred at a period when the auxin content had dropped to a low value. These data give further evidence that flower initiation is accompanied by a drop in auxin content and that this drop in auxin is actually associated with flower initiation and not with the change in photoperiod.

Effect of Sudden Changes in Day Length on the Auxin Content of Plants.

For the first day or two after the beginning of induction of short-day plants there is an increase in the auxin content. This increase may be associated with flower initiation or it may be the result of the sudden change from long days to short days. There is some evidence to support the latter view. Gustafson (28) found that when tomato plants were moved from the greenhouse and placed in continuous darkness there was an increase in auxin content for a period of 2 to 3 days. This same relationship was also found for corn. Avery, Burkholder, and Creighton (2), however, found that the auxin content decreased when *Nicotiana* plants were kept in the dark for some days.

In order to determine the effect of a sudden change from long days to short days on the auxin content in the plant,

a series of experiments was set up to measure the auxin content under these conditions. The plants were grown under 16 hour photoperiods and then placed under 8 hour photoperiods. The auxin content was measured for the next day or two. A sample from the control plants, which remained under 16 hour photoperiod, was also taken at the same time. The results are shown in Table 6.

TABLE 6.

Effect of a sudden change from long to short days on the auxin content of plants.

<u>Species</u>	<u>No of short photoperiods</u>	<u>Auxin content in degrees curvature of plants under:</u>		<u>Percent increase</u>
		<u>Long days</u>	<u>Short days</u>	
Xanthium (short day plant)	1	14.1	23.2	64.5
	2	13.4	17.8	25.4
Soybean (short day plant)	2	5.0	10.4	108.0
	3	9.5	22.1	145.0
	5	6.5	7.6	16.2
Barley (long day plant)	2	7.2	9.6	25.0
	2	7.9	9.7	18.5

The plants used in this series of experiments were young seedlings that had not yet reached the stage where they could be easily induced. Therefore, the shift observed in auxin content is the result of changes in day length and is not associated with the flower initiation process. In all instances, there was an increase in hormone content when the

plants were transferred from long days to short days. The magnitude and duration of this increase varied with the experimental plant. This increase in auxin may be associated with the light destruction of the growth hormones (57). A change to short days would bring about a decrease in destruction of the hormone before any decrease in synthesis was evident. After a length of time under short days, however, less auxin might be synthesized and hence a decrease in auxin content would result.

In order to better interpret the change in auxin during the first few days of induction of long-day plants, a similar study was made on the effect of a sudden change from short days to long days on the auxin content of young vegetative plants. The plants were grown under 8 hour photoperiods and then some were placed under a 16 hour photoperiod. For the next day or two the auxin content was measured in these plants. The results are shown in Table 7.

TABLE 7

Effect of a change from short days to long days on the auxin content of plants.

<u>Species</u>	<u>No. of long photoperiods</u>	<u>Auxin content in degrees curvature of plants under:</u>		<u>Percentage change</u>
		<u>Long days</u>	<u>Short days</u>	
Soybean (short-day plant)	2	6.3	10.0	-37.0
	3	15.6	11.0	41.8
Barley (long-day plant)	2	4.7	6.4	-26.6
Snapdragon (long-day plant)	1	13.7	11.2	22.3
	1	5.6	14.4	-61.1
	2	4.8	9.4	-49.0
	1	8.0	4.5	77.8
	2	5.7	7.3	-22.0
	1	9.8	12.3	-20.3

The results of these experiments, unlike those in which the plants were transferred from long days to short days, are very variable. In the majority of the determinations there is a decrease in auxin content for the first day or two after the change from short days to long days, although in some instances the results are just the opposite. Burkholder and Johnston (9) found that when they removed plants from darkness to light there was a loss of hormone for the first few days. This decrease in auxin can be explained in terms of light destruction of auxin. For the first day or two more hormone is destroyed than is synthesized. After a few long days, however, auxin synthesis is increased so that

these plants actually contain more auxin than the controls in short days. An explanation of the cases where an increase in auxin was noted when the plants were changed to long days is not so clear. The light and temperature conditions existing the day before and the day after the change seem to play an important part in determining the hormone level in the plant. It appears that if the weather was cloudy the day after the change the auxin level was more likely to increase than to decrease. However, further experiments are necessary before any definite statements can be made to this effect.

Attempts to Induce or Prevent Flower Initiation by Changing the Auxin Content of the plant.

Effect of decapitation.

As the previous experiments have shown, there is a change in auxin content during the photoinduction of both long-day and short-day plants. In general, there is a decrease in auxin content associated with the appearance of flower primordia. If this drop in auxin was the primary factor controlling the initiation of flowering, then it should be possible to initiate flower primordia under unfavorable photoperiods by artificially lowering the auxin content of the plant.

It has been known for many years that removing the apex of a plant results in the development of lateral buds. The growth of these laterals is thought to be due to the removal

of excess growth hormones produced in the tip. In fact, application of auxins to the decapitated surface suppresses the growth of the laterals. A search of the literature, however, failed to reveal any direct measurements of the growth hormone changes accompanying decapitation. It was therefore necessary to determine if decapitation did actually lower the hormone content of the plant. The apices were removed from a group of *Xanthium* plants on November 16th and the auxin content followed in these plants for a period of several days. The stem of the plant, which included the axillary buds, was used for analysis. The results are shown in Table 8.

TABLE 8

The effect of decapitation on the auxin content of *Xanthium* plants. The auxin content is given in degrees curvature of the *Avena* coleoptiles.

<u>Date</u>	<u>Photoperiod</u>	<u>Control</u>	<u>Decapitated</u>	<u>Percentage change</u>
11/21/52	LD	14.4	10.0	-30.6
11/21/52	SD	16.3	12.4	-23.9
11/23/52	LD	17.4	14.2	-18.4
11/23/52	SD	20.9	15.1	-27.8

Decapitation lowers the auxin content in the stem of the plant under both long days and short days. There was always considerable development of the lateral buds in the decapitated plants. Dissection of the buds on November 21st, five days after the beginning of the treatment gave the fol-

lowing results. The control plants under long days were vegetative as were the decapitated plants under long days. The control plants under short days had initiated terminal inflorescence primordia. The decapitated plant under short days had developed an inflorescence primordium in the top axillary bud. Other lateral shoots on the plants had grown to a considerable extent. The decapitated plants under long days never did initiate flower primordia. The development of the flower primordia on the plants under short days proceeded at the same rate in both the control and the decapitated groups. The same experiment was repeated with the short day plant Maryland Mammoth tobacco on November 21st. Neither the control nor the decapitated plants initiated flower primordia under long days. The control plants under short days developed apical flower primordia while the decapitated ones developed flower primordia only in the upper two axillary shoots. The primordia appeared at the same time in the two groups of plants under short days.

Decapitation, even though it does lower the auxin content, does not bring about flower initiation under inappropriate photoperiods. Decapitation also does not appear to hasten the flowering response of plants under a favorable photoperiod. It will, however, allow the growth of the lateral buds, and if the plant is under a favorable photoperiod, these lateral shoots will initiate flower primordia. Decapitation will, therefore, increase the number of flowers

formed on a plant during a given time, but will not hasten their development, nor cause them to be formed under an unfavorable photoperiod.

Effect of treating plants with auxins and antiauxins.

The auxin level within the plant can also be controlled by spraying them with auxins and antiauxins. Considerable attention has been directed to this experimental technique in an attempt to cause flower initiation under unfavorable photoperiods. In an attempt to determine the role of auxin in flower initiation a few of these experiments were repeated and extended.

In these experiments the auxin indoleacetic acid and the antiauxins triiodobenzoic acid, 2,4,6-trichlorophenoxyacetic acid, maleic hydrazide, coumarin and β -methyl umbelliferone were used. On March 29th, some Biloxi soybean plants were sprayed with several concentrations of indoleacetic acid, triiodobenzoic acid and maleic hydrazide. A total of 5 plants were used for each treatment. The plants were induced under short days for a period of 9 days and then put back under long days. Eleven days after the beginning of the treatment the plants were dissected. Indoleacetic acid inhibited flower initiation at concentrations of 10^{-2} M to 10^{-6} M. A 10^{-3} M concentration of triiodobenzoic acid increased the size of the flower primordia over that on the untreated controls, 10^{-4} M triiodobenzoic acid produced no

noticeable results, while 10^{-5} M and 10^{-6} M inhibited the development of flower primordia. A 0.25 % concentration of maleic hydrazide completely inhibited the development of flower primordia and 0.1 % to 0.05 % maleic hydrazide delayed the development. These results are in general agreement with those obtained by Bonner (7), Galston (21) and Naylor (49).

On September 20th, A group of *Matthiola* plants, a long-day species, were sprayed with 2,4,6-trichlorophenoxyacetic acid, triiodobenzoic acid, maleic hydrazide, coumarin and β -methyl umbelliferone. Two plants were used for each treatment. The plants were kept under long days until October 23rd, when they were examined. At this time the control plants had large flower buds. Flower formation in plants sprayed with 10^{-3} M 2,4,6-trichlorophenoxyacetic acid, 10^{-3} M triiodobenzoic acid and 10^{-2} M maleic hydrazide was completely inhibited. On the other hand plants treated with concentrations of 10^{-2} M coumarin and β -methyl umbelliferone produced very small flower primordia. It is thus seen that the compounds that have been reported as having antiauxin activity, inhibited or delayed flower initiation even under a favorable photoperiod. At no time was there a hastening or an increase in flower initiation as a result of treatment with antiauxins.

Effect of irradiation of plants with gamma and X-rays.

Skoog (55) and Gordon and Weber (26) have shown that X-irradiation will lower the growth hormone content of plants. Therefore, another method of investigating the influence of auxin in flower initiation is to expose the plants to ionizing irradiation. In such experiments it must always be kept in mind that the irradiation treatment may be affecting more than just the auxin level in the plant. A few reports have been made indicating that irradiation of plants did produce an effect on the flowering response. Johnson (33) found that X-irradiation hastened the time of flowering in Kalanchoe tubiflora. This species is a short-day plant and it could be that the drop in auxin content resulting from X-irradiation, hastened the time of flowering. Denffer and Schlitt (16) tested a large number of species as to their reaction to ultraviolet light. A positive reaction was shown only by Linum usitatissimum and Statice bonduellii. In these two species, short daily periods of ultraviolet irradiation hastened the average date of flowering by 17 days. Longer periods of irradiation inhibited growth and in some species completely inhibited flowering.

During the summer of 1952 the writer had a fellowship at the Brookhaven National Laboratory at Upton, Long Island, N. Y. At this laboratory it was possible to study the effect of continuous gamma irradiation as well as short durations of X-irradiation on flowering. For the continuous gamma

irradiation studies *Nicotiana*, *Xanthium*, *Calendula*, *Cosmos* and *Anthirrinum* plants were grown in concentric circles around the gamma source. The source consisted of radioactive Co^{60} delivering 300 curies attached to a pulley arrangement so that the source could be lowered into the ground when it was necessary to work in the gamma field and raised when it was desired to irradiate the plants. Various dosage levels, ranging from 5000 r/day to 3 r/day, were obtained by placing the plants at the appropriate distance from the source. High dosages of acute X-rays were obtained from a Westinghouse X-ray machine. This instrument was operated at 250 KV. and 30 ma. Filters consisting of 1 mm. aluminum plates were used in all X-ray experiments to filter out the soft X-rays. The X-ray dosage could be accurately measured and was usually applied at the rate of 1000 r/min.

The results of preliminary experiments on the effect of continuous gamma irradiation on the auxin content of plants are given in Table 9.

TABLE 9

The effect of continuous gamma irradiation on the auxin content of plants.

Species	No. of days of irradi- ation.	Distance from source in meters.	Dosage in r/day	Auxin content in degrees curvature.
Xanthium	41	3.	960.	7.2
"	41	4.	531.	3.4
"	41	5.	333.	10.5
"	41	6.	227.	9.7
"	41	14.	39.4	12.2
Calendula	21	3.	960.	10.8
"	21	4.	531.	5.6
"	21	5.	333.	3.3
"	21	6.	227.	3.0
"	21	10.	78.	8.2
"	21	14.	39.4	15.4
"	21	20.	19.3	16.0
Cosmos	71	5.	333.	9.6
"	71	6.	227.	4.7
"	71	7.	165.	5.9
"	71	10.	78.	9.3
"	71	40.	4.4	10.1

These results on the effect of continuous gamma irradiation on auxin content show that there is a decrease in growth hormones with increasing dosage of irradiation. This decrease in hormone, however, continues up to a certain dosage and then, with further increase in dosage, there is actually an increase in auxin content. In all the auxin determinations the main veins were removed from the blade of the leaf and only the mesophyll and spongy layers were used for auxin analysis. The increase in auxin at the higher dosages is probably not due to differences in morphology of the plants

as the same type of material was used for analysis in all cases. Further investigations will be necessary to determine what factors cause this increase. Dosages less than 30 to 40 r/day had practically no effect on the auxin level of the plants. The decrease in auxin with higher dosages was also accompanied by a decrease in growth. As the dosage increased, there was a stunting of terminal growth with a corresponding increase in the growth of lateral shoots. At the highest dosages, there was a complete cessation of growth. The general effect of irradiation on the growth of these plants is shown in Figures 6 and 7.

Continuous gamma irradiation had very little effect on the flowering response of the plants tested. Calendula plants, a long-day species, were set out in the gamma field so as to receive various dosages of gamma irradiation. The natural day length was long enough to induce flowering. All of the plants in the gamma field came into flower at the same time. Dosages of 4.4 r/day to 960. r/day neither stimulated nor delayed flowering. Snapdragon plants, variety Afterglow, were somewhat affected by the same dosages of gamma irradiation. The plants receiving 156 r/day had fewer flowers than the controls, at 213 r/day there were only a few flowers in the top axils, at a dosage of 300 r/day there was one abnormal flower and at 460 r/day the plants remained vegetative. Gamma irradiation, in this case, delayed or prevented flower initiation at the higher dosages. This

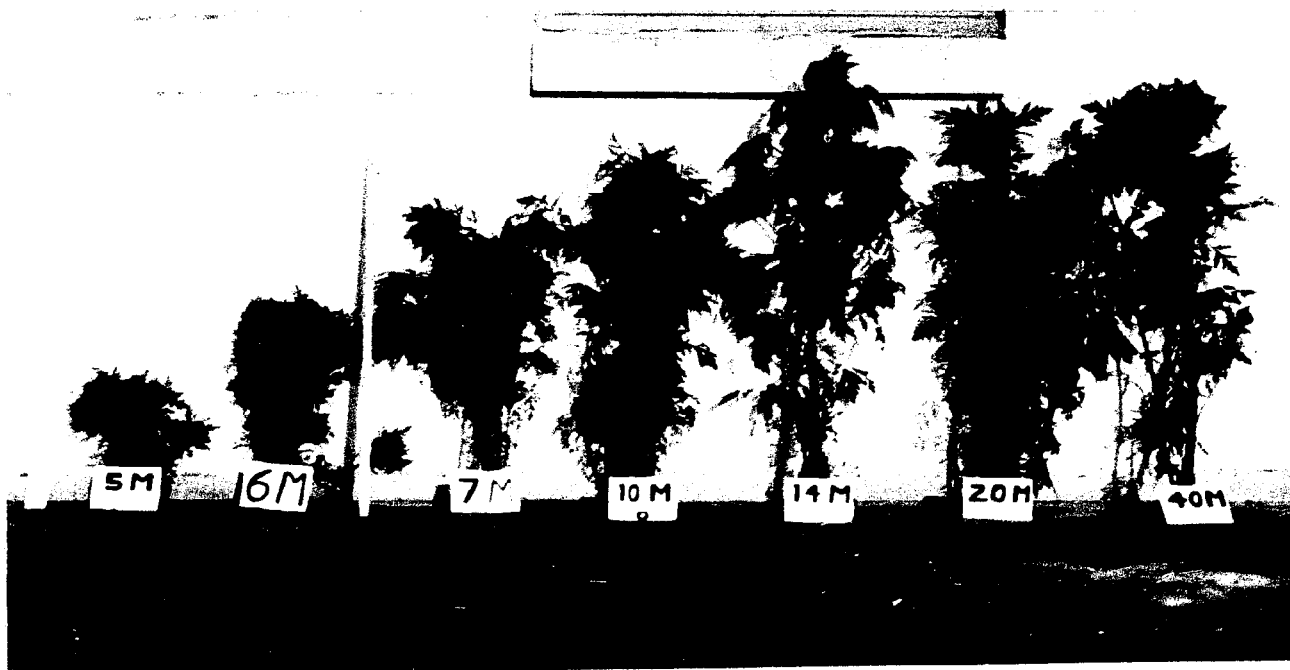


Fig. 6. Effect of continuous gamma irradiation on the growth of Cosmos sulfureus plants. The plants received 71 days of irradiation.

<u>Distance from source.</u>		<u>Dosage in r/day.</u>
5	meters	333.
6	"	227.
7	"	165.
10	"	78.
14	"	39.4
20	"	19.3
40	"	4.4



Fig. 7. Effect of continuous gamma irradiation on the growth of Xanthium plants. The plants received 41 days of irradiation.

<u>Distance from source.</u>	<u>Dosage in r/day.</u>
3 meters	960.
4 "	531.
5 "	333.
6 "	227.
7 "	165.
14 "	39.4

effect was probably non-specific for flowering as the plants at the higher dosages were considerably stunted in their vegetative growth. *Xanthium* plants receiving a dosage of 300 r/day were killed while a dosage of 156 r/day delayed the time of flowering. In no instance was there a stimulating effect on flowering. *Cosmos* plants, another short-day species, remained vegetative at all dosage levels. The day length never did become short enough during July and August to initiate flowering in this species. Gamma irradiation, while it did affect vegetative growth, did not bring about flower initiation in *Cosmos*. In all of the above cases, the irradiation treatment either had no effect on the flowering response or else at the higher dosages, it delayed or prevented flowering.

In one instance, however, the effect of gamma irradiation was somewhat different. A few plants of the day-neutral *Nicotiana rustica*, variety Olsen 68, were set out in the gamma field. The plants receiving 77 r/day or more came into blossom sooner than the control plants. As only a few plants were available, there was not enough material to dissect at regular intervals. Whether there was any difference in the time of the appearance of flower primordia cannot be definitely stated although it was quite evident that the plants receiving the higher dosages of irradiation came into flower at an earlier date. The appearance of the plants on July 24th is shown in Figure 8.



Fig. 8. Effect of continuous gamma irradiation on the growth of Nicotiana rustica plants. The plants received 38 days of irradiation.

<u>Distance from source.</u>	<u>Dosage in r/day.</u>
4 meters	531.
5 "	333.
7 "	165.
10 "	78.
14 "	39.4
28 "	9.7

A series of experiments were also conducted to determine the effect of X-irradiation on flower initiation in Xanthium. In the first experiment Xanthium plants were induced for one day and then X-rayed the following morning. The plants receiving 2000 r or more were inhibited in their flowering. Lower dosages had no effect on flowering. In a second experiment, the plants were X-rayed first and then induced for one day. The plants receiving 2000 r or more were inhibited in their flowering as had been the plants that were induced before X-irradiation. The plants receiving 1000 r had fewer buds than the controls while all lower dosages were without effect. These effects appeared to be non-specific as far as flowering was concerned. Those plants that were stunted by the high dosages of X-irradiation were also delayed or inhibited in their flowering response.

DISCUSSION AND CONCLUSIONS

The concentration of a number of naturally occurring compounds has been shown to vary with the length of the photoperiod in which the plants are grown. Bonner (4) found more thiamine in plants grown under long days than in plants grown under short days. Langston and Leopold (38) have shown that biotin, niacin and pantothenic acid decreased in Wintex barley when these plants were exposed to photoinductive long days. By means of night interruption studies they showed the biotin and pantothenic acid reductions are associated with the photoinduction process, while niacin changes were more closely associated with the change in day length. Roberts (52) found that the carotene content varied during the flower initiation process. Not only have these and a number of other physiologically active compounds been shown to vary in concentration with the length of the photoperiod, but also enzyme activity has been shown to vary during the photoinduction of a number of plants. Hibbard (32) and Stout (58) found that catalase, invertase and peroxidase varied during photoinduction. In long day plants, Elliott and Leopold (17) found a decrease in malic dehydrogenase, ascorbic acid oxidase and peroxidase activity, during photoinduction. With short day plants, there was a large increase in ascorbic acid oxidase activity after three inducing photoperiods. Recently,

Elliott and Leopold (18) reported that respiration in both long day and short day plants varied during photoinduction. In spite of all these investigations showing that there are extensive changes in the chemical composition during photoinduction, no one compound can be picked out as causing the change from vegetative to reproductive growth.

Considerable attention has been given to the role of auxin in the photoinduction process. Most of these investigations, however, have dealt with the effects of treating plants with auxins and antiauxins (7,20,41). Very little information is available concerning the changes in auxin content that normally take place with variations in photoperiod and during the process of flower initiation (11). If these external treatments with auxins and antiauxins are directly correlated with flowering, then some natural change in auxin content should be expected to occur during flower initiation. However, any changes in auxin content may only be associated with flowering and are not necessarily the cause of flowering.

The first set of experiments reported in this thesis show that there is normally a change in auxin content, as measured by the *Avena* coleoptile test, during the photoinduction of both long and short-day plants. The change in auxin during the first day or two of induction depends upon a number of factors, especially light conditions and temperature. In all of the experiments with short-day plants there

was an increase in hormone for the first few days after the plants were placed under short days. Table 6 shows that there is generally an increase in auxin content in any plant moved from long days to short days. This was true of vegetative plants of both long and short-day species and hence, is associated with the change in photoperiod and not with any flower initiation process. This increase in auxin is probably due to decreased destruction under short-day conditions before there is a decrease in synthesis of auxin or auxin precursors. The change in auxin content accompanying a change from short photoperiods to long photoperiods is much more variable. The data in Table 7 indicate that generally a decrease in auxin accompanies such a change. This decrease could be accounted for by an increase in the light destruction of the hormone before there is a significant increase in the synthesis of auxin or auxin precursors. In several cases there was an increase in hormone when the plants were taken from short days and placed under long days. Exact data on temperature and light conditions accompanying these experiments are not available. It appeared, however, that this increase took place when the day following the change was cool and cloudy. This could be explained by considering that there might be less destruction of the hormone by the lower light intensities, while at the same time there is an increase in auxin synthesis with the longer day length. In any event, this change in auxin content during the first few days

of induction can be accounted for by the change in photoperiod and therefore, is probably of little importance in the flower initiation process.

After the first few days of induction the auxin content of the induced plants begins to drop in both long-day and short-day species. In the case of short-day species, it might be argued that the decrease was due to the change in photoperiod and not to the flower initiation process. In fact, a series of experiments showed that plants grown under long photoperiods contained more auxin than plants grown under short photoperiods. However, if the plants are induced for a few days and then returned to day lengths that are unfavorable for flowering, the plants will still come into flower; and associated with this flower initiation there is also a decrease in auxin. It is possible to initiate flowering in *Xanthium* by exposing a single leaf to short photoperiods while the rest of the plant is maintained under long days. Here the effect of photoperiod itself would be unimportant in any overall changes in the auxin content of the plant. But here also, a decrease in auxin is associated with flower initiation when the plants are induced in this manner.

The appearance of flower primordia can first be detected during the end of the period of decreasing auxin content. This is further evidence that the decrease in hormone is closely associated with the flower initiation process. After the inflorescence and flower primordia are well developed

and are starting to differentiate floral parts, there is an increase in auxin content. The further growth of the floral parts can, therefore, be correlated with an increasing auxin content in the plant.

While it has been shown in these experiments that flower initiation is associated with a decrease in auxin, it has not been shown whether flowering is caused by this decrease or whether the flower initiation process itself brings about the decrease in auxin. It was for this reason that a number of attempts were made to induce flower initiation by lowering the auxin content of the plant. In the first of these experiments it was shown that removing the apex of the plant resulted in a decrease in auxin. This decapitation procedure, however, did not result in flower initiation under non-inductive photoperiods, nor did it hasten flowering under inductive photoperiods. The decapitated plants initiated many more axillary inflorescences than the controls. This is due to the growth of more lateral shoots which do not develop to any degree in the non-decapitated plants. Treatment with a number of antiauxin compounds such as 2,4,6-trichlorophenoxyacetic acid, 2,3,5-triiodobenzoic acid and maleic hydrazide did not bring about flower initiation under unfavorable photoperiods. Under inducing photoperiods, however, triiodobenzoic acid did increase the flowering response in Biloxi soybeans. This agrees with the results obtained by Galston (21). Treatment with auxin suppressed the flowering response

in soybeans. *Matthiola*, a long-day species, was not stimulated in its flowering by treatment with antiauxins. In fact, treatment with antiauxins delayed flowering in most plants.

In later experiments it was demonstrated that exposure to continuous gamma irradiation lowered the auxin content of the plant. These experiments with gamma irradiation were carried on during the summer months under long photoperiods. However, short-day plants were not induced nor were they hastened in their time of blooming. There was, however, a delay or actual suppression of flowering at the high dosage levels of irradiation. Only in one species did the irradiation treatment appear to aid in the flower initiation process. This was with *Nicotiana rustica*, var. Olsen 68, a day-neutral plant, which came into blossom sooner at the higher dosages of irradiation. In all these experiments where the auxin content was lowered by various treatments, no clear cut case of flower initiation was ever noted. This type of evidence lends support to the idea that the decrease in auxin is only correlated with flower initiation and is not by itself the cause of flower initiation.

In photoperiodism there is, no doubt, a chain of events leading to the appearance of flower primordia and it seems probable that auxin is involved in one of these steps. This would account for the many effects of auxin on flowering that have been reported in the literature. However, if auxin

is controlling a process and is not itself directly involved as a precursor of the flowering stimulus, then it would be difficult for a change in auxin by itself to bring about flower initiation. The action of auxin under these conditions would depend upon the formation of some precursor in response to a favorable photoperiod. If auxin does function as a cofactor in some enzyme, then its presence or absence at a certain concentration might affect the formation of some product involved in flowering. Once the precursor has been formed under the inducing photoperiod, then a change in auxin concentration may in some manner aid in the conversion of this precursor to the flower hormone.

It is also possible that auxin is working in another manner in controlling the change from vegetative to reproductive growth. A number of experiments (47) have been reported in which conditions of poor growth have hastened the time of flowering. This would appear to offer some evidence that vegetative growth is antagonistic to reproductive growth. A high concentration of auxin would favor rapid vegetative growth. With a decrease in auxin, however, conditions would be more favorable for reproductive growth. Once the reproductive growth system is active, it may perhaps be able to control the metabolism of the plant so that the plant keeps on growing reproductively even though returned to photoperiods favoring vegetative growth. Much more information on vegetative metabolism and reproductive

metabolism is needed before it can definitely be said that one is antagonistic to the other.

It can be concluded from these experiments that flower initiation in both long-day and short-day plants is associated with a decrease in auxin content. This decrease in auxin is not in itself the cause of flower initiation, as lowering the auxin content of the plant by various methods has not brought about flower initiation under unfavorable photoperiods. Further work is needed to determine just what part auxin does play in the flower initiation process.

SUMMARY

1. The auxin content of several plants as measured by the standard Avena method was followed during the photo-induction period. The tissue was first quick dried in an oven utilizing an infra-red lamp and then extracted overnight with ether at 5° C. Quick drying gave comparable results to lyophilization.
2. A growth inhibitor was noted in Xanthium leaves which was extracted in ether along with the auxin. The inhibition by this extract could be neutralized by the addition of IAA.
3. The inhibiting action of the extract was not destroyed by acid or alkaline hydrolysis, nor was it destroyed by oxidation with H₂O₂. The molecular weight of the inhibitor is approximately equal to that of IAA as it cannot be separated from IAA by the standard diffusion process.
4. The inhibitor gave a pH-fluorescence curve similar to that for coumarin derivatives but could not be identified with that of any of the published curves for coumarin derivatives.
5. The inhibitor was not present in the stems of Xanthium plants and hence the stem tissues of Xanthium were used for auxin analysis.

6. In the experiments dealing with photoperiodism it was discovered that there is a decrease in auxin content in both long-day and short-day plants associated with the appearance of flower primordia.
7. This decrease occurs regardless of the method of induction. If the plants are induced for only 3 days or if only a single leaf is used in induction, there is still a decrease in auxin accompanying the appearance of flower primordia.
8. A plant suddenly changed from long days to short days has an increase in auxin content for the first few days, but this change is associated with the change in photoperiod and not with any photoinductive process.
9. Likewise, a plant suddenly changed from short days to long days has, generally, a decrease in auxin content for the next few days, but this decrease is not associated with photoinduction.
10. After the plants have come into flower, their auxin content depends upon the photoperiod under which they are growing. The plants under long days, whether they are flowering or vegetative, contain more auxin than plants growing under short days.
11. Lowering the auxin content of the plant will not bring about flower initiation under unfavorable photoperiods.
12. Decapitation lowers the auxin content but has no effect on the appearance of flower primordia.

13. Continuous gamma irradiation lowers the auxin content of plants but in general, has no effect on flower initiation.
14. High dosages of gamma irradiation that stunt the plant also suppress flower initiation. The plants receiving these high dosages of irradiation contained more auxin than the plants receiving lower dosages of irradiation.
15. However, high dosages of gamma irradiation hasten the flowering of Nicotiana rustica, var. Olsen 68, although the auxin response to gamma irradiation of these plants is the same as that of other plants used in this study.
16. Treatment with antiauxins hastens the flowering response in Biloxi soybeans but will not bring about flower initiation under unfavorable photoperiods.
17. Treatments with antiauxins may decrease the flowering response of long-day plants.
18. Treatment with auxin suppresses flower initiation in short-day plants.
19. A drop in auxin content is associated with flowering but this decrease in auxin has not been shown to be the cause of flower initiation.

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