PARTIAL REACTIONS IN THE FORMATION OF THE FLORAL STIMULUS IN XANTHIUM [1]

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The formation of the flower-inducing principle in the leaves of *Xanthium pensylvanicum* Wallr. has been shown by Hamner (1) to consist of at least two partial reactions: a preliminary high intensity light process, followed by a process requiring for its completion a minimum period of continuous darkness. Recently it was reported (3) that the flowering response of Xanthium may be suppressed when the inductive dark period is followed (after a brief interruption by light) by a second dark period of 4 to 6 hours duration. The effectiveness of the second dark period is greatly enhanced by treatment of the plants with indoleacetic acid (IAA). These results suggest that certain processes necessary for the formation of the flowering stimulus must take place in the leaf following an inductive dark period. Experiments were, therefore, conducted in an attempt to further characterize these processes.

The flower inducing principle has not been identified as a single chemical compound, therefore we prefer not to refer to it as a hormone. In this paper it will be referred to as an internal stimulus, which is formed in the leaves and moves to the terminal meristem, where it changes the environment of the growing point causing the differentiation of the floral inflorescence.

The results reported here will show that a photochemical process stabilizing the floral stimulus takes place after the inductive dark period and before the flowering stimulus is transported from the leaf. This process may be measured by a decrease in the effectiveness of the second dark period resulting from exposure of the leaves to light after termination of the inductive dark period. The photochemical reaction which stabilizes the stimulus requires high intensity light for approximately 5 hours and in the absence of such light treatment the stimulus remains sensitive to destruction by a second dark treatment for at least several hours.

GENERAL METHODS AND MATERIALS

The methods and materials employed were similar to those reported previously (3). All older leaves of the experimental plants were removed within 24 hours before treatment, leaving only the 2 youngest fully expanded leaves and the expanding leaves during treatment. The stage of flowering was determined by dissecting the plants 3 weeks after treatment. Stages were assigned according to the morphological development of the inflorescence, according to a method developed in this laboratory (6). The relative stages seem to reflect accurately the quantity or intensity of flowering stimulus reaching the terminal meristem. From 8 to 10 plants were used in each experiment and the differences found here were highly significant. All data reported are representative of two or more separate experiments giving similar results.

Low temperatures were obtained in refrigerated rooms maintained at $\pm$ 2° C of the desired temperature. In these rooms, cool white fluorescent lights were installed providing illumination of 1200 fc at the leaf surface. The temperature of the illuminated cold room was measured by hanging a mercury thermometer under the lights at the level of the leaves. Some difficulty was encountered in preventing wilting of the plants kept at 5° C in high intensity light in excess of 4- to 5-hour periods. In some cases where wilting did occur the subsequent flowering response was greatly decreased and certain treatments were discarded when it seemed clear that wilting critically interfered with the results obtained.

It has recently been found in this laboratory (2), that if the second dark period is given at elevated temperatures (40° C) the effectiveness of this dark period is greatly enhanced. The application of IAA, which was done in previous work (3) in order to attain maximum destruction of the stimulus, would have introduced undesirable complications in some cases. Therefore, in certain experiments, the plants were exposed to the second dark treatment, 3 hours darkness at a temperature of approximately 40° C. To give this treatment, an insulated room was equipped with heating units wired through a bimetallic thermoregulator and a mercury relay. A fan was used to circulate the air and the temperature was maintained at 39.5 $\pm$ 0.1° C. All data reported here are representative of two or more separate experiments giving similar results.

EXPERIMENTAL RESULTS

EXPERIMENT 1: A number of experiments have demonstrated that, following a 12-hour inductive dark period, if the plants are exposed to 10 minutes of light, treated with IAA and given a second dark period of 5 to 6 hours, the flowering response is reduced to a value of 0 to 20 % of the control plants. Experiments were undertaken to determine whether the stimulus remained sensitive to the IAA and second dark period treatment as long as it remained in the leaf. Plants were placed under fluorescent lights (1000 fc) immediately following a 12-hour inductive dark period and, at various time intervals thereafter, treated with IAA and given a second dark period of 5 hours. To determine whether the stimulus had moved out of the leaf, control plants were given the same dark induction, placed under the fluorescent lights and groups defoliated (detailed below) at the

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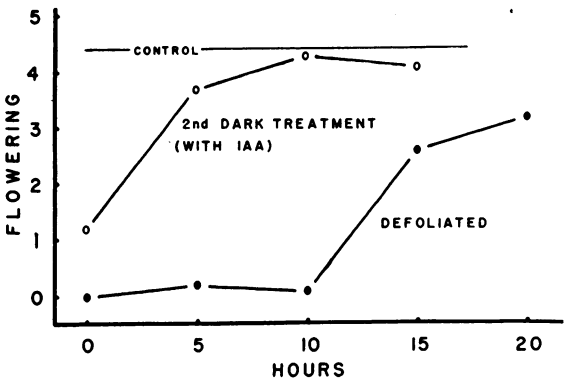


FIG. 1. The stability of the stimulus to a second dark treatment compared to the time of export of the stimulus from the leaf. Following a 12-hour inductive dark period the plants were placed under fluorescent lights (1000 fc) for the specified times then given either a second dark treatment (5 hrs darkness with IAA treatment of 50 mg/l) or defoliated as indicated.

time each treated group of plants was removed from the second dark period. These controls remained vegetative, demonstrating that the stimulus was still in the leaves after the second dark treatment.

Figure 1 shows that if the second dark treatment is given immediately following the inductive dark period, nearly complete inhibition is observed. If the second dark treatment is delayed its effectiveness is reduced until, after exposure of the plants to 6 to 8 hours of light, the flowering response can no longer be affected by the second dark period. Since the stimulus was still in the leaves but was no longer destroyed by the second dark treatment, some change must have taken place in the nature of the stimulus or the leaf during the time it was exposed to the light making the second dark treatment no longer effective. This change will be referred to as the stabilization of the stimulus.

EXPERIMENT 2: In an attempt to define more clearly the nature of the destruction of the stimulus, the effect of temperature on the second dark period was examined. The plants were given the usual 12-hour inductive dark period, terminated by 10 minutes of light. Some of the plants were then treated with IAA, treated and untreated groups were placed in the usual dark room (20–25° C) and similar groups in a refrigerated dark room (3–5° C). After 5 hours all groups were returned to the fluorescent lights.

It may be seen (table I) that at low temperatures the effectiveness of the second dark period is markedly reduced as compared to the usual temperatures used, even with IAA application. These results are interpreted to mean that the second dark treatment results in the destruction of the stimulus by a thermochemical reaction.

EXPERIMENT 3: In a number of experiments, it was desirable to determine the time of movement of the stimulus from the leaves. This was accomplished by removing all the leaves of a size greater than 0.5 cm in length from groups of plants at specified times after the end of the inductive dark period. In order to insure that the small leaves often present on the lateral buds would not contribute stimulus, the lateral buds in the axils of all mature leaves were removed at the same time. The plants were kept under the fluorescent lights from the end of the dark period until after defoliation and later returned to the long day greenhouse.

TABLE I

INFLUENCE OF TEMPERATURE ON THE SECOND DARK PERIOD EFFECT *

TEMP. ° C	AVERAGE STAGE OF FLOWERING	
	NO IAA	IAA (50 MG/L)
20–25	3.0	1.0
3–5	3.9	3.1

Control: Average stage of flowering—4.4

* Following a 12-hr inductive dark period (terminated by light), the plants were given a 5-hr second dark period under the conditions indicated.

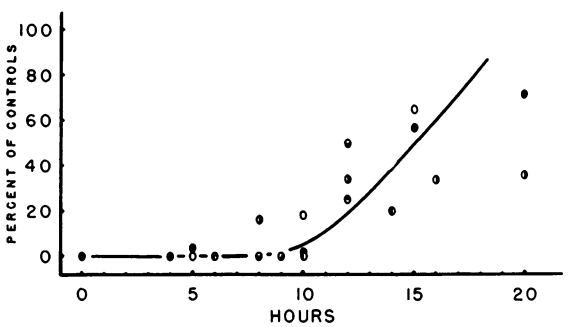


FIG. 2. The time of movement of the flowering stimulus out of Xanthium leaves following a 12-hour inductive dark period. The plants were placed under fluorescent lights (1000–1200 fc) following the dark period and groups of plants were defoliated at various intervals thereafter. The results are expressed as percentage of the average stage of flowering compared to the controls. The results presented here are from seven separate experiments.

In certain experiments defoliation of some plants was delayed 7 days following the dark period and it was found that, in these cases, the flowering response was fully equal to that of the undefoliated controls. Since there is little morphological change in the growing point before such defoliations, it may be concluded that a continuous supply of metabolites from mature leaves is not a necessary factor during the 3-week period allowed for development of the floral primordia. It also supports the contention that injury which would interfere with floral initiation and development is not caused by the defoliation procedure.

The results of 7 separate experiments are presented in figure 2, demonstrating that the stimulus ordinarily does not begin to move out of the leaves

TABLE II

THE EFFECT OF TEMPERATURE ON THE PHOTOCHEMICAL STABILIZATION OF THE FLORAL STIMULUS *

TEMP. °C	HRS OF LIGHT						
	0	0.5	1	2	4	5	8
30	32	32	50	36	64	..	81
5	..	21	50	29	69	..	**
Control: Average stage of flowering—4.2							
30	27	..	..	..	..	88	..
5	..	..	..	..	..	92	..
Control: Average stage of flowering—4.9							
30	0	..	22	..	59	..	..
5	..	..	15	..	73	..	..
Control: Average stage of flowering—4.1							

* The results are expressed as a percentage of the average stage of flowering of the control which received no second dark treatment. Treated plants were given a 12-hr inductive dark period, treated with light (1200 fc) at the temperature indicated, then given a second dark treatment (5 hrs darkness with IAA). The results of 3 separate experiments are presented.

** Plants badly wilted.

until approximately 10 hours after the termination of a 12-hour dark period. A level of flowering approaching that of the controls may be obtained if the leaves are allowed to remain on the plant for about 24 hours after the end of the inductive dark period.

EXPERIMENT 4: In further experiments the exposure to light of high intensity was given some plants at a low temperature (4 to 6° C) and compared to the effectiveness of the stabilization process at normal temperatures (ca. 29° C). After an inductive dark period, plants were placed at the desired temperatures under fluorescent lights giving intensities at the leaf surface of approximately 1200 fc. After various time intervals groups of plants were removed and the remaining non-stabilized stimulus was destroyed by exposure to a second dark treatment (IAA and 5 hours darkness at about 25° C). They were then returned to fluorescent lights at normal temperatures.

These experiments (table II) seem to indicate that the stabilization process progresses equally rapidly at these low temperatures, giving a Q_{10} of approximately one for temperatures between 5° and 30° C for the stabilization process. This would indicate that the rate-limiting reaction in this process is not a thermochemical reaction.

EXPERIMENT 5: It has been shown above that the presumed stimulus is stabilized in light even at low temperatures, while the second dark treatment was ineffective in destroying the stimulus under low temperature conditions. This response makes it possible to determine whether light is necessary to stabilize the stimulus or whether the light prevents its destruction while the stabilization process itself proceeds independently of light (e.g., a diffusion process). If the second possibility is the correct one, then 5 hours of darkness at low temperature would allow stabilization of the stimulus and the stimulus would be unaffected by a subsequent dark treatment at an elevated temperature. If, on the other hand, the light is directly required to stabilize the stimulus (a photochemical reaction), then a dark treatment at high temperature following the low temperature dark period should result in a destruction of the stimulus. The destruction observed should be essentially equal to that obtained when the second dark treatment is given immediately following the inductive dark period.

An experiment to test these possibilities was designed as shown in figure 3, and the results are tabulated in table III. The results show that, after 5 hours darkness or low intensity light (50 to 100 fc), the stimulus is still completely destroyed by a dark treatment at the elevated temperature (3 hours at 40° C). If the plants instead were exposed to 5 hours of high intensity light (1000 fc), at either low or normal temperatures, the stabilization was essentially complete at the end of such treatment. In this experiment the 5 hours of darkness at normal temperature (treatment V) failed to give destruction of the stimulus. This demonstrates the variability in

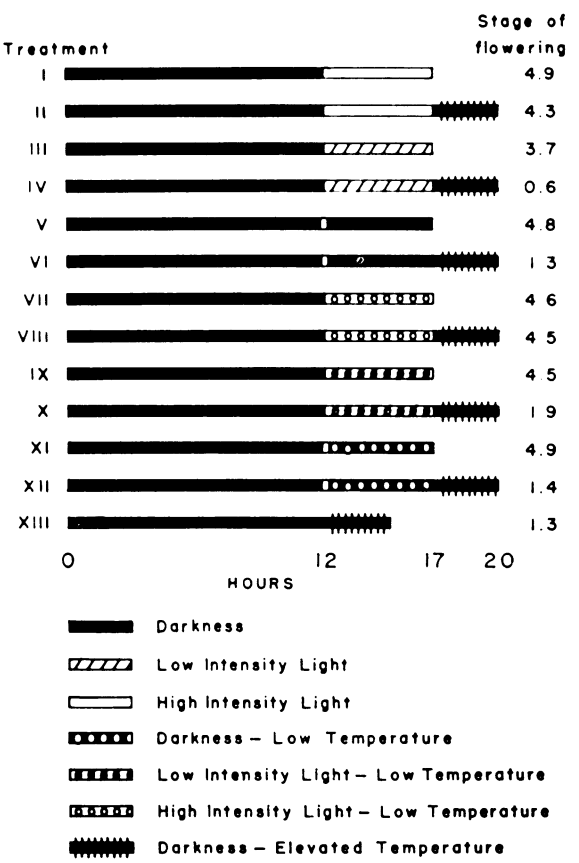


FIG. 3. The effect on the stabilization of the flowering stimulus of various light and temperature treatments for 5 hours immediately following induction. The non-stabilized stimulus was destroyed by exposure to a 3-hour dark treatment at 40° C. See also table III.

TABLE III

THE EFFECT ON THE STABILIZATION OF THE FLOWERING STIMULUS OF VARIOUS LIGHT AND TEMPERATURE TREATMENTS FOR 5 HOURS IMMEDIATELY FOLLOWING THE INDUCTIVE DARK PERIOD *

TREATMENT DURING 5-HR STABILIZATION PERIOD		% STABILIZATION **
TEMP. ° C	LIGHT (FC)	
25–30	1000	83
	100	-13
	0	0
4–6	1000	98
	100	22
	0	2

* The non-stabilization stimulus was destroyed (73 %) by exposure to a dark treatment at elevated temperature (3 hrs at 40° C). See also figure 3.

** Percent stabilization: $\frac{A - (A - B) \times \frac{4.9 - 1.3}{4.9}}{A} \times 100$

where A is the average stage of flowering observed as a result of the experimental treatment without a second dark treatment (see fig 3), and B is the average stage of flowering observed as a result of the experimental treatment followed by the second dark treatment (40° C for 3 hrs). The figures 4.9 and 1.3 are the average stage of flowering observed respectively in the control and in those plants where the second dark treatment immediately followed the inductive dark period.

the effectiveness of the second dark treatment at room temperature, as discussed previously (3). In this treatment as well, subsequent exposure to elevated temperature (treatment VI) demonstrated that the stimulus had not been stabilized in the dark, even though no destruction had occurred. Thus, the conclusion that light is directly necessary for the stabilization of the stimulus is reached independently of the validity of the experiments on the temperature coefficient of the stabilization process.

Included in the above experiment were treatments under conditions of relatively low intensity light, obtained by placing sets of plants at some distance from the fluorescent lights used to provide the light of high intensity. It has been pointed out previously (3), that low intensity light and darkness exert qualitatively the same effect when they follow an inductive dark period. In this experiment, darkness following the inductive dark period resulted in no apparent destruction of the stimulus. The low intensity light, however, gave a marked reduction of the flowering response at normal temperature (treatments III and IV). The destruction of the stimulus precursor by exposure to low intensity light accounts for the negative percentage of stabilization in treatment IV. Few direct comparisons may be made, but low intensity light has not been found previously to be more effective than darkness in causing inhibition.

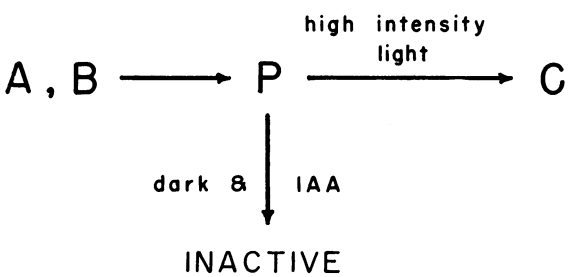
Control plants placed, following induction, under normal temperature fluorescent lights and defoliated 8 hours after the end of the dark period were all vegetative.

DISCUSSION

The length of the critical period of Xanthium is independent of the amount of leaf tissue present (5), and of the number of cycles of treatment (4). This demonstrates that two distinct processes, necessary for the formation of the floral stimulus, are taking place during the dark period. The first may be referred to as the "timing mechanism" which will reach a critical level after a standard length of time, determining the critical period. This process could not represent the total effect of the dark period since if this were the case the effect of darkness on flowering would be quantitative. In order to explain a critical period with the characteristics indicated above it must be postulated that a second process, resulting in the accumulation of the stimulus, will begin upon the attainment of a critical level of the first. In Xanthium this second process usually attains a maximum after approximately 15 hours of darkness, as indicated by the fact that a dark period of approximately this length will usually give maximum flowering. It seems likely that the optimum length of the dark period is governed by the amount of substrate available from the previous high intensity light treatment, shown by Hamner (1) to be required immediately prior to the inductive dark period. Thus at the end of an inductive dark period some condition has been attained which, upon the return of the plant to light, will result in the formation of the floral stimulus in the leaf.

The results presented here indicate that a further reaction, requiring light, normally takes place in the leaf subsequent to the inductive dark period and before the stimulus is translocated from the leaf. Prior to this photochemical reaction the stimulus produced in the inductive dark period may be considered to be present in the form of a precursor, which is changed as a result of the action of light to the final form in which it is exported from the leaves.

These observations may be schematized in the following form in which the A and B represent the



initial high light requirement and the long (inductive) dark period requirement respectively, as defined by Hamner (1). Retaining Hamner's definition of C as the final stimulus transported from the leaf, it has been shown here that an intermediate condition P must be included. The P is the precursor of the final stimulus, present at the end of an inductive dark period which, in the presence of high intensity light,

will give rise to the stable flowering stimulus, but which may be destroyed by a second exposure to darkness, especially in the presence of IAA.

The reaction P → C represents the stabilization of the precursor present at the end of the inductive dark period. It has been shown that this reaction requires relatively high intensity light for rapid (6 hours) completion, although as has previously been pointed out, the light is not an absolute requirement. If the inductive dark period is not interrupted and the plants kept in continuing darkness for 3 weeks they will initiate flowers at approximately the same rate as if they had received a single photoinductive cycle (6). In this case the inductive dark period is not broken and an interruption of induction seems to be necessary before the second dark period is effective in destroying the precursor P.

An alternative explanation would be that, at the end of an inductive dark period, the leaf is capable of destroying the stimulus in the absence of high intensity light. Then, as a result of exposure to light for several hours, the leaf is rendered incapable of destroying the floral stimulus. The P would represent the floral stimulus under conditions in which destruction was possible. The conversion to C would be a change in the leaf to a condition in which destruction of the stimulus would no longer occur under conditions of darkness and IAA treatment.

The overall stabilization process has been shown to have a temperature coefficient approaching unity. This would seem to indicate that a photochemical reaction, requiring high intensity light, was the rate limiting step in the stabilization process. The rate of stabilization has only been adequately established at 5° and 30° C, and studies at intermediate temperatures would be required to definitely establish the effect of temperature on the rate of the reaction. A single experiment, in which an intermediate temperature (16–18° C) was included, supported a temperature coefficient approaching unity. However, as indicated above, the conclusions reached in this paper do not depend on the validity of the temperature coefficient of the stabilization process.

The reaction P → inactive, the reduction of the flowering response as a result of exposure to a second dark period, has been reported previously (3), and the increased effectiveness of the second dark treatment at high temperatures will be reported in greater detail at a later date. It is possible that the inactivation of the precursor might represent a reversal of the reaction A, B → P. Since it is not as yet known whether this is the case, it is necessary to separate the two processes until their identity may be established. Whether the second dark period and IAA act on the same reaction is also uncertain, although the demonstration of an apparent interaction (3) makes it appear likely that this is the case.

It should be emphasized that the partial processes indicated here do not necessarily represent single chemical or physical reactions but may include a series of reactions. The partial processes would then represent only those steps in the overall process affected by the particular environmental conditions to which the plants have been exposed. The separation of the various partial processes as reported here should help make possible more discriminating experiments designed to elucidate the various chemical steps involved in the formation and transport of the flowering stimulus.

Summary

1. The flowering response of Xanthium may be markedly reduced by treatment with IAA and a second dark period following a 12-hour inductive dark treatment.

2. Several hours of high intensity light following the inductive dark period renders the inhibitory dark treatment no longer effective. This is referred to as the stabilization of the floral stimulus.

3. It is postulated that a precursor of the floral stimulus is present at the end of a 12-hour inductive dark period which may be destroyed by IAA and darkness or converted to the floral stimulus by high intensity light.

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