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INDUCTION OF FLOWERING IN LONG DAY PLANTS BY APPLIED INDOLEACETIC ACID[1,2]

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In short day plants the promotion of flowering by photoinductive cycles may be nullified by the application of auxin (2) and by interruption of the dark period with red light (17). Both of these inhibitory effects can be reversed by the application of an anti-auxin (1, 2, 12). On the other hand light interruption of the dark portion of the photoinductive cycle promotes floral induction in long day plants (3, 8). On the basis of these results we might expect either that the flowering of long day plants would be inhibited by auxin applied during photoinduction, or that auxin would replace or supplement low intensity light and actually cause flowering of plants kept in a short day regime. The experiments reported in this paper were undertaken to try to discover the role of auxin in the flowering of long day plants. The results demonstrate for the first time that auxin given in addition to low intensity light may cause flowering of long day plants under conditions in which the non-auxin treated controls remain vegetative.

MATERIALS AND METHODS

Two long day plant species, *Hyoscyamus niger* (annual variety) and *Silene Armeria,* were used for the experiments reported here. Experiments with a third long day plant, *Crepis capillaris,* also indicate a flower promoting effect of auxin applied to plants grown under threshold conditions. This material, however, proved so variable, that the experiments, taken by themselves, would not be conclusive and are not presented here. The photoperiodic response of Hyoscyamus was studied by Lang and Melchers (9). The action spectrum for the light flash reaction in this species was determined by Parker and co-workers and was found to be identical with the photoperiodic action spectrum of other day-length-dependent plants of both the long day and short day types (16). The strain used in our experiments is the same as that used by earlier workers (annual yellow flowered variety). The photoperiodic behavior of Silene, a species not previously used for photoperiodic work, was studied by Liverman and Lang (12, 14) and was found to be typical for a long day plant. This strain[5] was collected in the yard of one of us (J. L.) in San Gabriel, California where it had grown spontaneously for a number of years. A majority of the experiments reported below were carried out in the con-

[1] Received November 22, 1955.

[2] Report of work supported in part by the Lalor Foundation, the National Science Foundation, and the Frasch Foundation for Agr. Chemistry. Data in this paper were first presented at Cornell meetings, Amer. Soc. Plant Physiol., Sept., 1952.

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trolled environmental conditions of the Earhart Plant Research Laboratory (19). Hyoscyamus was grown in a gravel-vermiculite mixture, Silene in a gravel-sand-vermiculite mixture. All plants were watered daily with Hoagland's nutrient solution.

Experiments with both species were conducted under threshold conditions consisting of a basic 8-hr period of high intensity light given either in the greenhouse or in rooms lighted by panels of fluorescent and incandescent lamps followed by 16 hours of supplementary light of different intensities. In the early experiments the variations in intensity of the supplementary light were achieved by placing the plants at different distances from a 15-watt incandescent bulb suspended well above the level of the plants. In some later experiments with Hyoscyamus the plants were given supplementary light in individual rooms where the intensity was adjusted with the aid of variable voltage regulators (powerstats). In all experiments each container of plants at a given intensity station was shifted daily according to a schedule to insure uniform supplementary light treatment of all plants at that station. This light regime was selected since it is to be expected that the effects of various experimental treatments on the photoperiodic response become most apparent if treatment is applied under conditions wherein the plants are just below or just above the boundary which separates inductive from non-inductive photoperiods, or flowering from non-flowering. The intensity of the supplementary light was measured with a photometer which had been calibrated against a standard lamp. In the experiments with the powerstats possible differences in spectral characteristics of the light were not considered.

Immediately after the end of the 8-hr period of high intensity light the leaves were thoroughly wetted by spraying with various concentrations of indoleacetic acid (IAA) containing approximately 0.001 % Atlas Tween 20 or Tween 80 as a wetting agent.

Tween 20 and Tween 80 are the monolaurate and the mono-oleate respectively of polyoxyethylene sorbitan. The controls were sprayed with water alone containing Tween. New IAA solutions were made every two or three days and were stored in a cold room at approximately 5° C between sprayings.

Experiments with Hyoscyamus niger

The plants used in the first experiment were grown in Earhart Plant Research Laboratory under an 8-hr day until they were 10 weeks old. Prior to the experimental treatment the plants were grown at a temperature of 23° C day and 20° C day–17° C night, being shifted at regular intervals from one temperature to the other. At the end of this growth period the plants were randomized into 40 treatment groups and put under threshold conditions. During the 17 to 25 days of threshold treatment, the plants received high intensity light at 23° C in the greenhouse and were then sprayed daily with various concentrations of IAA before being moved to the darkroom for supplementary light. Five different intensities of supplementary light were used, the plants receiving a total of 218, 71, 34, 20 or 16 fc min/night. After 17 days, all plants receiving the highest level of supplementary light had elongated and their treatment was discontinued. The other series were treated for a total of 25 days. At the end of the experimental treatment those plants which did not show visible buds were returned to the greenhouse for growth under short day conditions for an additional 11 to 19 days prior to being examined for the presence or absence of microscopic flower buds.

Results: All of the control and experimental plants at the two highest intensities initiated flowers with the single exception of 2 of 6 plants at the second intensity which were sprayed with 30 mg/l of IAA. The results for the 3 lowest intensities are presented in figure 1. At these intensities non-auxin treated controls remained vegetative whereas auxin treated plants showed a marked inductive response with 45 to 100 % of the treated plants initiating flowers. The optimum indoleacetic acid concentration in this experiment appears to lie between 3 and 10 mg/l, with 30 mg/l being less effective. The data in table I, in which the number of leaves produced during the experimental period is given, shows that this promotive effect of auxin is quite specific. The plants treated with IAA and induced to flower produced only about one half as many leaves as the plants which did not initiate flowers, thus, auxin did not simply accelerate the growth of the plants, but actually caused the initiation of flowers. Only at the highest auxin concentration does there appear to be a discrepancy to this conclusion. This discrepancy may possibly be attributed to the reduced growth rate of the plants treated with such a high auxin concentration.

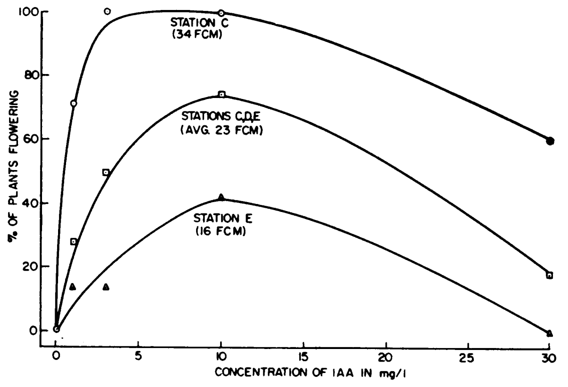
These results were confirmed in all essential respects in four additional experiments. Data of one of these experiments are summarized in figure 2. A comparison with figure 1 shows that the results of the

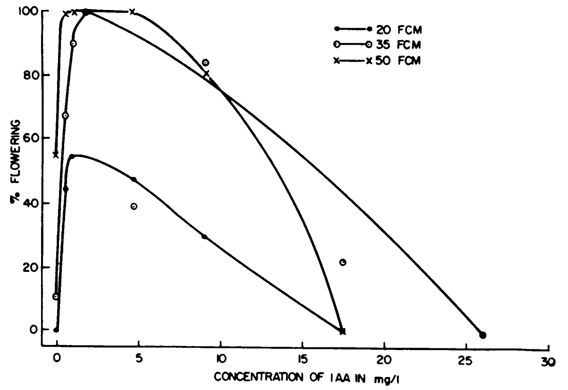
Table I

Average Number of New Leaves Formed by Hyoscyamus Plants from Start of Treatment to Flower Initiation or Dissection

Amt of suppl light, fc min	State	Conc IAA, mg/l				
		0	1	3	10	30
218	Flowering	7.3	7.9	10.4	10.9	11.8
	Non-flowering	. . .	. . .	. . .	. . .	. . .
71	Flowering	13.9	13.6	13.9	12.9	15.4
	Non-flowering	. . .	. . .	. . .	. . .	20.6
34	Flowering	. . .	17.2	16.5	16.6	19.9
	Non-flowering	25.0	30.5	. . .	. . .	19.1
20	Flowering	. . .	. . .	. . .	17.4 †	. . .
	Non-flowering	33.3 *	33.0 *	23.9 **	36.0 ** †	23.8 **
16	Flowering	. . .	13.0 †	17.0 †	19.6	. . .
	Non-flowering	34.8	32.4	30.1	27.1	30.8

* Dissected 11 days after end of treatment.
** Dissected 9 days after end of treatment.
† One plant only.





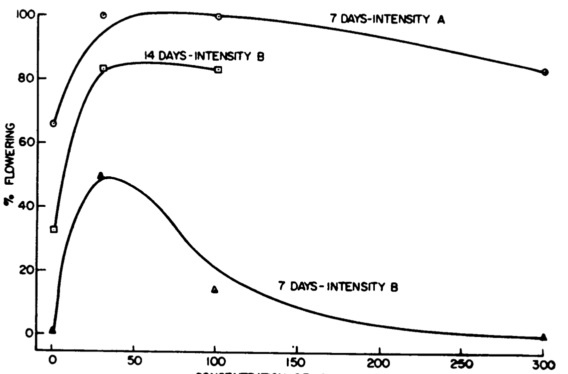


FIG. 1 (*upper*). Floral induction in *Hyoscyamus niger* as a function of light intensity and IAA conc.

FIG. 2 (*center*). Additional experiment showing effect of light and auxin on floral induction in *Hyoscyamus niger*.

FIG. 3 (*lower*). Floral induction in *Silene Armeria* by light and IAA. Plants at station A received approximately 2000 fc min of supplementary light, at station B approximately 400 fc min.

two experiments are quite similar except that the optimum auxin concentration for flower promotion in the last experiment is lower, and that the higher auxin concentrations completely suppress flowering. It was found that the optimum auxin concentration may vary between approximately 3 and 30 mg/l and also that the light intensity at which the auxin effect becomes evident may vary from one experiment to another over an approximately 2-fold range. These differences from experiment to experiment may be related to the age and general condition of the plants. This difference in auxin concentration needed to give the maximum response is particularly noticeable when comparing plants given their high intensity light as artificial light with those receiving their light period in the greenhouse. Plants receiving high intensity light from the artificial source (fig 2) have uniformly given a maximum response at lower auxin concentration than greenhouse grown plants. In any case the important fact is that auxin shows a marked inductive effect in both these experiments as well as in other similar experiments not reported here.

EXPERIMENTS WITH SILENE ARMERIA

The type of experiment reported above for *Hyoscyamus niger* has been completed with another long day plant, *Silene Armeria*. All experiments reported below were conducted in Earhart Plant Research Laboratory under controlled conditions of temperature and light intensity. Prior to the experimental treatment, the plants were grown under a regime of eight-hour days in the greenhouse at 20° C and 16 hours of darkness at 14° C. When the plants were 2.5 months old, they were randomized and placed in a dark room maintained at 23° C. Here they received 450 to 750 fc of light from a fluorescent source plus light from four 75-watt incandescent bulbs from 8 A.M. until 4 P.M. for 3 additional short days during which they were sprayed with IAA or water solutions containing Tween 80. The plants were then placed under threshold conditions and the spraying continued. These threshold conditions consisted of a basic period of high intensity light as described above from 8 A.M. to 4 P.M. and supplementary light from a 15-watt frosted incandescent bulb from 4 P.M. until 8 A.M. One-half of the plants from each treatment group were removed to short day conditions in the greenhouse after 7 days of threshold treatment and the remaining one-half at the end of 14 days. The plants were dissected 30 days from the beginning of the threshold treatment.

RESULTS: The results of the experiments with Silene are shown in figure 3. It is obvious that Silene is not as responsive to low intensity light as is Hyoscyamus since it takes approximately 2000 fc minutes of light for even partial induction. It is also obvious that plants treated with IAA show a marked increase in flowering.

DISCUSSION

So far as the authors are aware this report presents the first evidence that auxin applied to a long day plant causes flowering under conditions in which the controls remain vegetative, i.e., this is the first evidence for a qualitative effect of auxin on the flowering of a long day plant. It should be noted, however, that at least 3 day-neutral plants, the pineapple, the sweet potato, and the litchi have been induced to flower by auxin application (5, 6, 18). Previous experiments with long day plants, which were carried out by various workers (7, 10, 11), have shown that the flowering of a long day plant may be quantitatively increased by low concentrations of auxin. This effect, however, was observed only under strict long day conditions and it is not clear whether it is specific. No effect of auxin was observed under short day conditions. Only Claes (4) has conducted experiments under semithreshold conditions and even under these conditions no effect of auxin was observed. Our results show that induction by applied auxin becomes apparent only under the very specific conditions wherein auxin has been made the limiting factor. These conditions were achieved by bringing the plants very near the threshold for flowering by exposure to small amounts of supplementary light. Under conditions where neither auxin nor light alone are effective in inducing flowering, the two are able to interact in such a way as to cause flowering. The exact mechanism of this interaction is not known although some, as yet, unverified suggestions have been made (13, 15). A thorough understanding of this interaction should lead us closer to a solution of the mechanism of floral induction.

Summary

It has been shown in 7 separate experiments that 2 long day plants, *Hyoscyamus niger* and *Silene Armeria*, can be induced to flower by the application of IAA to the leaves of plants grown under threshold conditions.

The authors wish to express their thanks to Dr. F. W. Went for his kind permission to use the facilities of the Earhart Plant Research Laboratory, and to Dr. James Bonner for his generous advice and criticisms during the course of these experiments and in preparation of the manuscript. The senior author also wishes to thank Mrs. Lucy Mason Clark for the generous support provided by the Lucy Mason Clark Predoctoral Fellowship during the last two years of graduate study, 1950–1952.

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